FEEDING PEA TO MODULATE POST-PRANDIAL METABOLIC RESPONSE: IMPACT ON WELFARE, HEALTH AND PERFORMANCE OF FEED RESTRICTED BROILER BREEDERS

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By

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ABSTRACT

An experiment was conducted for 244 d to investigate the impact of feeding a pea or wheat based diet on post-prandial metabolic status, production, health and welfare of feed restricted broiler breeders. A wheat- or pea-based diet was fed on an every-other-day basis from 3-15 wk of age and every-day basis thereafter (15-21 wk). At 21 wk of age, broiler breeder pullets were fed either a pea- or wheat-based diet once (OAD; 08:00) or twice (TAD; 08:00, 15:00) a day until the end of experimental period. At 84 d of age, uniformity was improved by feeding a pea-based diet. Broiler breeders fed a pea-based diet resulted in a reduced post-prandial glucose peak and relative liver weight and fat content over the period between meals. A treatment by time interaction showed that less fat was stored in the liver over the period of 48 h as a result of feeding a pea-based diet. Over the 48 h period between feeding, the expression of acetyl CoA carboxylase and VLDL-apolipoprotein was reduced for birds fed pea. Interaction effects of dietary treatment and time demonstrated a reduced degree of change (over the period of 48 h) in the expression of malic enzyme genes in pullets fed a pea-based diet. Birds fed a wheat-based diet demonstrated increased drinking over the 48 h period. Interactions between diet and day showed that pullets fed a pea-based diet expressed increased comfort and resting behavior, and reduced foraging and walking behavior during the off-feed day (24-48 h). Feeding a pea-based diet resulted in reduced levels of serum non-esterified fatty acids and β-hydroxybutyrate starting at 26 to 28 h post-feeding. During the laying phase, feeding the wheat-based diet on TAD basis improved hen-day egg production as compared to the OAD feeding program, but feeding frequency did not affect production of hens fed the pea-based diet. Hens fed the pea-based diet once-a-day had a lower average egg weight than other diet by feeding frequency subclasses. Feeding a pea-based diet reduced total embryonic mortality. In conclusion, feeding a pea-based diet resulted in improved performance and welfare of feed restricted broiler breeders.

ACKNOWLEDGEMENTS

DEDICATION

"This thesis is dedicated to my parents and family, who have always encouraged me to follow my

dream"

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LIST OF ABBREVIATION

ACC	Acetyl-CoA carboxylase
AME	Apparent Metabolizable Energy
AOAC	Association of Official Analytical Chemists
apoVLDL- II	Apolipoprotein VLDL-II
CCK	Cholecystokinin
CP	Crude Protein
CS	Compound Symmetry
CV	Coefficient of Variation
DM	Dry Matter
ED	Every-Day
EOD	Every-Other-Day
FLHS	Fatty Liver Hemorrhagic Disease
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GIP	Gastric Inhibitory Polypeptide
GLP-1 and 2	Glucagon-like Peptide-1 and 2
GLPH	Glycogen Phosphorylase
GLSY	Glycogen Synthase
GPCR	G-Protein Coupled Receptor
H/L Ratio	Heterophil/Lymphocytes Ratio
HDEP	Hen Day Egg Production
LSD	Least Square Difference
LYF	Large Yellow Follicle
ME	Malic Enzyme
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NEFA	Non-Esterified Fatty Acids
NRC	National Research Council
OAD	Once-a-Day
PRDX6	Peroxiredoxin-6
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SCFA	Short Chain Fatty Acid
SD	Standard Deviation
SDS	Slowly Digestible Starch
SEM	Standard Error of Mean
SGLT-1	Sodium Glucose Co-transporter-1
SREBP-1	Sterol Regulatory Element Binding Protein
SYF	Small Yellow Follicle
TAD	Twice-a-Day
VLDL	Very Low Density Lipoprotein
YFBM	Yolk Free Body Mass
β-HBA	β-hydroybuyrate

1   INTRODUCTION

Genetic selection of broilers for faster growth for the last six decades has resulted in a

massive increase in appetite, attributed to changes in mechanisms (central and peripheral)

responsible for regulating hunger (Lacy et al., 1985; Denbow, 1989). In turn, ad-libitum

feeding of broiler breeders results in excessive weight gain, which has a negative impact

on production, health and welfare. More specifically, previous studies have shown

increased mortality (skeletal and metabolic), disrupted follicular hierarchies, and reduced

reproductive performance (Robinson et al., 1991; Mench, 2002; Renema and Robinson,

2004) during the laying phase. Feed restriction is an important management tool to

control body weight of rapidly growing broiler breeders to optimize reproductive

performance. Advantages of feed restriction include lower mortality, a lower incidence of

double hierarchies and abnormal eggs, higher egg production and better persistency of

lay (Renema and Robinson, 2004). Despite the benefits of feed restriction, it is often

criticized from a welfare perspective because of the length of time birds are without feed

and as a consequence hungry. Previous studies have looked at various physiological

(glucose/NEFA ratio, corticosterone and H/L ratio) and behavioural measures (drinking,

spot pecking, time taken to consume meal and comfort behaviours) to quantify hunger

and found that feed restricted birds suffer from the state of hunger (Kostal et al., 1992;

Savory et al., 1993; de Jong et al., 2003).

Feed restriction in broiler breeders during the rearing phase is applied using various

feeding programs including feeding every-other-day (EOD), 5-2 (fed on five out of every

7 days) or every-day (ED). Feeding every 48 h is the most commonly used feeding

program with an objective to improve flock uniformity. The impact of various feeding

program on health and performance on broiler breeders was well studied. de Beer and Coon (2007, 2009) found that ED feeding resulted in higher egg production and better efficiency as compared to an EOD feeding regimen. Spradley et al. (2008) compared ED and twice-a-day (TAD) feeding and found that increased frequency of feeding resulted in increased egg production and egg weight. Based on these findings, it can be concluded that increased frequency of feeding results in improved efficiency in feed restricted broiler breeders. This effect is possibly attributed to the reduced degree of storage and mobilization of nutrients with increased frequency of feeding (de Beer and Coon, 2007). As EOD feeding program is still very popular in the commercial industry, nutritional strategies to further reduce the degree of nutrient storage and mobilization to improve health and productivity are warranted.

Extensive research has been conducted to reduce the degree of the negative impact of feed restriction on welfare of broiler breeders. Previous studies have looked at various managemental and nutritional strategies such as the use of a foraging feed program and use of diluted feed (Zuidhof et al., 1995; Hocking et al., 2006; de Jong et al., 2011; Nielson et al., 2011). Rationale for using scattered feeding or dietary dilution includes increased time spent undertaking eating behaviour and improved satiety related to more gut fill. Recently, the use of dietary appetite suppressants was studied to improve the satiety of feed restricted broiler breeders (Morrissey et al., 2014a, 2014b). The authors concluded that use of alternative diets resulted in reduced stereotypic feather pecking, thereby signaling improved satiety. Another nutritional alternative may be to feed a diet based on slowly digested feed ingredient such as pea.

The functional significance of slowly digestible starch (SDS) is mainly attributed to slow release of glucose which subsequently alters physiological and metabolic processes. Slowly digestible starch reduces the fluctuations in post-prandial glucose and insulin concentration and has been shown to reduce the incidence of health issues including oxidative damage, metabolic syndrome, cardiovascular problems and obesity (Lehmann and Robin, 2007). Furthermore, human research has shown that slowly digestible starch results in reduced hunger and improved satiety (Anderson et al., 2010). Improved satiety with SDS is attributed to slow and prolonged starch oxidation in comparison to rapid oxidation with rapidly digested starch (RDS) (Sparti et al., 2000). Another mechanism relates to the role of incretins including GLP-1. Wachters-Hagedoorn et al. (2006) demonstrated that SDS resulted in increased release of GLP-1, which has been related to reduced gastric emptying and SI transit, and as a consequence, improved satiety (Edholm et al., 2010). Therefore, sustained and slow release of nutrients by using slowly digestible starch has potential to reduce the degree of post-prandial nutrient storage and mobilization and the severity of negative effects related to feed restriction in broiler breeders.

Previous research showing the impact of SDS on performance and health of poultry species is limited. Weurding et al. (2003) demonstrated that the addition of some SDS to diets improves broiler performance. They suggested that improved efficiency with SDS might be attributed to improved synchronization of amino acid and energy digestion. Based on the physiological and metabolic effects in humans (Lehmann and Robin, 2007), incorporation of SDS into diets has potential to improve productivity of feed restricted broiler breeders by reducing the degree of post-prandial and post-absorptive nutrient

storage and mobilization, thereby improving efficiency. Furthermore, SDS has the potential to improve satiety in feed restricted broiler breeders. To our knowledge, no research has been conducted to see the impact of rate of starch digestion on metabolic status, performance, health and welfare of feed restricted broiler breeders.

An experiment was conducted for 244 d to investigate the impact of feeding a pea (SDS) or wheat (RDS) based diet on post-prandial metabolic response (glucose levels, liver weight, fat and glycogen content, and expression of genes encoding for synthesis and lysis of hepatic fat and glycogen), production (uniformity, weekly body weights, egg production, reproductive performance, chick yield and early post natal growth), health and welfare (behavioural and physiological indicators of hunger and satiety) of feed restricted broiler breeders. The relevant sections of the research are outlined in Figure 1.1. It was hypothesized that feeding a diet based on pea will reduce degree of nutrient storage and mobilization during the post-prandial phase, thereby improving the performance of feed restricted broiler breeders. Furthermore, a slower rate of starch digestion associated with feeding a pea-based diet will result in better welfare by improving satiety.

Feeding pea- or wheat-based diet to feed restricted broiler breeders from 21-244 d of age.

Chapter 3	Chapter 4	Chapter 5	Chapter 6
• Growth and productivty during rearing phase (body weight, feed intake and uniformity) during the rearing phase. • In-vitro starch digestibility. • Digesta and gut mass.	• Blood glucose levels. • Hepatic fat and glycogen levels. • Gene expression (hepatic fat and glycogen synthesis and lysis).	• Behavioural parameters of hunger. • Physiological paprameters of hunger.	• Growth during laying phase (body weight, feed intake and uniformity). • Laying and reproductive performance. • Post-natal growth.

Figure 1.1 A schematic depiction of the organization of the experimental data in the

thesis.

2   LITERATURE REVIEW

2.1   Broiler Breeders

Broilers have been selected for decades for a variety of characteristics including growth rate. The successful and extensive selection, while providing benefits for the production of chicken meat (Renema et al., 2007), make broiler breeders difficult to manage. Genetic selection for faster growth has resulted in a corresponding large increase in appetite, attributed to changes in mechanisms (central and peripheral) responsible for regulating hunger (Lacy et al., 1985; Denbow, 1989; Richards and Proszkowiec-Weglarz, 2007). In a recent review, Classen (2017) concluded that modern broiler chickens are unable to precisely alter the feed intake in response to dietary energy. As a consequence, if fed on an ad-libitum basis, broiler breeders can grow to an extent that negatively impacts bird health and reproductive performance (Savory and Maros, 1993; Mench, 2002). To prevent these negative effects, broiler breeders are only fed on an ad-libitum basis for the first three weeks after hatch and then are feed restricted for the remainder of their lives. Feed restriction is most severe during the rearing phase and is applied using various feeding programs including feeding every-other-day (EOD), feeding five out of every seven days (5-2) or every-day (ED; de Beer and Coon, 2007). Feeding EOD (every 48 h) is a commonly used feeding program with the objective of improving flock uniformity. As sexual maturity approaches, birds are fed limited amounts of feed on a daily basis, the feeding program that persists into the breeding phase. However, the degree of feed restriction is less severe as birds require more nutrients in preparation for sexual maturity and for the production of eggs.

2.1.1 Feed Restriction and Welfare

Feed restriction is an important management tool to control the body weight of rapidly growing broiler breeders and to optimize reproductive performance. However, feed restriction has both positive and negative impacts on broiler breeder production and welfare. Advantages of feed restriction include lower mortality, a lower incidence of double follicular hierarchies and abnormal eggs (un-settable eggs), higher total egg production and better hatchability of eggs set. Previous studies have demonstrated that providing ad-libitum access of feed to broiler breeders resulted in increased mortality due to metabolic and skeletal problems, decreased productivity during the laying phase, disrupted follicular hierarchies (multiple ovulations) and reduced fertility (Robinson et al., 1991; Mench, 2002; Renema and Robinson, 2004).

Despite the benefits of feed restriction, it is often criticized from a welfare perceptive because of the length of time birds are without feed and as a consequence hungry. Previous research has demonstrated that feed restricted broiler breeders have increased motivation to eat and expression of stereotypic behaviours including excessive drinking, object pecking, hyperactivity and pacing before a meal (Hocking, 1993; de Jong et al. 2002; Mench, 2002; Riber et al., 2017). The increased desire to eat and expression of stereotypic behaviours are considered to be a reflection of a state of hunger. Physiological (glucose/non-esterified fatty acids ratio, corticosterone and heterophil/lymphocyte ratio) and behavioural measures (drinking, spot pecking, time taken to consume meals and comfort behaviours) have been used to quantify hunger in feed restricted broiler breeders (Kostal et al., 1992; Savory et al., 1993; de Jong et al., 2003).

2.2   Starch

Starch is the major storage polysaccharide in plants and considered a vital source of energy for humans and animals including poultry. The structure and shape of starch granule varies with sources, organs of the plant and stages of the development. The starch granule can be of various shapes including elongated, polygonal, oval, disk or spherical with diameter ranges from submicron to more than 100 microns (Briones et al., 1968; Jane et al., 1997; Vrinten and Nakamura, 2000; Li et al., 2007). Starch granules are composed of linear amylose and highly branched amylopectin molecules. Amylose is the linear polymer composed of glucose units bound with α 1, 4 glycosidic linkage. The chain length of amylose polymers varies from 200 to 700 glucose molecules with only 9-20 branches per molecule (Oates, 1997; Tester et al., 2004a, b). The amylose content of starch varies from very low in waxy starch to more than 50% in high amylose starch with a range of approximately 15 to 30% in most grains (Hovenkamp-Hermelink et al., 1987; Nakamura et al., 1995; Regina et al., 2006; Li et al., 2008). In contrast, amylopectin is a highly branched polymer made up of α 1, 4 linked glucose chains that are connected by α 1, 6 branch linkages (Oates, 1997). Based on the differences in the crystallization of the amylopectin molecule, starch can be categorised as A, B or C type. The double helix in A type starch is very compact with no space in the granule centre for water or any other molecules (Imberty et al., 1991). In B type starch, hydrogen bonds connect double helices to form a channel (Gallant et al., 1992). The C type starch is a mixture of A and B type. Generally, A type starch is present in cereal grains and tapioca, B type starch in tuber and high amylose cereals, and C type starch in legumes (Gallant, 1992; Eliasson and Gudmundsson, 2006; Jane et al., 1997).

2.2.1 Starch Digestion and Glucose Absorption

Digestion of starch in poultry starts with soaking of feed in the crop and subsequent grinding in the gizzard. Previously, it was demonstrated that saliva of chicken may contain some α-amylase, however the majority of starch digestion occurs in the small intestine (Riesenfeld et al., 1980; Osman, 1982). Starch digestion in the small intestine includes lumen digestion by the action of pancreatic amylase and further digestion in brush border membranes by the action of membrane proteins with saccharidase activity. Pancreatic amylase in the small intestine converts starch to maltose and some branched oligosaccharides (Gray, 1992). Thereafter, the enzymes, maltase and iso-maltase, convert maltose and other oligosaccharides to glucose. The digestion of complex dietary carbohydrates is completed by the various disaccharidases located in the brush border membrane of the jejunum (Leeson and Summers, 2001). Glucose released after the digestion of starch is absorbed into the blood stream using active transporters such as sodium dependent glucose transporter (SGLT-1), and insulin independent glucose transporters (Gray, 1992; Engelking, 2011).

2.2.1.1 Glucose Homeostasis in Poultry

The key differences in glucose homeostasis between poultry and mammals are hyperglycemia and the relative insensitivity to insulin (Vasilatos-Younken, 1986; Scanes, 2009) in poultry. The glucose level is considerably higher and ranges from 156 to 330 mg/dl in meat type commercial chickens (Scanes, 2008). Blood glucose levels are highly regulated in chickens and the impact of fasting on peripheral glucose is short term (Scanes, 2009; Edwards et al., 1999). Glucagon is the key hormone involved in glucose homeostasis and it increases glucose synthesis from protein and fat during events such as

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fasting (Kurima et al., 1994; Edwards et al., 1999). Chickens are relatively insensitive to insulin, however it plays an important role in maintaining glucose concentration in the fed state (Simon et al., 2000; Dupont et al., 2008).

2.2.1.2 Rate and Extent of Starch Digestion

The structure of starch (granule size, amylose and amylopectin content) influences the rate and extent of starch digestion. The digestion of starch increases with decreasing granule size, attributed to increase in surface area for enzymatic action (Franco et al., 1992; Tester et al., 2004b). The ratio of amylose and amylopectin also plays an important role in determining the rate and extent of starch digestion. The digestion of starch decreases with increasing amylose content due to a smaller surface area and the presence of hydrogen bonds linking amylose molecules, thereby making amylose less susceptible to amylase. Amylose can form complexes with compounds such as lipids, thereby reducing the exposure between enzyme and starch molecules (Zobel, 1988; Svihus, 2006). Furthermore, the lipid and protein content of starch granule negatively affects digestibility by reducing the exposure to digestive enzymes (Classen, 1996; Svihus et al., 2005). Based on a large granule size and higher amylose content, pea starch is considered to be slowly digestible in comparison to wheat with its small granule size and lower amylose content (Ratnayake et al., 2002; Weurding, 2002). Recently, Ebsim (2013) demonstrated that pea starch is slowly digestible in chickens with 19 to 26% of starch digested in the ileum.

The digestive characteristics of starch have been estimated using in vitro models with the rate of digestibility classified as either rapidly or slowly digestible. Further, the indigestible starch fraction is considered resistant starch. In-vitro starch digestibility

experiments categorized rapidly (RDS) or slowly digestible starch (SDS) as fractions digested from 0-20 or 20-120 min post incubation, respectively (Englyst et al., 1992). The rapidly digestible starch is digested and absorbed in the duodenum and proximal small intestine and produces higher post-prandial blood glucose levels. The rapid increase and decrease in blood glucose after consumption of rapidly digestible starch is undesirable in human nutrition as it, among other things, results in oxidative damage to organ and tissues by stimulating the overproduction of reactive oxygen species (Brownlee, 2001). Resistant starch is not digested in the small intestine and therefore is available for microbial fermentation and the production of short chain fatty acids (SCFA). Short chain fatty acids are absorbed and provide energy, and have a beneficial impact on colon health in humans (Topping and Clifton, 2001). Slowly digestible starch is digested slowly in the small intestine, thereby resulting in reduced post-prandial glucose and insulin levels.

2.2.1.3 Functional Significance of Slowly Digestible Starch

Research on the importance of starch digestion characteristics has been focused on mammalian species such as humans, rodents and pigs and work focused on poultry species is limited. Due to limited research focused on poultry species, this section is based on the studies conducted in humans and pigs. The functional significance of SDS is mainly attributed to the slow release of glucose, which subsequently alters the physiological and metabolic processes. Post-prandial hyperglycemia and insulinemia related to rapidly digestible starch is responsible for oxidative damage, metabolic syndrome, cardiovascular problems, obesity, diabetes, increased blood triglyceride concentration and fat deposition (Ludwig, 2002). Slowly digestible starch reduces the

fluctuation in post-prandial glucose and insulin concentrations, and has been shown to reduce the incidence of above stated health issues (Lehmann and Robin, 2007).

Research showing the impact of SDS on the production and health of poultry species is limited. Based on the physiological and metabolic effects in humans and pigs, it has the potential to improve production and welfare of poultry species. For example, SDS might help to improve welfare and production of feed restricted broiler breeders by improving satiety and reducing post-prandial and post absorptive nutrient storage and mobilization. The metabolic impact of SDS in rapidly growing broilers could be related to improved efficiency due to synchronization of available energy from glucose with digestion and absorption of amino acids (Weurding et al., 2003). Research focused on the use of SDS in poultry species is warranted to validate physiological and metabolic findings in other species.

2.2.1.3.1 Slowly Digestible Starch and Satiety

Studies have shown that SDS results in reduced hunger and improved satiety in humans. Improved satiety with SDS is attributed to slow and prolonged starch oxidation in comparison to rapid oxidation with rapidly digestible starch (Sparti et al., 2000). This finding aligns with the glucostatic theory of appetite control where an increase in blood glucose results in improved satiety. Another mechanism might involve the role of gut hormones including GLP-1. Wachters-Hagedoorn et al. (2006) demonstrated that SDS resulted in increased release of GLP-1, which has been related to slow gastric emptying and small intestine transit, and as a consequence, improved satiety (Edholm et al., 2010).

In addition to SDS, resistant starch (RS) also improves satiety and has a beneficial impact on gastrointestinal tract health. The functional significance of RS is attributed to

the release of SCFA as a result of microbial fermentation in the hindgut. Short chain fatty acids act as source of energy for hind gut enterocytes and have been related to improved gut health (especially the colon). Furthermore, SCFA result in the increased secretion of GLP-1 and PYY responsible for slowing gastric emptying and small intestinal transit rate, further increasing satiety (Bosch et al., 2009).

Although SCFA production is limited in poultry species, its functional significance can't be ruled out due to the presence of caeca (Svihus et al., 2013). In review of the functional significance of avian caeca, Svihus et al. (2013) concluded that avian caeca play a role in the re-absorption of water and salt, and fermentation of carbohydrate and uric acid to SCFA and ammonia, respectively.

2.2.1.3.2 Slowly Digestible Starch and Immune System

Dietary ingredients can modulate the host immune system either directly or indirectly by affecting the gut microbiota. Bioactive compounds, including but not limited to polyunsaturated fatty acids (PUFA), amino acids (arginine and lysine), vitamins (Vitamin E and D) and minerals (selenium and zinc), help modulate the immune system, which in turn improves production and health of animals. Furthermore, the use of prebiotics and probiotics has been shown to alter gut microbiota, thereby affecting immune status (reviewed by Patterson and Burkholder, 2003). In conclusion, dietary composition plays an important role in modulating gut microbiota and the immune system.

In humans, use of legumes (SDS) results in the decreased secretion of pro-inflammatory plasma proteins (C-reactive protein (CRP) and complement C3). Over expression of these proteins might have a negative impact on growth and efficiency of

animals, attributed to the diversion of energy towards an immunological response (Korver and Klasing, 1997). The continual exposure to non-pathogenic environmental allergens during commercial rearing conditions might stimulate inflammation, the release of these proteins and a negative impact on growth. Based on this concept, use of SDS has the potential to modulate the immune system to improve growth and efficiency of poultry species.

2.2.1.3.3 Slowly Digestible Starch and Gut Health

The rate and extent of starch digestion affects the colonization of specific bacterial species in the gut, thereby affecting the mucosal immune system and subsequently health and production. Brown et al. (1997) found an increased concentration of *Bifidobacterium* spp. in the faeces of pigs fed high amylose maize starch in contrast to low amylose corn starch. Recently, Regmi et al. (2011) found that starch with high amylose content and low in-vitro digestibility resulted in increased digestive tract *Bifidobacterium* spp. in the digestive tract of pigs. *Lactobacillus* spp. and *Bifidobacterium* spp. are thought to provide a beneficial impact to gut health and host immune status by preventing the colonization of pathogenic organisms and enhancing the immune response in pigs (Gibson and Roberfroid, 1995). The majority of studies focusing on effect of starch digestion kinetics on gut microbiota are based on a pig model and research with chickens is lacking. Only one study reported that feeding SDS to broilers resulted in decreased colonization of clostridium in the hind gut (Weurding, 2002). Based on the promising effects of starch kinetics on gut microbiota in humans and pigs, more research is warranted to investigate the impact of slowly digestible starch on colonization of gut microbes in poultry species.

The integrity and functionality of the digestive tract is negatively affected by management practices (feed withdrawal or restriction) in poultry species, and therefore ways to maintain a healthy digestive tract is worthy of investigation. Thompson and Applegate (2006) found changes in structural components of the jejunum and ileum (decreased ileal villus width and crypt depth, increased jejunal villus height, and decreased mucosal content) with short-term feed (ranging from 0 to 24 h) withdrawal in broilers and suggested that it might affect gut integrity. Improved barrier function may reduce the risk of translocation of commensal bacteria (*Campylobacter and Salmonella*) and contamination of meat, thus improving bird health and food safety. The integrity and structure of the gut wall might be affected by the nature of feed restriction in broiler breeders. Yamauchi et al. (1996) demonstrated decreased duodenal and jejunal villus height within first 24 h of fasting in laying hens. Similarly, Chappell et al. (2003) found small intestine atrophy characterized by decreased villus height and cell proliferation for birds feed restricted for the same period of time. The sustained and slow release of nutrients as a result of using SDS has the potential to reduce the severity of negative effects of feed restriction on gut health. In conclusion, it can be speculated that feeding a starch with a low rate and extent of digestibility may improve productivity and health of poultry species by enhancing gut health.

2.2.1.4   Use of Slowly Digestible Starch (pea) in Poultry

Research focussed on the use of SDS in poultry is limited. Previous studies have demonstrated that pea is a suitable feed ingredient for poultry mainly attributed to moderate levels of crude protein and metabolizable energy. Pea is deficient in methionine, but is a good source of lysine (Sosulski and Holt, 1980). Igbasan and

Guenter (1997) evaluated the impact of feeding yellow, green and brown pea on the production performance of laying hens. They demonstrated that feeding pea up to 400 g/kg did not affect the productivity of the laying hens. Recently, Ebsim (2013) confirmed that pea starch was slowly digestible as compared to cereal grains and that the nature of pea starch digestion was significantly impacted by feed processing. Furthermore, Ebsim (2013) conducted a series of experiment to determine the impact of feeding pea on the production parameters of laying hens, broilers and broiler breeders. The research concluded that feeding of pea up to 300 g/kg to laying hens resulted in increased body weight and egg weight. In broilers, research demonstrated that pea can be successfully fed up to 300, 600 and 750 g/kg during starter, grower and finisher phases, respectively, without having detrimental effects on growth and productivity. Weurding et al. (2003) demonstrated that feeding pea to broiler resulted in improved performance, which they attributed to protein sparing impact of slowly digestible starch. The protein sparing effect of slowly digestible starch is attributed to slow and sustained release of glucose, thereby reducing the use of amino acids as an energy source for the gut wall. Additionally, SDS results in a slow and prolonged release of insulin, which may lead to increased protein deposition. An experiment conducted with broiler breeder pullets demonstrated that feeding a pea-based diet resulted in reduced post-prandial glucose levels and changes in the liver weights between meals in an EOD feeding program. Furthermore, the blood corticosterone concentration of pea fed pullets was numerically lower as compared to birds fed wheat (Ebsim, 2013).

2.3   Hepatic Lipid Metabolism

The liver is the key site of lipid synthesis in chickens as compared to adipose tissue in mammals. O'Hea and Leveille (1968) noted that the capacity of chicken adipose tissue to synthesise fatty acids by incorporating acetate, pyruvate or glucose carbons is considerably lower in contrast to rats. However, chicken adipose tissue has the capacity to synthesize triglycerides using free fatty acids. Experiments conducted using labelled substrates such as pyruvate or glucose revealed that the liver is the key site for lipid synthesis in chickens (O'Hea and Leveille, 1969b). Furthermore, Goodridge and Ball (1967) concluded that approximately 96% of lipid synthesis in pigeons takes place in liver. The important role of chicken liver in lipogenesis was further supported by the higher expression of a key transcriptional factor of lipid synthesis (Sterol Regulatory Element Binding Protein-1; SREBP-1) in chicken liver as compared to adipose tissue (Gondret et al., 2001). In chickens, glucose derived from starch digestion is considered as key substrate for lipid synthesis. Hepatic lipid synthesis involves the conversion of glucose to triglycerides and involves reactions of glycolysis, the citric acid cycle, and fatty acid synthesis (Figure 2.1). Key enzymes include acetyl CoA carboxylase, malic enzyme and fatty acid synthase (Richards et al., 2003). Acetyl CoA carboxylase is responsible for the conversion of acetyl CoA to malonyl CoA and is a rate limiting step. Malic enzyme is responsible to provide reducing power via NADPH. Fatty acid synthase is responsible for converting malonyl CoA to palmitate (Richards et al., 2003). The triglycerides synthesized in the liver are transported to adipose tissue where they are stored for future use during periods of food deprivation, such as fasting or starvation (O'Hea and Leveille, 1969a). Apolipoprotein B is the primary lipoprotein and is required

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for the formation of very low density lipoproteins (VLDL) in the liver; very low density lipoproteins are responsible for transferring triglycerides to adipose tissue. The hydrolysis of triglycerides by lipoprotein lipase results in the production of fatty acids, which may be released into plasma as non-eserified fatty acids (NEFA) or taken up into tissue, such as adipose for re-esterification and storage (Richards et al., 2003).
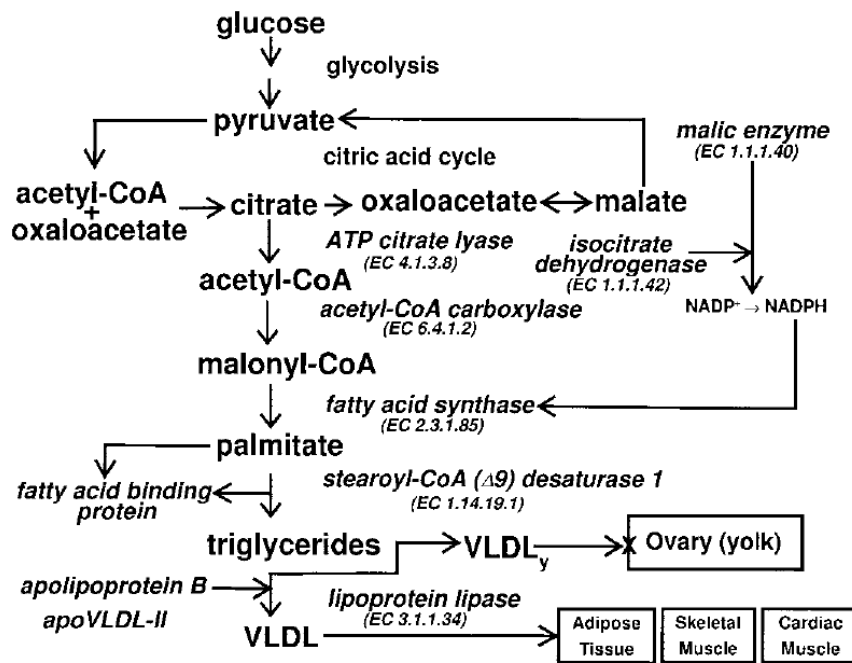
Figure 2.1 Hepatic lipid synthesis in chickens (Taken from Richards et al., 2003).

2.3.1 Intermediary Metabolism during Fasting and Starvation

After a meal, available glucose is either used directly to meet immediate needs or stored as glycogen or triglycerides for future use during an absence of exogenous nutrient supply. Triglycerides are transported from the liver to adipose tissue via VLDL for storage. The post-prandial increase in insulin level plays a vital role to modulate this process (Figure 2.2; Engelking, 2011).
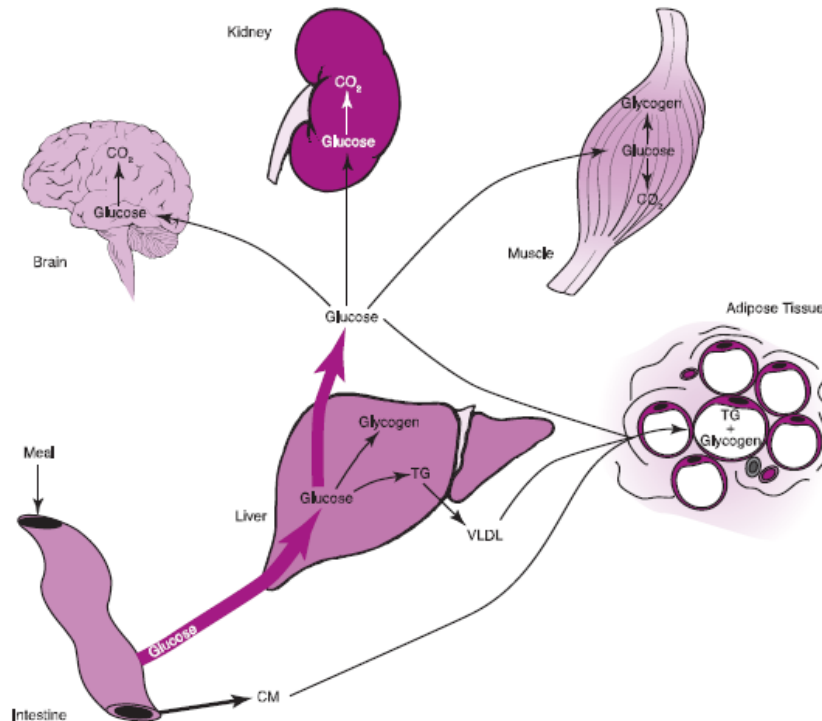
Figure 2.2 Uptake and utilization of glucose released after a meal in animals (Taken from Engelking, 2011).

The early phase of fasting starts with a decrease in absorption of glucose at the end of intestinal phase, thereby resulting in reduced levels of insulin and activating conversion of glycogen to glucose in the liver to support vital bodily functions such as the kidneys and central nervous system. However, due to limited glycogen reserves, this step is short and therefore metabolism shifts to utilization of fat reserves as an energy source. This phase is characterized by hepatic gluconeogenesis and adipose tissue lipolysis. The free fatty acids released from adipose tissues are used as energy source by muscles, kidneys and the liver, and glycerol is used as substrate for the glucose synthesis in the liver. During this phase, glycogen levels decrease and blood free fatty acids increase (Figure 2.3; Engelking, 2011).
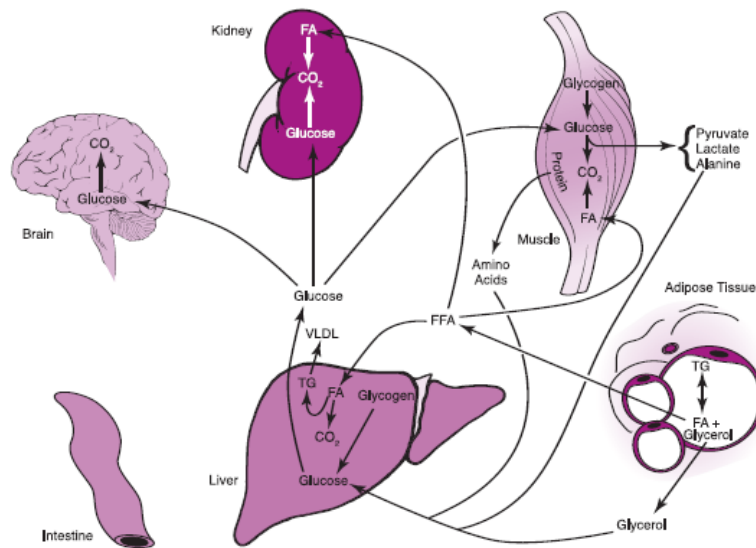
Figure 2.3 The intermediary metabolism during early phase of fasting in animals (Taken from Engelking, 2011).

The intermediate phase of fasting is characterised by the oxidation of fats to spare carbohydrate and proteins. During this phase, free fatty acids and ketone bodies act as the main source of energy to support various organs such as muscle, kidneys, pancreas and central nervous system. The concentration free fatty acids further increase and ketone bodies start to rise during this phase of fasting (Figure 2.4; Engelking, 2011). Research has demonstrated the impact of fasting on levels of free fatty acids and ketone bodies in various animal species. Armstrong et al. (1993) observed increased NEFA concentrations with feed restriction in heifers. Furthermore, Cheryl et al. (1988) demonstrated that levels of NEFA and β-hydroxybutyrate (a ketone body) start increasing during the early phase, remained at high levels during the intermediate phase and decreased during the late phase of fasting in geese. Previous studies have concluded that short term fasting in birds results in increased levels of NEFA and β-hydroxybutyrate (Brady et al., 1978; Le Maho et al., 1981). In contrast to mammals, levels of acetoacetate do not increase with fasting,

which is attributed to low levels of β-hydroxybutyrate dehydrogenase in the avian liver (Nehlig et al., 1980). The late phase of fasting is characterised by the utilization of the protein in order to maintain blood glucose levels. Based on the physiological changes during these stages of fasting, feed restriction in broiler breeders with every-other-day feeding may be best categorized as early to intermediate stage of fasting.
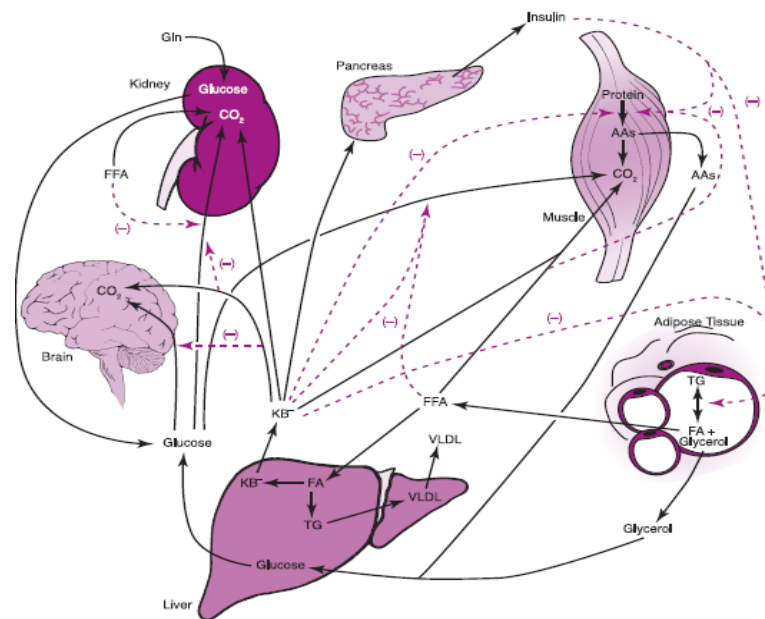
Figure 2.4 The intermediary metabolism during intermediate phase of fasting in animals (Taken from Engelking, 2011).

2.4   Quantification of Satiety and Hunger

To study the impact of management and nutritional strategies on welfare of broiler breeders, quantification of hunger is an important consideration. To accomplish this goal, both physiological and behavioural parameters have been used as indirect measures to quantify degree of hunger. Physiological parameters include blood levels of NEFA, β hydroxy-butyrate, corticosterone and heterophil: lymphocyte (H/L) ratio. de Jong et al. (2003) found a linear relationship between the glucose/NEFA ratio and degree of feed

restriction in broiler breeders. Similarly, Van Itallie and Hashim (1960) demonstrated this effect in human subjects, suggesting that it could be used an indicator. Corticosterone levels also increased in feed restricted broiler breeders as compared to those provided ad-libitum access to feed at 7 wk of age (de Jong et al., 2003). However, the level of corticosterone has been shown to increase only in more severe feed restriction situations ($\sim < 50 \%$ of ad-libitum), and as a consequence does not demonstrate a linear relationship with degree of feed restriction (de Jong et al., 2003). The increased concentration of corticosterone with feed restriction might be either related to the bird's metabolic status or chronic stress associated with hunger (de Jong et al., 2002). The relationship between H/L ratio and degree of feed restriction has been inconsistent, and therefore is not considered a good measure (D'Eath et al., 2009). Additionally, expression of genes encoding for neuropeptide Y or Agouti related protein (both stimulate feeding by activating the release of orexin and melanin concentrating hormone) has also been measured to quantify hunger during feed restriction (Boswell et al., 1999; Dunn et al., 2012; Toolkamp and D'Eath, 2016). It was concluded that a positive correlation exists between the degree of feed restriction and the expression of genes encoding for these peptides. Biomarkers such as leptin and ghrelin have been used to assess satiety in humans (de Graaf et al., 2004). In contrast to mammals, ghrelin inhibits feed intake in poultry species. The role of leptin in controlling feed intake in birds is not conclusive with evidence of reduced feed intake to no effect. Furthermore, studies have suggested that impact of leptin is lower in rapidly growing chickens. Therefore, modern day broilers breeders might be less responsive to leptin (Cassy et al., 2004; Richard and Proszkowiec-Weglarz, 2007).

In addition to physiological indices, behaviours reflecting feeding motivation and frustration have been used to quantify hunger. Behavioural expression is an important tool as it reflects negative affective states related to hunger. Savory and Maros (1993) found that expression of drinking, litter pecking and walking increased with feed restriction in comparison to birds with ad-libitum access. In a systematic review by D'Eath et al. (2009), increased expression of drinking, litter and empty feeder pecking, and decreased expression of comfort behaviours were found in the majority of reviewed studies on feed restricted broiler breeders in comparison to *ad-libitum* or alternative diets such as those with high fiber or appetite suppressants. In addition, time taken to consume a meal, compensatory feed intake and operant tests can be used to measure the degree of hunger; however their usefulness is limited under practical broiler breeder rearing conditions (D'Eath et al. (2009). In conclusion, assessment of hunger in feed restricted broiler breeders cannot be achieved by monitoring a single parameter, but the collective use of behavioural and physiological indices provides insight into the satiety status of these birds.

2.5   Ways to Improve Welfare of Feed Restricted Broiler Breeders

Extensive research has been conducted to explore management and nutritional strategies to reduce the degree of hunger, and thereby improve the welfare of feed restricted broiler breeders. Multiple mechanisms may be utilized to improve the welfare of feed restricted animals by reducing the degree of hunger.

2.5.1 Increased Feeding Time

Previous studies have looked at various management strategies to increase feeding time with the intent to improve the welfare of feed restricted broiler breeders. This includes use of a scattered feeding program. de Jong et al. (2005) demonstrated reduced object pecking in feed restricted broiler breeders with a scattered feeding program. The rationale for scattered feeding includes birds spending more time eating and foraging to satisfy the need of natural feeding behaviour.

2.5.2 Increased Gut Fill

The use of dietary diluents to reduce the negative impact of feed restriction has been studied in the past. Dietary diluents include but are not limited to wheat bran, oat hulls, potato pulp, sugar-beet pulp (Zuidhof et al., 1995; de Jong and Guémené, 2011; Nielson et al., 2011). The primary intent of using dietary diluents is to increase the bulkiness of feed, thereby increasing feeding time and gut fill, and potentially reducing the degree of hunger (Zuidhof et al., 1995; Savory et al., 1996; Savory & Lariviere, 2000; Hocking et al., 2004; de Jong et al., 2005; Nielsen et al., 2011).

2.5.3 Chemical Mediation

Use of appetite suppressants (such as calcium propionate) with a high fiber diet was studied to improve the satiety of feed restricted broiler breeders (Morrissey et al., 2014a, b). The authors concluded that the use of a diet containing an appetite suppressant resulted in reduced stereotypic feather pecking, thereby signaling towards improved satiety. Furthermore, authors concluded that some level of hunger was present and that it is impossible to fully prevent the chronic hunger in feed restricted broiler breeders.

2.5.4   Changing Digestion and Metabolism

Feeding slowly digestible ingredients (pea) has a potential to increase satiety in broiler breeders. Previous research to study the impact of SDS on health and productivity of poultry species is limited. Weurding et al. (2001) investigated the impact of SDS on broiler performance and concluded that its addition to diets improved productivity. The functional significance of SDS is mainly attributed to the slow release of glucose, which subsequently alters physiological and metabolic processes (Lehmann and Robin, 2007). Previous work in our lab (Ebsim, 2013), found that feeding pea to broiler breeders resulted in reduced post-prandial glucose levels, and changes in relative liver weight over the period of 48 h between meals. No effect of feeding a wheat- or pea-based diet was observed on the serum corticosterone, however, pea fed birds had numerically lower values for corticosterone during the off-feed day (24-48 h post feeding). Based on these results and the functional significance of SDS, it can be speculated that feeding SDS to broiler breeders may result in improved animal welfare by decreasing the severity of hunger. The positive impact of feeding SDS may be related to altered post-prandial intermediary metabolism, thereby increasing feed clean-up time and gut-fill, and reducing nutrient storage and mobilization.

2.6   General Comments

Body weight of broiler breeders is controlled by restricting access to feed during rearing and laying phases. Advantages of feed restriction include improved ovarian morphology, a lower incidence of double hierarchies and abnormal eggs, lower mortality, higher egg production and better persistency of lay. Despite the benefits of feed restriction, it is often criticized for the long periods without feed. Multiple strategies have

been studied to reduce the degree of impact of feed restriction. The functional ability of SDS to improve satiety, reduce fat deposition and modulate metabolic status is well demonstrated in humans (Lehmann and Robin, 2007). Previous work in our lab found that feeding pea to broiler breeders decreased the post-prandial glucose peak and increases in liver weights between meals as compared to birds fed a wheat-based diet (Ebsim, 2013). Based on the review of literature, this study was conducted to investigate the impact of feeding pea- or wheat-based diets on broiler breeder welfare, production, intermediary metabolism, reproductive capacity, and offspring quality and growth.

3 EFFECT OF FEEDING PEA OR WHEAT BASED DIETS ON GROWTH, DIGESTA AND GUT MASS OF BROILER BREEDERS

Preface

The objective of this chapter was to study the effects of feeding a pea- or wheat-based diet during the rearing phase on growth, uniformity, digesta content (DM basis) and gut mass of feed restricted broiler breeders.

Author Contributions

Aman Deep (Department of Animal and Poultry Science, University of Saskatchewan) planned, designed and conducted the experiment, analyzed the data, and drafted the manuscript.

Henry Classen (Department of Animal and Poultry Science, University of Saskatchewan) provided guidance and scientific input, reviewed and edited the manuscript, and provided funding for the research.

3.1 Abstract

Feed restriction is applied to broiler breeders during rearing and laying phases to maintain body weight targets essential for good health and production. It is common practice to feed on an every-other-day (EOD) basis during the rearing phase to achieve body weight goals. As a consequence birds are hungry and without feed for considerable periods of time. The rate of starch digestion may affect hunger and therefore this experiment compared feeding diets based on slowly (pea) or rapidly (wheat) digested starch to broiler breeder pullets. Ross 308 broiler breeder pullets (384) were housed in 24 pens (16/pen; 12 replications per diet). Pea- or wheat-based diets were fed from 21 to 89 d on an EOD (48 h) basis. Differences in the extent and rate of starch digestibility of diets were confirmed using an *in-vitro* assay. Body weight was monitored on a weekly basis and feed allocation was adjusted accordingly. Individual pullets were weighed at 84 d of age to determine uniformity. At 89 d of age, 4 pullets/ treatment were randomly selected and euthanized at 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding; digesta content and digestive tract mass were monitored. Feeding a wheat-based diet resulted in heavier pullets at 4, 5, 6, 7, 8 wk of age, but differences were small. At 84 d of age, uniformity was improved by feeding a pea-based diet. Digesta content and gut mass were increased by feeding a pea based diet over a 48 h period. In conclusion, feeding a diet based on pea resulted in improved uniformity, and increased digesta and gut mass.

3.2 Introduction

The selection of broilers for rapid growth for the last six decades has resulted in a continuous increase in feed intake and weight gain. As a consequence, if fed ad-libitum, the body weight of broiler breeders increases to a level that has a negative impact on health and productivity. Previous studies have demonstrated that providing ad-libitum access of feed to broiler breeders results in increased mortality due to metabolic and skeletal problems, decreased productivity during the laying phase, disrupted follicular hierarchies (multiple ovulations) and reduced fertility (Robinson et al., 1991; Mench, 2002; Renema and Robinson, 2004). Feed restriction is an important tool for maintaining body weight targets of rapidly growing broiler breeders. The degree of feed restriction varies with age with a maximum occurring during the rearing phase. Beneficial effects of feed restriction include reduced losses due to metabolic and skeletal problems, and improved productivity and reproductive capacity (Mench, 2002). Improved health and production with feed restriction is mainly attributed to the control of body weight and excessive accumulation of fat. Chen et al. (2006) suggested that ovarian dysfunction related to over eating in broiler breeders might be explained on the basis of toxic effects of excessive body lipids in non-adipocytes including ovarian tissue.

In contrast, feed restriction during the rearing phase results in long periods without feed, which has been associated with hunger. Physiological and behavioural measures have been used to quantify hunger and these parameters have indicated that feed restricted birds suffer from the state of hunger (Kostal et al., 1992; Savory et al., 1993; de Jong et al., 2003). As a consequence of feeding restricted amounts every 48 h, broiler breeder pullets need to store and re-utilize nutrient between meals. de Beer et al. (2007a)

found that feeding broiler breeders on an every-other-day (EOD) basis resulted in extensive changes in relative liver weight, and glycogen and fat content over the period of time between meals. It is possible that the storage and mobilization process results in metabolic stress to broiler breeders, which might in turn negatively affect efficiency of growth and the bird uniformity. Additionally, due to the state of hunger, breeders have a tendency to eat feed quickly, providing an opportunity for heavier and aggressive birds to consume more than their share of feed (Bennet and Leeson, 1989). A reduction in the time required to consume feed with increasing levels of restriction has been associated with reduced bird uniformity (Blair et al., 1976). Furthermore, feed restriction might have a negative impact on gut morphology. Yamauchi et al. (1996) demonstrated decreased duodenal and jejunal villus height within first 24 h of fasting in White Leghorn hens. Similarly, Chappell et al. (2003) found small intestine atrophy characterized by decreased villus height and cell proliferation with different level of feed restriction in rats.

Management and nutritional strategies have been investigated to lessen the negative impact of feed restriction; examples include the use of a foraging feed program that increases the time for birds to find their feed, and the use of diluted feed to again extend bird feeding time (de Jong et al., 2011). Another alternative may be to feed a slowly digested feed ingredient such as pea. Weurding et al. (2001) conducted an experiment with broilers and demonstrated that pea based diets were slowly digested. Sustained and slow release of nutrients by using slowly digestible starch (SDS) has potential to reduce the severity of negative effects related to feed restriction. Based on this possibility, the present study was designed to study the effects of feeding a pea- or wheat-based diet during the rearing phase on growth, uniformity, digesta content (DM basis) and gut mass

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of feed restricted broiler breeders. It was hypothesized that feeding a diet based on SDS will result in improved growth and uniformity of feed restricted broiler breeders.

3.3   Material and Methods

Experiments were approved by the University of Saskatchewan's Animal Research Ethics Board and were performed in accordance with recommendations of the Canadian Council on Animal Care (1993, 2009).

3.3.1   Dietary Treatments

Feed was formulated based on the Aviagen recommendations for a four stage rearing program (Aviagen, 2007). For the first 3 wk, all chicks were provided with a common starter (I) and thereafter fed either a pea- or wheat-based diet on an every-day (ED) basis for a week (21-27 d), followed by EOD feeding for the rest of the experimental period (28-89 d). A second starter (II) and a grower feed were provided for 21-41 d and 42-89 d, respectively. Diets were formulated using pea or wheat as main ingredients and were approximately equalized for energy, protein and fat levels (Table 3.1). Digestible amino acid content was formulated according to Aviagen (2007) specifications. Ground oat hulls (3 mm slotted screen in a flail type hammer mill) were used to dilute the wheat based diets and assist in equalizing diet energy levels. The decision to use oat hulls was based on better suitability as compared to other high fiber material such as pea fiber.

Table 3.1. The ingredient and nutrient composition of pea- or wheat-based diets fed to broiler breeders.

Ingredients	Starter-I	Starter-II (Pea)	Starter-II (Wheat)	Grower (Pea)	Grower (Wheat)
Pea	50.64	69.40	-	64.74	-
Wheat	37.13	-	36.54	-	42.15
Barley	-	20.80	25.00	25.00	25.00
Soybean meal	5.74	2.84	22.43	-	12.96
Canola oil	2.00	2.54	2.53	2.24	2.17
Limestone	1.46	1.57	1.60	1.45	1.48
Dicalcium phosphate	1.69	1.39	1.30	1.18	1.16
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.33	0.44	0.38	0.45	0.38
DL-Methionine	0.27	0.26	0.10	0.15	0.05
Choline chloride	0.10	0.10	0.10	0.10	0.10
Oat hulls	-	0.08	9.44	4.12	13.96
Biocox[2]	0.05	0.05	0.05	0.05	0.05
Enzyme[3]	0.025	0.03	0.03	0.03	0.03
Calculated nutrients (%)					
AME (Mcal/kg)		2.700	2.700	2.600	2.600
Ca		1.00	1.00	0.90	0.90
Non phytate P		0.45	0.45	0.40	0.40
Total P		0.64	0.61	0.58	0.54
Analyzed nutrients (%)					
DM	-	89.67	89.84	89.93	90.06
CP	-	19.06	19.61	17.74	16.24
Total fat	-	3.56	3.62	3.34	3.12
Total starch	-	40.09	37.26	37.15	38.69
TDF	-	19.70	20.95	20.67	21.43

[1] Vitamin-mineral premix (units per kg of feed) contained the following: vitamin A, 11000 IU; vitamin D_3, 2200 IU; vitamin E, 30 IU; menadione, 2.0 mg; riboflavin, 6.0 mg; pantothenic acid, 10 mg; vitamin B_{12}, 0.02 mg; pyridoxine, 4.0 mg; thiamine, 1.5 mg; folic acid, 0.6 mg; niacin, 60 mg; biotin, 0.15 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[2] Salinomycin Sodium; Alpharma Canada Corp, Mississauga, ON, Canada.
[3] Endofeed W; GNC Bioferm, Cobble Hill, BC, Canada.

3.3.2 Birds and Housing

Ross 308 day old broiler breeder chicks (384) were reared as a group in a large pen at the Poultry Centre, University of Saskatchewan. At 2 wk of age, all chicks were randomly placed in 24 equal size pens (16 chicks/ pen) in two identical rooms (12 pens/ room). For the first 3 wk, all chicks were provided with the same starter (I) feed containing pea and wheat as base ingredients, with an objective to acclimatize chicks to the key ingredients prior to feeding the experimental diets. Each of the two dietary treatments was assigned in a randomized complete block design to 12 pens (6/room) at 3 wk of age. An equal amount of straw (7-10 cm) was placed in each pen. Pen size was 2.3×2.0 m, which provided a floor space of 0.27 m^2 per bird. Each pen was equipped with a drinker with 6 nipples and a trough feeder with a feeding space of 230 cm (14.4 cm/bird). Temperature was set at 31°C at d-0 and decreased gradually thereafter to 21°C by d-24. All chicks were provided with 23 h of light (20 lux) for the first week, followed by 8 h of light and 16 h of dark (10 lux) for the remainder of the trial.

3.3.3 Data Collection

Average body weight for each pen was monitored on a weekly basis and feed allocation was adjusted accordingly to achieve target weights. Individual body weights were recorded to assess uniformity at the start of experiment (21 d) and again at 84 d of age.

At 89 d of age, 4 birds (1/pen) were randomly selected at 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding and humanely euthanized using cervical dislocation technique. The crop, jejunum and ileum segments of the digestive

tract were weighed full and empty and contents were collected on dry ice and stored (-20°C) for measurement of dry matter.

3.3.4 Chemical Analysis

An *in-vitro* assay, based on methodology of Englyst et al. (1992), but modified to reflect the chicken digestive tract, was used to test the rate and extent of starch digestibility of diets. The starter-II diet was not included in this assay as it was fed for only 3 wk and ingredients (wheat or pea) were the same source as used in the grower diet. In contrast to Englyst et al (1992), the assay was maintained for 360 min post-incubation. Additionally, absolute ethanol was used to stop the enzymatic reaction instead of 66% ethyl alcohol, based on the study conducted by van Kempen et al. (2010).

In the present study, samples were ground (Retsch mill with a screen size of 1.0 mm) to simulate the grinding action of the gizzard during *in-vivo* digestion. Approximately, 0.1 g of samples were weighed in triplicate. To mimic the action of the proventriculus, 1.5 ml of HCl-pepsin (2000U/ml; Sigma ref. P-7125; St. Louis, MO. USA) solution was added and samples were incubated in a shaking water bath maintained at 41°C for 30 min. After this, 20 ml of sodium acetate buffer (0.1 M; ph-5.2) was added to maintain the pH conditions similar to small intestine, followed by 5 ml of enzyme cocktail containing 3 g of pancreatin (8 × USP specifications, 3000 rpm for 10 min; Sigma ref. P-7545; Louis, MO. USA), 22.5 ml amyloglucosidase (200 U/ml; Megazyme, Bray Business Park, Bray, Ireland), and 9 ml of invertase (1500 U/ml; Megazyme, Bray Business Park, Bray, Ireland) in water. A 0.5 ml of aliquot was taken at 15, 30, 45, 60, 120, 180, 240, 300 and 360 min of the small intestine phase and added to the tube containing 20 ml of absolute ethanol. Clear supernatant (3000 rpm for 2 min) solution obtained after

centrifugation was analyzed for glucose using a glucose oxidase kit (K-GLUC; Megazyme, Bray Business Park, Bray, Ireland).

Dietary and digesta moisture was determined by method 930.15 of the Association of Official Analytical Chemists (AOAC; 1990). Crude protein content of the diets was analyzed using the combustion method (984.13; AOAC, 1995) using a Leco FP-528 protein analyzer (St. Joseph, MI). Crude fat were analyzed according to the method from AOAC (1990; method 920.39). Dietary starch levels were determined using a commercial kit (amyglucosidase/α-amylase method) developed by Megazyme (K-TSTA, Bray Business Park, Bray, Ireland).

3.3.5 Statistical Analysis

In-vitro starch digestibility data were analyzed as repeated measures. Compound symmetry covariance structure was used. Growth and uniformity data were analyzed using Proc Mix of SAS (SAS Institute, 2002) as a randomized block design with room serving as a block. Repeated measures with compound symmetery covariance structure was used to analyse digesta and gut mass. Pen was considered as the experimental unit.The level of significance was fixed at $P \leq 0.05$ unless otherwise stated.

3.4 Results

3.4.1 In-vitro Starch Digestibility

The rate of starch digestibility was low for pea as compared to wheat based diets, but the extent of digestibility was same for both treatments (Table 3.2). Starch digestibility at 15, 30, 45, 60, 120 min was low for pea based diet but no differences were noted thereafter.

Table 3.2. In-vitro starch digestion rate (% of total starch) of the grower diets fed to broiler breeder pullets during the rearing phase ($n = 3$).

Item	Treatment		Incubation time (min)									SEM
	Pea	Wheat	15	30	45	60	120	180	240	300	360	
Diets	68.78[b]	75.90[a]	25.86[f]	42.87[e]	60.34[d]	75.06[c]	87.49[ab]	89.75[ab]	93.40[a]	84.51[b]	91.78[ab]	1.363
					Treatment×Time							
Pea	-	-	23.81[G]	35.85[F]	63.60[E]	65.62[D]	81.08[C]	88.45[ABC]	95.92[A]	83.91[BC]	90.80[ABC]	-
Wheat	-	-	27.92[FG]	49.88[E]	67.09[D]	84.51[ABC]	93.91[AB]	91.04[ABC]	90.87[ABC]	85.11[ABC]	92.75[ABC]	-

[a,b] Means within a row with different superscripts differ significantly ($P < 0.05$).
[A,B] For the interaction, means with different superscripts differ significantly ($P < 0.05$).
P values: Treatment = <0.0001; Time = 0.0007; Treatment x Time = 0.05.

3.4.2 Body Weight and Feed Allocation

Broiler breeders fed the wheat-based diet were heavier at 28, 35, 42, 49 and 56 d of age as compared to those fed the pea-based diet (Table 3.3). Feed allocation was the same for both treatments until 35 d of age and thereafter increased for breeders fed a pea-based diet to equalise and maintain body weight targets (Table 3.3). Mortality was very low and unaffected by dietary treatment (data not presented).

Table 3.3. Effect of feeding pea- or wheat-based diets on feed allocation and weekly body weights (kg; $n = 12$) of broiler breeder pullets.

Age (d)	Feed allocation		Body weight		SEM	*P* Value
	Pea	Wheat	Pea	Wheat		
21	35.0	35.0	0.338	0.339	0.005	0.90
28	42.3	42.3	0.436	0.448	0.003	0.007
35	48.8	48.8	0.527	0.552	0.003	<.0001
42	49.8	47.9	0.723	0.770	0.003	<.0001
49	56.0	54.3	0.753	0.779	0.004	0.0002
56	56.9	55.9	0.885	0.900	0.004	0.02
63	59.1	58.1	0.989	0.997	0.005	0.20
70	59.1	58.7	1.110	1.120	0.005	0.53
77	62.1	61.4	1.200	1.200	0.006	0.26
84**	62.6	62.6	1.320	1.330	0.006	0.08

** Feed intake was equalized for collection of data on digestive tract size and content.

3.4.3 Uniformity

Body weight uniformity as depicted by coefficient of variation (CV) and standard deviation (SD) was same for both treatments at the start of experimental period (21 d; Table 3.4). Feeding a

diet based on pea resulted in improved uniformity at 84 d of age in comparison to breeders fed the wheat-based diet.

Table 3.4. Effect of feeding pea- or wheat-based diets on uniformity of broiler breeder pullets at 21 and 84 d of age ($n = 12$).

Age (d)	Item	Treatment		SEM	*P* Value
		Pea	Wheat		
21	SD*	0.04	0.04	0.002	0.67
	CV**	11.8	11.4	0.677	0.64
84	SD	0.16	0.20	0.009	0.01
	CV	12.64	15.30	0.747	0.02

* SD – Standard deviation.
** CV – Coefficient of variation.

3.4.4 Digesta and Gut Mass

Crop content was unaffected by dietary treatment (data not presented). Broiler breeder pullets fed a pea-based diet had a higher jejunal content (DM basis) at 4, 8, 12, 16, 20, 24, 26 and 40 h after feeding (Table 3.5). Similarly, ileal content was also increased at 8, 12, and 16 h after feeding in birds fed a diet based on pea as compared to wheat (Table 3.6). Average jejunal and ileum content over 48 h was also higher for the pea-based diet (Table 3.6). No treatment differences were found in regards to the percent digesta moisture.

In comparison to wheat, feeding a pea-based diet resulted in an increased empty jejunum and ileum weight (Table 3.7).

Table 3.5. Effect of feeding pea- or wheat-based diets on jejunum content (g; DM basis) of broiler breeders at 89 d of age ($n = 4$).

Item	Treatment		SEM
Time[***]	Pea	Wheat	
1 h before feed	1.234	0.554	0.384
1 h after feed	1.284	1.366	0.384
2 h after feed	2.243	1.742	0.378
4 h after feed	4.602	2.638	0.384
8 h after feed	3.082	1.809	0.384
12 h after feed	4.083	1.621	0.378
16 h after feed	4.439	2.444	0.384
20 h after feed	3.652	1.913	0.384
24 h after feed	3.453	2.516	0.378
26 h after feed	3.712	2.155	0.384
28 h after feed	1.919	2.365	0.384
32 h after feed	1.298	0.569	0.378
36 h after feed	1.233	1.205	0.256
40 h after feed	1.960	0.635	0.384
44 h after feed	1.372	0.555	0.378

$P = 0.006$ (Treatment x Time); $P = <0.0001$ (Treatment); $P = <0.0001$ (Time).

Table 3.6. Effect of feeding pea- or wheat-based diets on ileum content (g; DM basis) of broiler breeder pullets at 89 d of age ($n = 4$).

Item	Treatment		SEM
Time[***]	Pea	Wheat	
1 h before feed	1.077	0.328	0.440
1 h after feed	1.044	1.515	0.440
2 h after feed	1.596	0.789	0.439
4 h after feed	3.094	2.505	0.440
8 h after feed	3.305	1.845[b]	0.440
12 h after feed	3.998	1.556	0.439
16 h after feed	4.938	1.959	0.440
20 h after feed	3.041	2.032	0.441
24 h after feed	3.695	2.579	0.439
26 h after feed	3.146	2.439	0.440
28 h after feed	2.430	2.581	0.440
32 h after feed	1.648	0.669	0.439
36 h after feed	1.386	1.801	0.440
40 h after feed	0.970	0.380	0.440
44 h after feed	0.193	0.427	0.441

$P = 0.01$ (Treatment x Time); $P = <0.0001$ (Treatment); $P = <0.0001$ (Time).

Table 3.7. Effect of feeding a pea- or wheat-based diets on relative jejunum and ileum weight (%) of broiler breeders at 89 d of age ($n = 4$).

Item	Treatment		SEM	*P* Value
	Pea	Wheat		
Relative jejunum weight	0.80	0.72	0.013	<0.0001
Relative ileum weight	0.66	0.57	0.012	<0.0001

3.5 Discussion

The present research was conducted to study the concept of feeding a diet based on slowly digestible ingredient to broiler breeders and its effects on growth, uniformity, digesta content and gut mass. Limitations of this study need to be considered before making a conclusion. First, diets were formulated to be equal on the basis of AME values from previous research (National Research Council, 1994). However, these values might be different from the actual AME of the ingredients used in the current experiment. This may have resulted in slightly higher energy intake for the pea than the wheat fed birds after 35 d of age. The *in-vitro* assay demonstrated that the starch in the pea-based diet was slowly digestible as compared to wheat, which is an agreement with Weurding et al. (2001). However, it is less easy to judge the extent of starch digestibility. At 360 min of the small intestine phase no differences in cumulative starch digestibility were found between the pea- and wheat-based diets, but a direct comparison between time in the small intestine phase in the *in vitro* assay and actual starch digestion in the bird is lacking. Regardless, the similarity of feed intake value suggests any difference in digestible nutrient content in the diets were minimal. Secondly, whole ingredients were used as a starch source instead of purified starch, therefore effects of dietary associated compounds such as protein, fat and fiber cannot be ruled out. Lastly, previous research in our lab demonstrated differences in the starch digestibility of different pea cultivars collected from different location

and time of year (Ebsim, 2013). Considering all these factors and lack of research in this area, this manuscript provides preliminary findings and concepts on the use of SDS in feeding broiler breeders, which might be useful for the development of future research.

Body weight of broiler breeders fed a pea-based diet was lower at 28, 35, 42, 49 and 56 d of age, but differences were very minor. As noted above, this may relate to the digestible nutrient content of the two diets. Weurding et al. (2003) demonstrated that broilers fed a pea-based diet were more efficient as compared to rapidly digestible ingredients such as wheat. This suggests that pea is an acceptable feed ingredient for broiler breeders. In conclusion, high levels (55-60 %) of pea can be successfully fed to feed restricted broiler breeder pullets without having a major impact on growth during the rearing phase. Research is required to explore beneficial effects such as improved satiety, reduced weight gain and fat deposition as seen when slowly digestible starch was fed in human and rat models (Pawlak *et al*., 2001; Lehmann and Robin, 2007; McCrory et al., 2010). These mechanisms might provide improved welfare of feed restricted broiler breeders related to hunger and metabolic stress.

Body weight uniformity plays a vital role in optimizing production and reproductive capacity of broiler breeders. Robinson et al. (2003) reviewed the importance of flock uniformity with a focus on reproductive performance. Increased variability in body weights has an impact on age of sexual maturation with fewer birds reaching a threshold at a given point in time, subsequently decreasing and delaying the peak rate of production. Hudson et al. (2001) found increased egg production with higher uniformity at 20 wk of age in broiler breeder hens. Furthermore, an increased proportion of birds that are too light and heavy might have an impact on egg size variability, thereby decreasing the percentage of settable eggs. Based on previous research, effects of variation in body weight on hatchability and fertility have been inconsistent. Petite et

al. (1982) compared flocks with uniformities of 80 or 89% within 10% of the flock mean and found that fertility was unaffected. Overall, maintaining a high uniformity is an essential component of modern broiler breeder production system.

Uniformity of a flock can be affected by different managemental and nutritional practices including stocking density, feeder and drinker space, environmental variables (temperature, ventilation and non-uniform light intensity), quantity and quality of food, egg size and genetic variability in the parent stock (Costa, 1981; Robinson and Robinson, 1991). The practice of feed restriction during the rearing phase of broiler breeders further poses a negative impact on flock uniformity. Based on the importance of flock uniformity, different strategies were used to decrease body weight variability. Most importantly, use of grading based on body weight at different stages of the rearing phase resulted in a significant improvement in uniformity (Petitte et al., 1981). Zuidhof et al. (1995) studied the effects of qualitative feed restriction and revealed that uniformity was improved with feed containing diluents. Furthermore, use of different feeding programs was found to affect flock uniformity. Bartov et al. (1988) showed that uniformity was improved by a EOD feeding program as compared to feeding ED. Despite the presence of measures to decrease variation in body weight, maintaining ideal uniformity is still a major concern for broiler breeder producers.

In the present research, uniformity was improved be feeding a pea-based diet in comparison to wheat. Improved uniformity with a diet based on pea might be related to improved satiety, subsequently increasing the feed clean up time (time taken to consume the allocated feed), thereby reducing the degree of competition between small and large size pullets. Improved uniformity with EOD feeding in contrast to ED was explained on a similar basis (de Beer and Coon, 2007). Although no research has been conducted to determine the impact of slowly

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digestible ingredients on hunger and satiety in broiler breeders, human studies suggest such an effect (Lehmann and Robin, 2007; McCrory et al., 2010). Another explanation for improved uniformity by feeding pea may be based on reduced degree of nutrient storage and mobilization (Deep et al., 2012), attributed to slow release of nutrients; in turn this may reduce associated metabolic stress. Nutrient storage and mobilization is a less efficient process as suggested by de Beer et al. (2007). A less efficient process in a highly feed restrictive state, superimposed on variation in individual bird efficiency, might make some pullets more susceptible to body weight change as compared to others, thereby affecting uniformity. For an example, environmental or nutritional stress also increases variability in body weight. Corzo et al. (2004) demonstrated that a reduction in dietary amino acids resulted in decreased body weight uniformity of broilers. More research is required to accurately determine this impact.

Jejunal and ileal digesta content on a DM basis was higher for pullets fed a pea- as compared to those fed a wheat-based diet. This effect may relate to the changes in feed passage rate and/or differences in the release of endogenous secretions, but more research is required to precisely determine the cause of increased digesta content. Although feed passage rate was not measured in this experiment, previous research in humans demonstrated that feeding a low glycemic index diet resulted in delayed gastric emptying. Holt et al. (1992) found an inverse relationship between glycemic index and the cholecystokinin (CCK) response. CCK is a hormone responsible for delaying gastric emptying (Liddle et al., 1986). Furthermore, Wachters-Hagedoorn et al. (2006) demonstrated that slowly digestible starch resulted in increased release of GLP-1, which has been related to slow gastric emptying and SI transit (Edholm et al., 2010). Therefore, it can be speculated that feeding a pea based diet (low glycemic index) might have resulted in a decreased feed passage rate. Regardless, increased digesta content between meals may improve satiety in

feed restricted broiler breeders by affecting gut fill. Increased gut fill by feeding a pea-based diet has potential to improve satiety by interacting with mechanoreceptors present in gastrointestinal tract (reviewed by Houpt, 1982).

Maintaining adequate gut health is critical for broiler breeders as they are subjected to high degree of feed restriction with long periods of fasting. Previous research has demonstrated the negative impact of fasting on gut health. Mucosal sloughing and depression in the villi tips was observed with short term fasting of broilers (3, 5 or 7 d). Yamauchi et al. (1996) found decreased duodenal villus height even with 24 h fasting in laying hens. Thompson and Applegate (2006) found that feed restriction in broilers resulted in reduced intestinal mucus and altered intestinal morphology, and suggested these changes were consistent with reduced barrier function. Chappell et al (2003) studied the impact of different levels of feed restriction on gut morphology in rats and found that villus height decreased with increasing level of restriction. de Beer et al. (2008) found that the crop of broiler breeders fed on EOD basis was empty after 24 h of feeding. Based on these studies, it could be speculated that gut morphology of feed restricted broiler breeders might be changing over a 48 h period, subsequently affecting gut health. More research is required to characterize changes in gut morphology that result from feeding a pea-based diet.

In conclusion, feeding a pea-based diet resulted in improved uniformity without having a major impact on growth. High levels of pea can successfully be fed to broiler breeders during rearing phase. Ileum and jejunum contents (DM basis) and gut mass were also increased with a pea-based diet, but more research is required to elucidate the underlying cause. Silimarly, more research is needed to study the effects of diets based on slowly digestible ingredients on hunger and satiety of feed restricted broiler breeders.

4 EFFECT OF FEEDING PEA- OR WHEAT-BASED DIETS ON POST-PRANDIAL METABOLISM OF FEED RESTRICTED BROILER BREEDERS

Preface

The objective of this chapter was to study the effects of feeding a pea- or wheat-based diet during the rearing phase of broiler breeders fed every-tother-day on the storage and mobilization of nutrients between meals.

Author Contributions

Aman Deep (Department of Animal and Poultry Science, University of Saskatchewan) planned, designed and conducted the experiment, analyzed the data, and drafted the manuscript.

Henry Classen (Department of Animal and Poultry Science, University of Saskatchewan) provided guidance and scientific input, reviewed and edited the manuscript, and provided funding for the research.

Andrew Van Kessel (Department of Animal and Poultry Science, University of Saskatchewan) provided scientific input and guidance in planning and desiging this experiment, laboratory infrastructure for gene expression analysis, and manuscript editing.

4.1 Abstract

Broiler breeders are often fed every 48 h during the rearing period, and therefore have to constantly store and re-utilize nutrients between meals. The rate of nutrient digestion (in particular starch) may impact the storage-mobilization cycle and therefore the present study was conducted to study the impact of feeding wheat (rapidly digested) and pea (slowly digested) on blood glucose, and liver fat and glycogen metabolism in feed restricted broiler breeder pullets. Ross 308 broiler breeders (384) were randomly assigned to twelve pens in each of two rooms. For 0-3 wk of age, all pullets were fed a common starter ration on an ad-libitum basis; thereafter (3-13 wk) birds were fed either a pea- or wheat-based diet on an every-other-day basis to acheive target body weights. At 89 d of age, 4 birds (1/pen) were randomly selected at 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding. Blood glucose was measured, and livers were weighed and samples were collected for fat, glycogen and gene expression analyses. Data were analyzed as repeated measures using a compound symmetry covariance structure. The level of significance was fixed at $P \leq 0.05$. Broiler breeders pullets fed a pea-based diet demonstrated a lower post-prandial glucose peak in comparison to those fed wheat. Over the period of 48 h, the relative liver weight of pullets fed a pea-based diet was lower compared to birds fed the wheat-based diet. A treatment by time interaction showed that less fat was stored in the liver over the period of 48 h as a result of feeding a pea-based in contrast to a wheat-based diet. Regardless of treatment, liver glycogen levels were near maximum from 2 to 20 h after feeding. Over the 48 h period between feeding, the expression of acetyl CoA carboxylase and VLDL-apolipoprotein were reduced for birds fed pea instead of wheat. Interaction effects of dietary treatment and time demonstrated a reduced degree of variation (over the period of 48 h) in the expression of malic enzyme and glycogen phosphorylase genes in pullets fed a pea-based diet

as compared to wheat. In conclusion, feeding a pea-based diet resulted in reduced need for nutrient storage and mobilization between meals in feed restricted broiler breeder pullets.

4.2 Introduction

Extensive genetic selection of broilers for rapid growth has increased their appetite and altered mechanisms (central and peripheral) responsible for regulating feed intake (Lacy et al., 1985; Denbow, 1989). Recently, Classen (2017) reviewed the relationship between dietary energy and feed intake in chickens and concluded that modern broiler chickens are unable to precisely alter their feed intake in response to dietary energy. As a consequence, if fed on an ad-libitum basis, broiler breeders become overweight, which subsequently results in increased mortality and reduced productivity and reproductive performance during the laying phase (Mench, 2002). Hocking and Duff (1989) found reduced skeletal problems in feed restricted as compared to fully fed broiler breeders. Furthermore, Renema and Robinson (2004) concluded that ad-libitum feeding resulted in increased mortality (mainly attributed to metabolic and skeletal problems), reduced vigour and reduced control over production and release of ovarian follicles, thereby resulting in multiple ovulations and fewer settable eggs (double yolked, poor shell quality). Feed restriction is applied during the rearing and laying phases of broiler breeders to achieve target body weights and reduce the above performance issues. The level of feed restriction varies with age and is maximum during the rearing phase (de Beer and Coon, 2007). Body weight control is universally practiced to improve broiler breeder performance and welfare (Mench, 2002).

Feed restriction in broiler breeders during the rearing phase is applied using various feeding programs including feeding every-other-day (EOD), fed on five out of every seven days (5-2) or every-day (ED). Feeding every 48 h is a commonly used feeding program with an objective to

improve flock uniformity (Savory and Kostal, 1996; de Jong et al., 2002; de Beer and Coon, 2007). The impact of various feeding programs on health and performance on broiler breeders is well studied. de Beer and Coon (2007, 2009) found that ED feeding resulted in higher egg production and better feed efficiency as compared to EOD feeding. Additionally, ED fed birds reached sexual maturity at an earlier age in contrast to the EOD progam. de Beer et al. (2007) investigated the impact of feeding broiler breeder pullets ED or EOD on hepatic lipid metabolism and concluded that ED feeding resulted in less change in liver weight, fat content and expression of genes encoding for enzymes involved in hepatic fat synthesis during the time period between feeding. Based on these findings, it can be concluded that increased frequency of feeding results in improved efficiency in feed restricted broiler breeders. This effect can possibly be attributed to reduced storage and mobilization of nutrients with increased frequency of feeding (de Beer and Coon, 2007). Since EOD feeding is still common, nutritional strategies are required to reduce the degree of nutrient storage and mobilization to improve efficiency. One potential strategy is the use of ingredients with slowly digested starch (SDS) to reduce post-prandial blood glucose and associated metabolic effects.

The functional significance of SDS is mainly attributed to the slow release and absorption of glucose, which subsequently alters physiological and metabolic processes. Post-prandial hyperglycemia and insulinemia related to rapidly digested starch (RDS) have been suggested to be responsible for oxidative damage, metabolic syndrome, cardiovascular problems, obesity, diabetes, increased triglyceride concentration and fat deposition in humans (for review see Ludwig, 2002). Slowly digestible starch reduces post-prandial glucose and insulin concentrations, and has been shown to reduce the incidence of the above stated health issues (Lehmann and Robin, 2007). Sustained and slow release of nutrients by using ingredients

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containing SDS has potential to reduce the severity of negative effects related to feed restriction in broiler breeders.

Weurding et al. (2001) conducted an experiment with broilers and demonstrated that starch in pea-based diets was slowly digested. Therefore, the impact of rate of starch digestion on the storage and mobilization of nutrients between meals of broiler breeder pullets was investigated using pea- and wheat-based diets fed on an EOD basis. Based on the functional significance of SDS, it was hypothesized that feeding a pea-based diet will result in reduced nutrient storage and mobilization between meals.

4.3   Material and Methods

This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use (Canadian Council on Animal Care, 1993, 2009).

4.3.1   Dietary Treatments

Feed was formulated based on the Aviagen recommendations for four stage rearing program (Aviagen, 2007). For the first 3 wk, all chicks were provided with the same starter (I) feed containing pea and wheat as base ingredients, with the objective to acclimatize chicks to the major ingredients prior to feeding experimental diets (Table 4.1). Thereafter, birds were fed either pea- or wheat-based diets on an ED basis for a week (21-27 d), followed by EOD feeding for the rest of the experimental period (28-89 d). A second starter (II) and a grower feed were provided for 21-41 d and 42-89 d, respectively. Diets were formulated using pea or wheat as main ingredients and were approximately equalized for energy, protein and fat levels (Table 4.1). Digestible amino acid levels met recommended minimum values. Ground oat hulls (3 mm slotted

screen in a flail type hammer mill) were used to dilute the wheat based diets and assist in

equalizing diet energy levels. Feed allocation was the same for both treatments until 35 d of age

and thereafter increased for breeders fed the pea-based diet to equalise and maintain body weight

targets (Table 3.3). Broiler breeders fed wheat-based diets were heavier at 28, 35, 42, 49 and 56 d

of age as compared to those fed a pea based diets, but otherwise body weights were equal (Table

3.3).

Table 4.1. The ingredient and nutrient composition of pea- or wheat-based diets fed to broiler breeders.

Ingredients	Starter-I	Starter-II (Pea)	Starter-II (Wheat)	Grower (Pea)	Grower (Wheat)
Pea	50.64	69.40	-	64.74	-
Wheat	37.13	-	36.54	-	42.15
Barley	-	20.80	25.00	25.00	25.00
Soybean meal	5.74	2.84	22.43	-	12.96
Canola oil	2.00	2.54	2.53	2.24	2.17
Limestone	1.46	1.57	1.60	1.45	1.48
Dicalcium phosphate	1.69	1.39	1.30	1.18	1.16
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.33	0.44	0.38	0.45	0.38
DL-Methionine	0.27	0.26	0.10	0.15	0.05
Choline chloride	0.10	0.10	0.10	0.10	0.10
Oat hulls	-	0.08	9.44	4.12	13.96
Biocox[2]	0.05	0.05	0.05	0.05	0.05
Enzyme[3]	0.025	0.03	0.03	0.03	0.03
Calculated nutrients (%)					
AME (Mcal/kg)		2.700	2.700	2.600	2.600
Ca		1.00	1.00	0.90	0.90
Non phytate P		0.45	0.45	0.40	0.40
Total P		0.64	0.61	0.58	0.54
Analyzed nutrients (%)					
DM	-	89.67	89.84	89.93	90.06
CP	-	19.06	19.61	17.74	16.24
Total fat	-	3.56	3.62	3.34	3.12
Total starch	-	40.09	37.26	37.15	38.69
TDF	-	19.70	20.95	20.67	21.43

[1] Vitamin-mineral premix (units per kg of feed) contained the following: vitamin A, 11000 IU; vitamin D_3, 2200 IU; vitamin E, 30 IU; menadione, 2.0 mg; riboflavin, 6.0 mg; pantothenic acid, 10 mg; vitamin B_{12}, 0.02 mg; pyridoxine, 4.0 mg; thiamine, 1.5 mg; folic acid, 0.6 mg; niacin, 60 mg; biotin, 0.15 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[2] Salinomycin Sodium; Alpharma Canada Corp, Mississauga, ON, Canada.
[3] Endofeed W; GNC Bioferm, Cobble Hill, BC, Canada.

4.3.2 Birds and Housing

For the first two weeks, Ross 308 day old broiler breeder chicks (384) were reared as a group in a large pen. At 2 wk of age, all chicks were randomly placed in 24 equal size pens (16 chicks/pen) in two identical rooms (12 pens/room). Each of the two dietary treatments was assigned in a randomized complete block design to12 pens (six/room) at 3 wk of age, thus resulting in 12 replications per treatment. An equal amount of straw (7-10 cm) was placed in each pen. Pen size was 2.3×2.0 m, which provided a floor space of 0.27 m^2 per bird. Each pen was equipped with a drinker with 6 nipples and a trough feeder with a feeding space of 230 cm (14.4 cm/bird). Temperature was set at 31°C at d-0 and decreased gradually thereafter to 21°C by d-24. All chicks were provided with a 23 h of light (20 lux) for the first week, followed by 8 h of light and 16 h of dark (10 lux) for the remainder of the trial.

4.3.3 Data Collection

Average body weight for each pen was monitored on a weekly basis and feed allocation was adjusted accordingly to achieve target weights. At 89 d of age, 4 birds (1/pen) were randomly selected at 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding. Glucose was measured in brachial vein blood using the One Touch UltraMini blood glucose monitoring system (LifeScan, Inc., Milpitas, CA, USA). Immediately after monitoring glucose, blood samples were collected from each bird and stored at room temperature for approximately 60 min and thereafter at 4°C overnight. Serum was extracted by centrifuging blood at 3,000 × g for 5 min and stored at -20°C until further analyses. After blood sampling, pullets were humanely euthanized by cervical dislocation. Liver samples were collected on dry ice and stored at -20°C and -80°C for fat and glycogen analysis, respectively. For gene expression analysis, liver samples were snap frozen in liquid nitrogen and stored at -80°C until further analysis. For consistency,

livers were divided into left anterior and posterior and right anterior and posterior sections and samples were collected from same location each time for hepatic fat (left anterior), glycogen (right anterior) and gene expression analysis (left posterior).

4.3.3.1   Blood Glucose Validation

The One Touch UltraMini glucose monitoring system is intended for human use, therefore validation was performed prior to use in this experiment. For validation, glucose levels in serum (collected immediately after glucose monitoring as described above) were analysed using a Glucose (HK) assay kit (Sigma, Saint Louis, Missouri, USA; Rudrappa and Humphrey, 2007) and these results were compared to the One Touch UltraMini values.

4.3.3.2   Hepatic Fat and Glycogen Analysis

Liver glycogen was determined using a hydrochloric acid method (Passonneau and Lauderdale, 1974) as modified by Dadgar et al. (2011). Briefly, liver samples were homogenized (PT 3100, Kinematica AG, Littau, Switzerland) with 10 mL of 0.03 N HCl for 30 s. Enzymatic reactions were stopped by placing homogenate in a boiling water bath for five min. For hydrolyzation, 1 mL of homogenate was boiled with 2 mL of 1 N HCl for 3 h; thereafter, 2 mL of 1 N NaOH was used to neutralize the mixture.

An aliquot (1 mL) of this sample was centrifuged for 10 min at 12,000 rpm and used for glucose analysis. Glucose concentration was measured before and after hydrolysis using a D-Glucose HK assay kit (Megazyme International, Bray Business Park, Bray, Co. Wicklow, Ireland). Liver glycogen content was calculated as a percentage on a dry matter basis.

Liver samples stored at -20°C were used for fat determination using method (# 1920.39) of Association of Official Analytical Chemists (AOAC, 1990). Liver fat levels are presented as a percentage on a dry matter basis.

4.3.3.3 Gene Expression Analysis

Liver tissue samples stored at -80°C were ground under liquid nitrogen using a mortar and pestle treated to inactivate RNase (RNase Away, Molecular Bio-products Inc., San Diego, CA, USA). Approximately 30-35 mg of sample was used to extract ribonucleic acid using the TRI Reagent® Solution (Applied Biosystems®). The quantification of extracted RNA was conducted using a NanoDrop 2000 UV-vis Spectrophotometer at 260/280 nm (Thermo Scientific, NanoDrop Products, Wilmington, DE, USA). Thereafter, quantified RNA (1 µg) was used to synthesize cDNA using a high capacity cDNA Reverse Transcription Kit (Applied Biosystems). The cDNA was stored at -80°C until further analysis. The primers (Table 4.2) for acetyl CoA carboxylase (ACC; accession # J03541), malic enzyme (ME; accession # AF408407) and apolipoprotein (apoVLDL- II; accession # M25774) were developed using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/; Ye et al., 2012). Previously validated primers (Yadgary and Uni, 2012) were used for glycogen synthase (GLPX; accession # XM_416432.2) and glycogen phosphorylase (GLPH; accession # AY271349). The relative abundance of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; accession # NM_204305) and peroxiredoxin-6 (PRDX6; accession # 001039329.1) were determined for use as housekeeping genes (normalization); however, only PRDX abundance was not affected by treatment and thus used for normalization. For all primers developed here, the PCR product was gel purified (QIAEX II Agarose gel DNA extraction kit, Qiagen), quantified spectrophotometrically and sequenced (Sanger et al., 1977) for target amplicon confirmation. A CFX96 real time PCR

detection system with a C1000 thermal cycler (BioRad, Guénette, QC, Canada) was used for quantitative PCR. For each reaction, 7.00 µL double distilled sterilized water, 1.0 µL of template cDNA and 10 µL SsoFastTM EvaGreen® Supermix (BioRad) and 1.0 µL each of forward and reverse primer (Table 4.2) were used. Thermal cycling conditions were 95°C for 3 min followed by 35 cycles of 95°C for 5 sec and appropriate annealing temperatures as listed in Table 4.2 for 5 sec. A dissociation curve analysis was conducted after amplification by increasing temperature from 65°C to 95°C in 0.5°C increments for 5 sec each. Standard curves prepared from a cDNA dilution series demonstrated reaction efficiencies in the range 0.97 - 1.20, and R^2 values of $\geq$ 0.98. Abundance of the genes of interest (arbitrary value) was interperlated from a standard curve and normalized for abundance of PRDX6 interprelated from a standard curve. Standard curves were included on each 96-well reaction plate for each target gene.

Table 4.2. Details for primers used in the gene expression analysis.

Gene[1]	Primer Sequence (5'-3')	Orientation	Annealing Temperature (°C)
ACC	ATGGGGATAAACAGGGACCA	Forward	61
	CCCAATCTCGTTTCCTCCTG	Reverse	
ME	GGAGGAAAGGCAACAACTGA	Forward	59.9
	GTCCCCGGTCATGGATAGTA	Reverse	
apoVLDL-II	CTCACTAAACTGGCGGAACA	Forward	60
	TGGTCTTTGATGCTGGGTTC	Reverse	
GLSY	CATCTGTACACTGTGCCCATGTG	Forward	60
	TTTGGAGTGACAACATCAGGATT	Reverse	
GLPH	CCGTCCTCCATGTTTGATGTG	Forward	60
	TCTTGATGCGGTTGTACATGGT	Reverse	
GAPDH	GTGAAAGTCGGAGTCAACGGA	Forward	60
	AAGGGATCATTGATGGCCAC	Reverse	
PRDX6	CGCTGATAAGGACCGAGAG	Forward	60
	AAAGGGATGTGGAGGATTGG	Reverse	

[1] ACC = acetyl-CoA carboxylase; ME = malic enzyme; apoVLDL- II = apolipoprotein II; GLSY = glycogen synthase; GLPH = glycogen phosphorylase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PRDX6 = peroxiredoxin-6.

4.3.4 Statistical Analysis

The data for validation of glucose monitoring was compared using correlation analysis. All other data were analyzed as repeated measures using Proc Mix of SAS (SAS Institute, 2002). Compound symmetry covariance structure was used. All data were checked for normality prior to analysis. Pen was considered as the experimental unit. Least Significant Difference method was used for mean separation.The level of significance was fixed at $P \leq 0.05$.

4.4 Results

4.4.1 Glucose Validation

The blood glucose levels monitored using One Touch UltraMini system were correlated with glucose levels analyzed by Glucose (HK) assay kit ($R^2 = 0.60$, $P = <.0001$). Therefore, it was concluded that One Touch UltraMini glucose monitoring system can be successfully used in poultry species.

4.4.2 Blood Glucose Levels

A significant treatment by time interaction was noted for blood glucose levels over the period of 48 h (Figure 4.1). Broiler breeder pullets fed pea-based diets demonstrated a lower post-prandial glucose peak in comparison to those fed wheat.
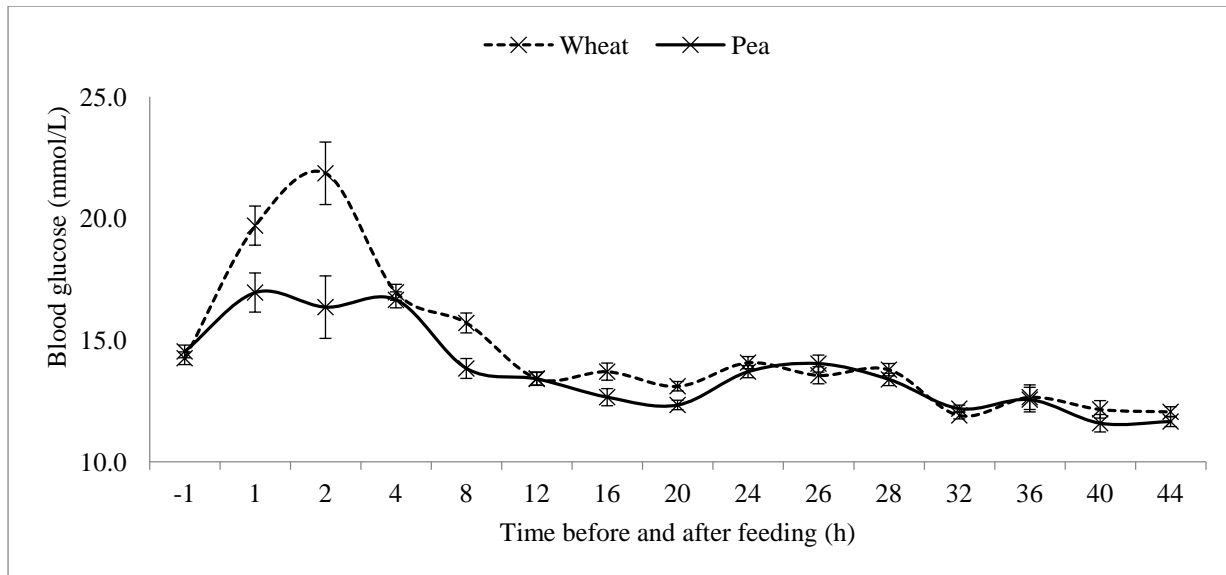
Figure 4.1. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on blood glucose levels (mmol/L) of broiler breeder pullets fed every-other-day (89 d of age). *P* Values: Treatment = <0.0001; Time = <0.0001; Treatment x Time = <0.0001.

4.4.3   Hepatic Weight and Fat and Glycogen Levels

Both the effect of diet and time affected relative liver weight, but the interaction between main effects was not significant (Figure 4.2). Overall, relative liver weight of pullets fed a pea-based diet was lower as compared to birds fed wheat. Furthermore, relative liver weight for both treatments increased post-prandially with a maximum reached at 16 h after feeding and then decreased to reach its lowest point. An interaction between diet and time was found for percentage liver fat content (Figure 4.3). Liver fat for birds fed both dietary treatments increased from feeding until reaching a maximum value 26 h later. The interaction effect was due to the larger increase in fat content for the birds fed the wheat-based diet. The percentage liver fat increased 3.4 and 2.4 fold for the birds fed wheat- and pea-based diets, respectively.
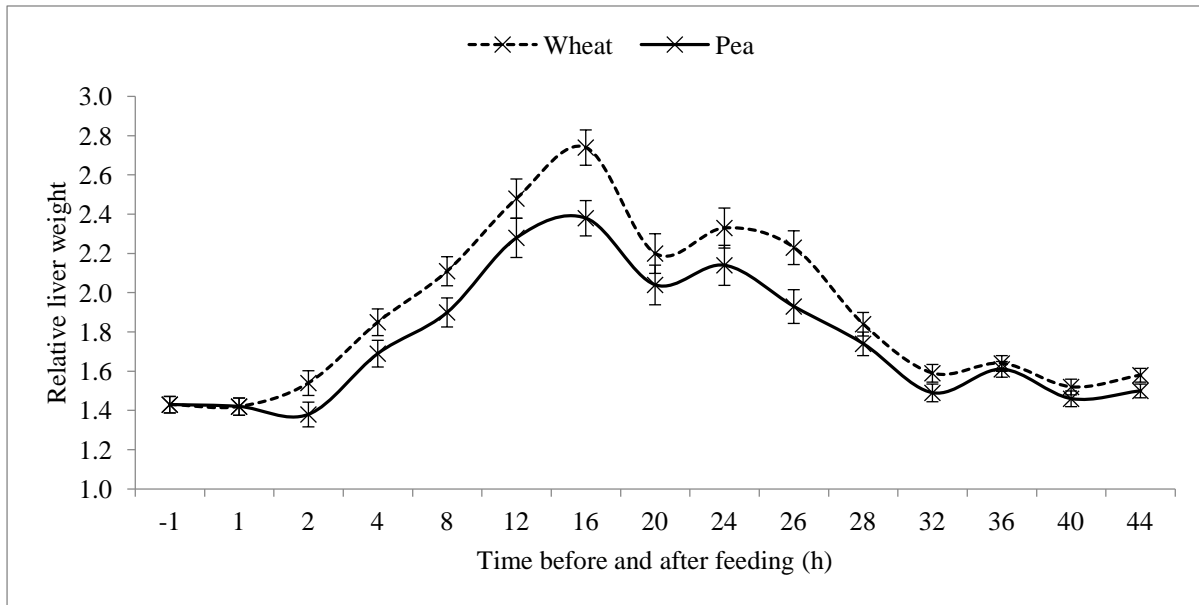
Figure 4.2. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on relative liver weight of broiler breeders at 89 d of age. *P* Values: Treatment = 0.005; Time = <0.0001; Treatment x Time = 0.29.
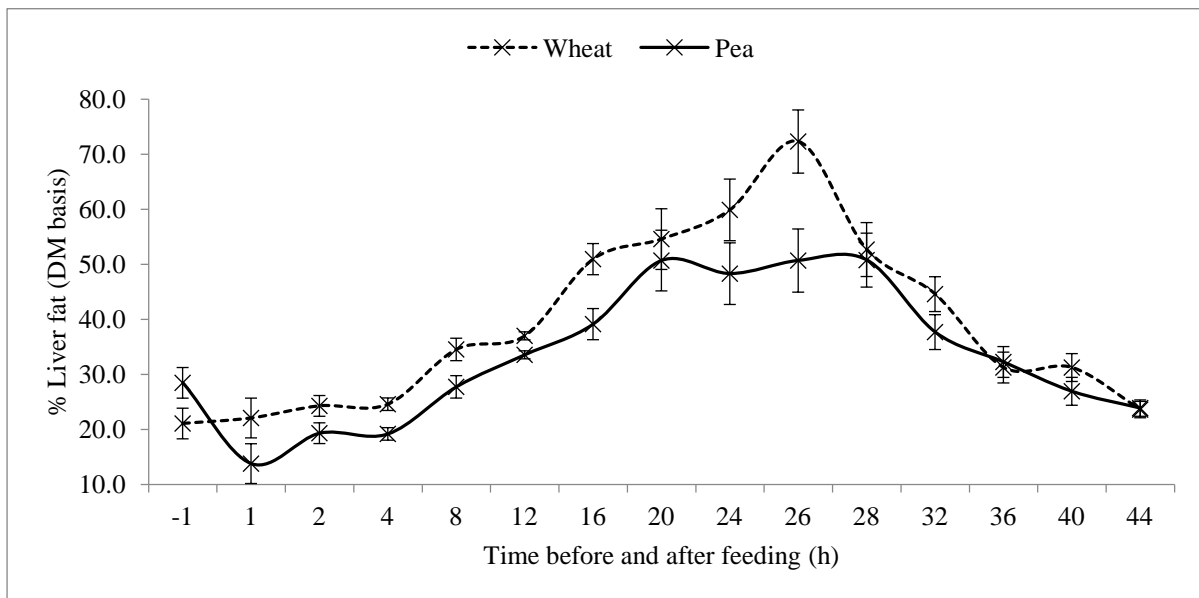
Figure 4.3. Effects of feeding a pea- or wheat-based diet and time in relationship to feeding on percent liver fat (DM basis) of broiler breeders at 89 d of age. *P* Values: Treatment = 0.0001; Time = <0.0001; Treatment x Time = 0.05.

Liver glycogen levels were not affected by dietary treatment or the interaction between diet and time (Figure 4.4). However, percent glycogen levels for both treatments increased post-prandially to reach near maximum from 2 to 20 h after feeding and thereafter reduced to lowest point by 44-47 h after feeding.
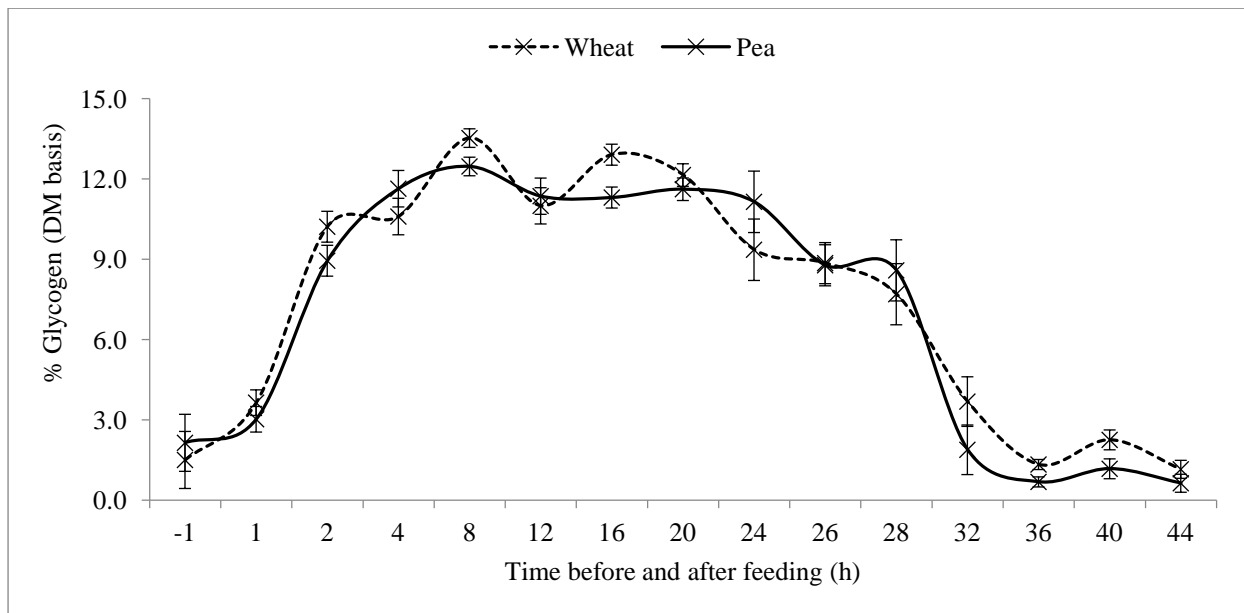
Figure 4.4. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on percent liver glycogen (DM basis) of broiler breeders at 89 d of age. *P* Values: Treatment = 0.43; Time = <0.0001; Treatment x Time = 0.90.

4.4.4   Gene Expression

Over the period of 48 h, the gene expression of ACC was higher for pullets fed a wheat-based diet as compared to birds fed pea (Figure 4.5). Expression of ACC increased after feeding for both treatments and reached to a maximum level by 12 (pea) or 16 (wheat) h after feeding, and then decreased to their lowest levels at 47 h. No interaction effect was observed. Over the period of 48 h, expression of ME was significantly affected by the diet and time interaction (Figure 4.6).

Expression of ME increased post-prandially and reached to a maximum level at 12 h and then

reduced to lowest point by 47 h after feeding. The significant interaction was due to higher

expression of ME during 2-16 h post feeding in pullets fed a wheat-based diet as compared to

pea. Broiler breeder pullets fed a wheat-based diet resulted in a higher expression of apoVLDL-II

in comparison to those fed pea over the period of 48 h (Figure 4.7). For both treatments,

expression of apoVLDL-II increased post feeding with a maximum level at 8 h. Time and

interaction effects were not significant.
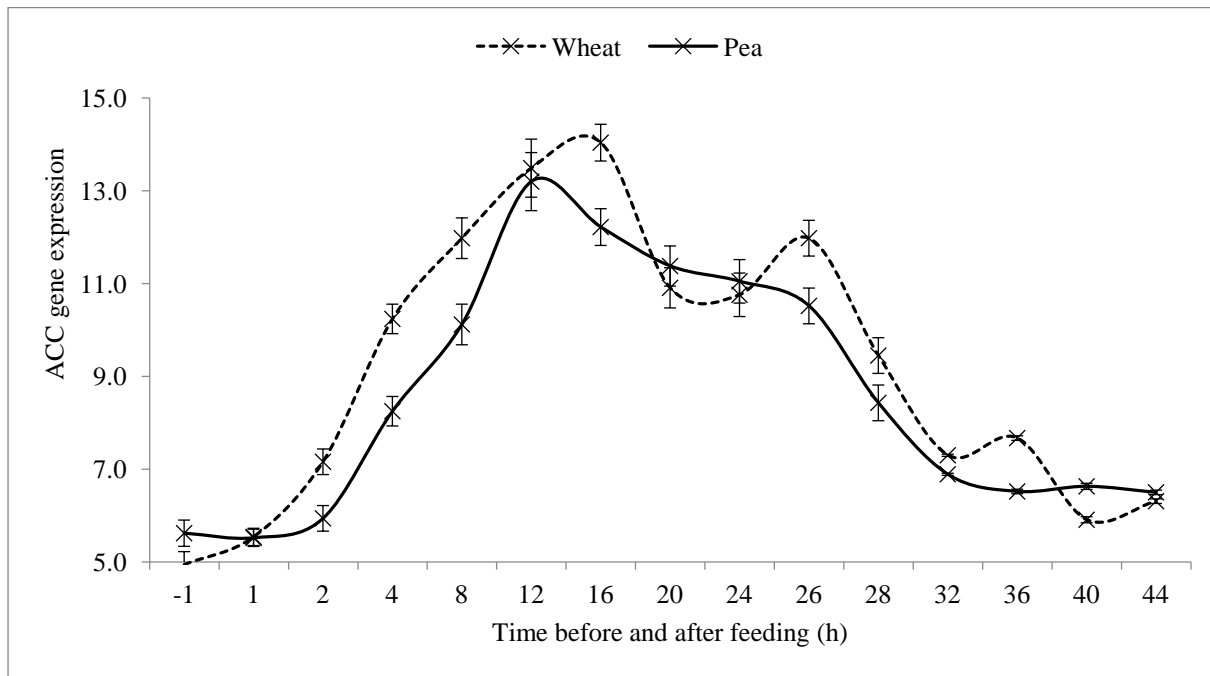
Figure 4.5. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on expression of hepatic acetyl-CoA carboxylase (ACC) of feed restricted broiler breeders at 89 d of age. *P* Values: Treatment = 0.05; Time = <0.0001; Treatment x Time = 0.66.
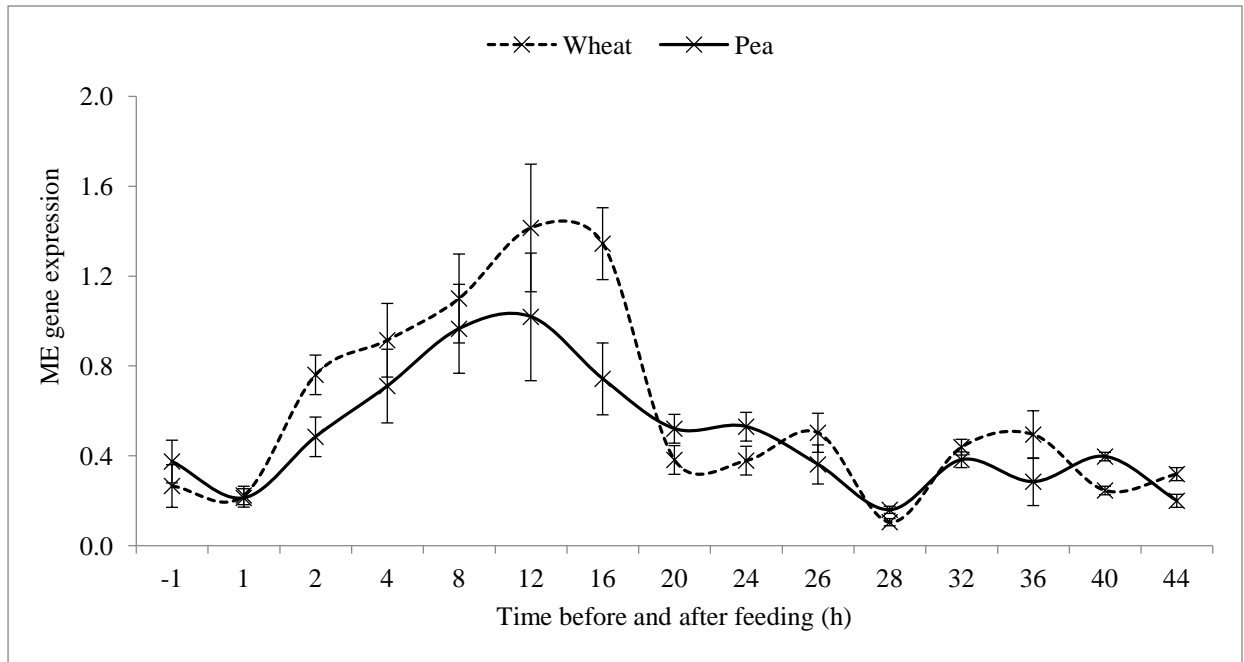
Figure 4.6. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on expression of hepatic malic enzyme (ME) of broiler breeders at 89 d of age. *P* Values: Treatment = 0.0001; Time = <0.0001; Treatment x Time = 0.02.
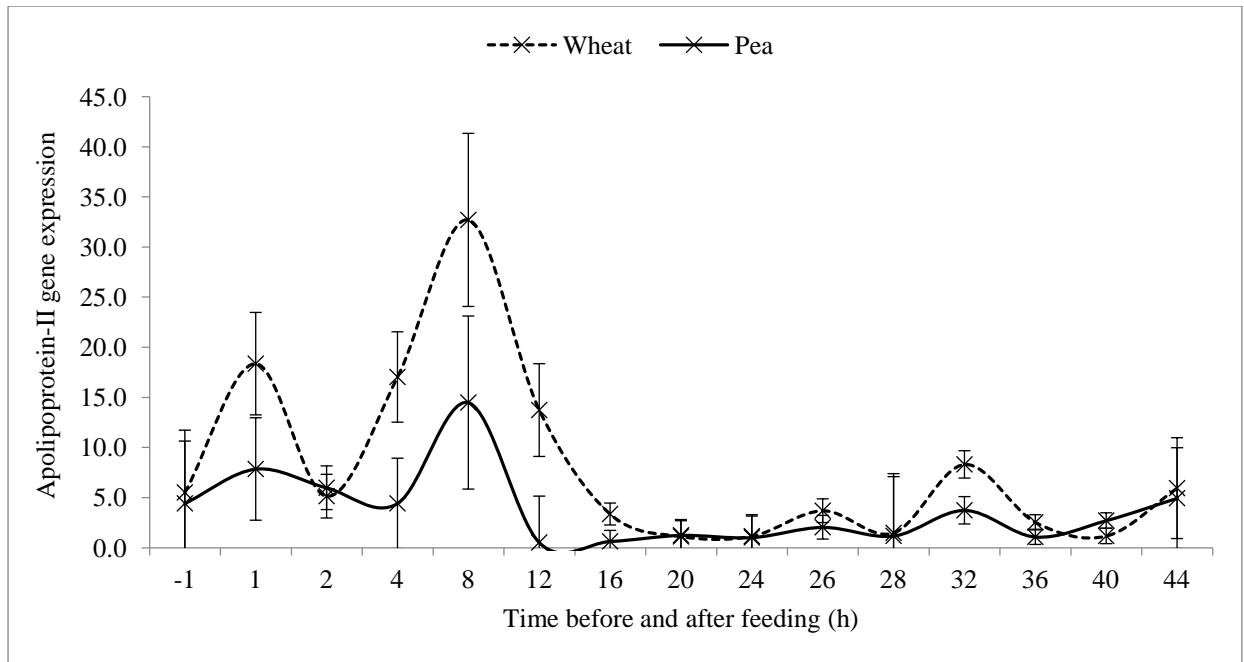
Figure 4.7. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on expression of hepatic apolipoprotein-II of broiler breeders at 89 d of age. *P* Values: Treatment = 0.002; Time = 0.0001; Treatment x Time = 0.45.

Over a period of 48 h, expression of GLSY tended to be higher (*P* = 0.08) for pullets fed the wheat based-diet in contrast to birds fed the pea-based diet (Figure 4.8). Expression of GLSY was not affected by time and the interaction between main effects. Interaction with time demonstrated that expression of GLPH was higher for pullets fed the wheat-based diet starting at 12 h after feeding (Figure 4.9).
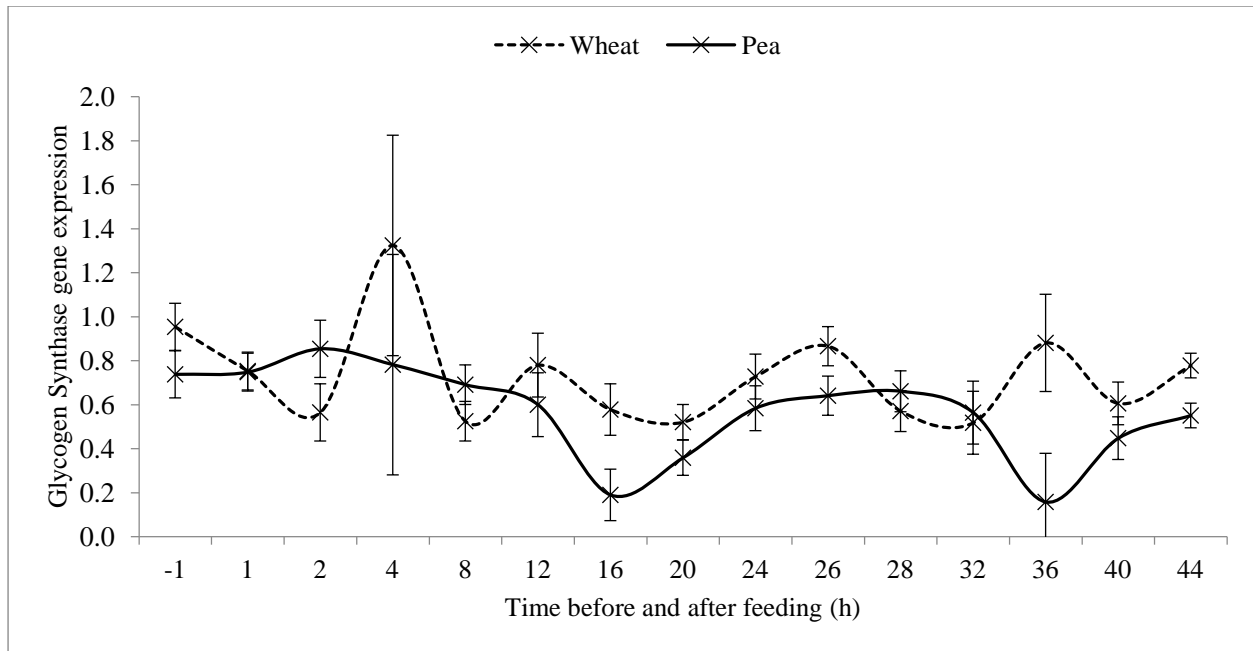
Figure 4.8. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on expression of hepatic glycogen synthase of broiler breeders at 89 d of age. *P* Values: Treatment = 0.08; Time = 0.58; Treatment x Time = 0.90.
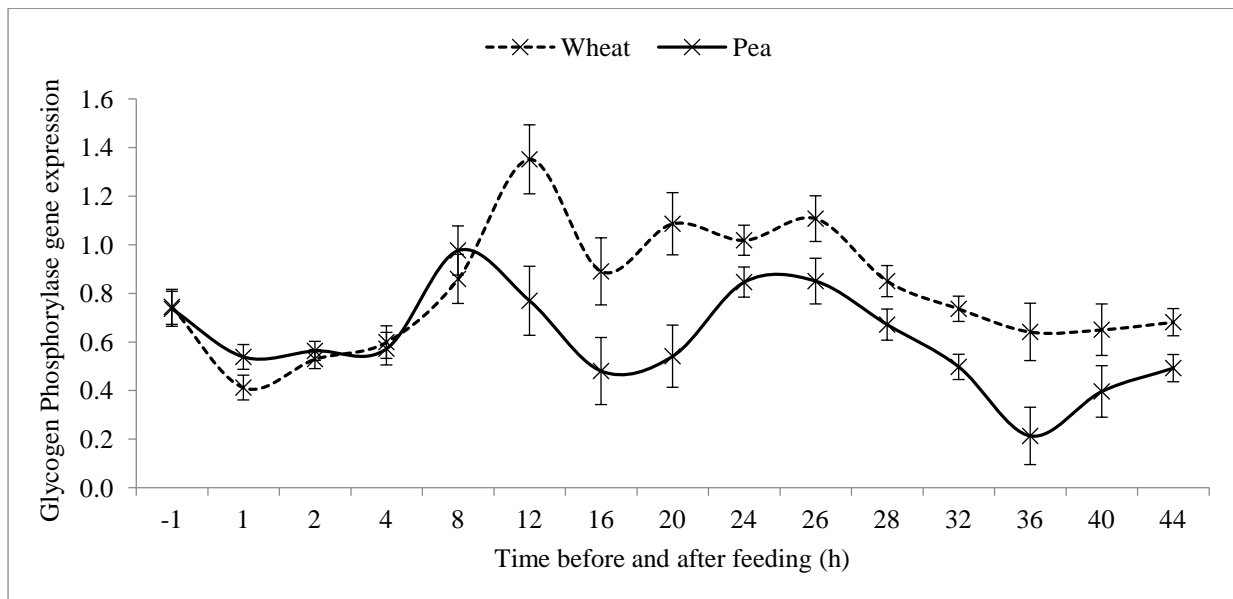
Figure 4.9. Effect of feeding a pea- or wheat-based diet and time in relationship to feeding on expression of hepatic glycogen phosphorylase of broiler breeders at 89 d of age. *P* Values: Treatment = <0.0001; Time = <0.0001; Treatment x Time = 0.03.

4.5   Discussion

The present study was designed with the objective to study the effects of feeding a pea-(SDS) or wheat-based diet (RDS) during the rearing phase on the storage and mobilization of nutrients between meals of broiler breeder pullets fed on an EOD basis. A significant treatment and time interaction was noted for blood glucose levels over the period of 48 h. Broiler breeder pullets fed a pea based diet demonstrated a lower post-prandial glucose peak in comparison to those fed wheat. However, the effect of diet was relatively short and glucose levels for both dietary treatments were similar after 4 h post-feeding. Peripheral glucose levels are tightly regulated in birds. More specifically, data have shown that peripheral glucose levels increased rapidly in birds after feeding and thereafter are reduced to base levels within a 1 to 1.5 h (Sinsigally, 1987; Beauchat and Chong, 1998). The impact of feeding RDS or SDS on post-prandial glucose levels was previously studied in human subjects. Seal et al. (2003) revealed that feeding SDS resulted in lower post-prandial glucose levels as compared to rapidly digestible starch in healthy subjects. Based on the large granule size and higher amylose content, pea starch is considered as slowly digestible as compared to wheat with small granule size and lower amylose content (Ratnayake et al., 2002; Weurding et al., 2002; Ebsim, 2013). As a consequence, the reduced post-prandial glucose peak after feeding a pea based diet is attributed to the slowly digestible nature of pea starch and as a consequence slow release and absorption of glucose.

Relative liver weight was not affected by the interaction of diet and time. Over the period of 48 h between feeding, relative liver weight of broiler breeders fed a pea-based diet was lower as compared to wheat. Furthermore, relative liver weights increased to a peak at 16 h after the start of feeding and then decreased to pre-feeding size by 44 h. At peak, weights increased

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approximately 92 and 66% for the wheat and pea fed treatments in comparison to 1 h before feeding. Similarly, an interaction between diet and time demonstrated that liver fat increased to a greater extent in pullets fed a wheat-based diet in contrast to those fed pea. After feeding, liver fat increased to reach maximum levels approximately 50% and 70% higher than the concentrations at 1 h before feeding in pea- and wheat-fed birds, respectively. Thereafter, differences between diets were smaller or non-existent as hepatic fat levels decreased. Changes in liver weight in broiler breeder pullets have been previously reported (de Beer et al., 2007) and attributed to changes in liver glycogen and fat content. The authors found a bigger change in liver weight and composition in broiler breeders fed on a EOD as compared to ED basis. Differences were attributed to reduced degree of nutrient storage and mobilization with a more frequent feeding regimen. In the present study, differences in liver weight between two meals are primarily attributed to changes in liver fat content. Furthermore, these findings reflect the reduced nutrient storage and mobilization between two meals by feeding a pea-based diet. Differences in relative liver weight and fat content might be explained on the basis that feeding pea resulted in the slow release and/or absorption of nutrients, and the consequential reduced need for nutrient storage and mobilization in comparison to the rapid release of glucose in birds fed the wheat-based diet.

Liver glycogen levels were not affected by dietary treatment or the interaction between diet and time. For both pea and wheat fed pullets, glycogen level reached near maximum values by 2h after feeding, stayed high until 20 h and then reduced gradually to 28 h before declining rapidly to minimum levels by 32 h. In research that occurred simultaneously to the current research, feed was found in the small intestine for approximately 26-28 hours after feeding (Chapter 3). Furthermore, de Beer et al. (2008) found that crop of broiler breeders fed on an EOD basis was empty after 24 h of feeding. Therefore, these data are supportive of the fact that glycogen is the

first source of glucose used during the initial phase of fasting (Engelking, 2011). Furthermore, research has demonstrated that hepatic glycogen level depleted rapidly during fasting in chickens and turkeys (Rosebrough et al., 1978; Tinker et al., 1986). Lack of a diet effect on glycogen levels might be explained on the basis that both pea and wheat starch provide more than enough glucose to maximize glycogen synthesis.

Experiments conducted using labelled substrates such as pyruvate or glucose revealed that the liver is the key site for lipid synthesis in chickens (O'Hea and Leveille, 1969b). This has been found to be true in other avian species as approximately 96% of lipid synthesis in pigeons takes place in liver (Goodridge and Ball, 1967). The important role of chicken liver in lipogenesis was further supported by the higher expression of a key transcriptional factor of lipid synthesis (Sterol Regulatory Element Binding Protein-1; SREBP-1) in liver as compared to adipose tissue (Gondret et al., 2001). In chickens, glucose derived from starch digestion is considered a key substrate for lipid synthesis. Hepatic lipid synthesis involves the conversion of glucose to triglycerides and involves reactions of glycolysis, the citric acid cycle, and fatty acid synthesis. Key enzymes include ACC, ME and fatty acid synthase (Richards et al., 2003). Acetyl CoA carboxylase is responsible for the conversion of acetyl CoA to malonyl CoA and is a rate limiting step. Malic enzyme is responsible for providing reducing power by converting $NADP^+$ to NADPH. Fatty acid synthase is responsible for converting malonyl CoA to palmitate (Richard et al., 2003). The triglycerides synthesized in the liver are transported to adipose tissue where they are stored for usage during periods of food deprivation such as fasting or starvation (O'Hea and Leveille, 1969a). Apolipoprotein B is the primary lipoprotein required for the formation of very low density lipoproteins (VLDL) in the liver, and VLDL are responsible for transferring

triglycerides to adipose tissue. Lipoprotein lipase is a key enzyme responsible for the hydrolysis of triglycerides to release fatty acids prior to uptake by adipocytes (Buyse and Decuypere, 2015).

In the present study, the expression of genes responsible for key enzymes involved in hepatic lipid and glycogen synthesis and lysis were studied. Feeding a pea-based diet resulted in reduced gene expression of ACC over the 48 h. Gene expression increased after feeding and was relatively high for both diets between 8 and 26 h after feeding before rapidly declining. The interaction between treatment and time demonstrated that feeding a pea- in contrast to a wheat-based resulted in lower ME expression after feeding. More specifically, the expression of ME was increased to a greater extent by feeding wheat- as compared to pea-based diet up to 12 h after feeding. The reduced expression of ACC and ME is in agreement with reduced liver weights and liver fat levels by feeding a pea-based diet. Previously, Goodridge (1968) demonstrated that fasting of chicks resulted in reduced hepatic lipid synthesis. Similarly, fasting resulted in reduced hepatic lipid synthesis and acetyl CoA levels in the liver of chicks (Yeh and Levielle, 1971). de Beer et al. (2007) compared two feeding regimens and found that expression of genes encoding for ACC, ME and fatty acid synthase were increased in pullets fed on EOD as compared to those fed daily. They concluded that broiler breeder pullets fed on an EOD basis were required to store and re-utilize more nutrients than birds fed ED. In the current study, the reduced expression of genes encoding for ACC and ME reflects the reduced degree of hepatic lipid synthesis in pullets fed a pea-based diet. This can be attributed to the slowly digestible nature of pea starch providing an exogenous source of glucose for an extended period of time.

The liver is the site of lipogenesis in birds and fat can be stored in the liver or body adipose sites. The transport of fats from the liver to adipose sites occurs via VLDL. Expression of apoVLDL-II was measured to understand the dissemination of fat to the remainder of the body.

Feeding wheat resulted in the increased expression of apoVLDL-II during the period between two meals as compared to pullets fed a pea-based diet. Furthermore, expression increased after feeding before declining to low levels at 16 h after feeding. No interaction was found. These results suggest that birds fed wheat transported more fat to extra-hepatic tissues such as adipocytes for further storage and mobilization during the off-feed day as compared to those fed a pea-based diet. Increased degree of mobilization of fat during off-feed day in pullets fed a wheat-based diet was demonstrated by increased level of serum NEFA and β-HBA (Chapter 5). This further supports the hypothesis that feeding pea (slowly digested starch) reduces the need for storage and mobilization of nutrients.

Over a period of 48 h, expression of GLSY tended to be higher ($P = 0.08$) for pullets fed a wheat-based diet in contrast to pea. However, expression of GLSY was unaffected by time and the interaction between diet and time. The interaction between diet and time significantly affected the expression of GLPH between meals. The interaction effect was attributed to the higher expression of GLPH by wheat-fed birds starting at 12 h post-feeding. This appears to be contrary to the lack of a dietary treatment effect on liver glycogen level, but it may suggest that glycogen turnover (synthesis and hydrolysis) was higher in birds fed wheat- than pea-based diets. Of interest, the expression of both GLSY and GLPH appeared to demonstrate a circadian rhythm in pea-fed birds with distinctively lowest gene expression at 16 and 36 h after feeding (both in the scotophase). In contrast, these trends were either missing (GLSY) or of lower amplitude (GLPH) for wheat-fed pullets. Kaminsky and Kosenko (1987) studied dirurnal rhythms in carbohydrate metabolism in rats and mice, and demonstrated that hepatic glycogen synthase and phosphorylase activities demonstrated diurnal rhythms with lowest activity during the dark period. Furthermore, the authors suggested that light-dark and feeding cycles act as key zeitgebers. In the present

study, all birds were exposed to the same light-dark and feeding cycle so dietary differences in gene expression were unrelated to an obvious zeitgeber. It can be speculated that non-existent or weak rhythms in wheat-fed pullets may reflect their energy status and increased need for nutrient storage and mobilization.

In conclusion, the reduced degree of change of relative liver weight and fat content over the period between meals coupled with reduced expression of genes encoding for ACC, ME and apoVLDL-II indicate that feeding a pea-based diet results in reduced nutrient storage and mobilization. This is attributed to the slow digested nature of starch in the pea-based diet.

5 EFFECT OF FEEDING PEA OR WHEAT BASED DIETS ON HUNGER OF FEED RESTRICTED BROILER BREEDERS

Preface

The objective of this chapter was to study the effects of feeding a pea- or wheat-based diet during the rearing phase on behavioural (drinking, foraging, resting, standing, walking, comfort, feeder pecking, object pecking) and physiological parameters (NEFA and β-HBA) indicative of hunger and satiety in feed restricted broiler breeder pullets.

Author Contributions

Aman Deep (Department of Animal and Poultry Science, University of Saskatchewan) planned, designed and conducted the experiment, analyzed the data, and drafted the manuscript.

Henry Classen (Department of Animal and Poultry Science, University of Saskatchewan) provided guidance and scientific input, reviewed and edited the manuscript, and provided funding for the research.

Andrew Van Kessel (Department of Animal and Poultry Science, University of Saskatchewan) provided scientific input and guidance in planning and desiging this experiment, laboratory infrastructure for gene expression analysis, and manuscript editing.

5.1 Abstract

Feed restriction in broiler breeders is applied to maintain target body weights, thereby improving health and reproductive performance, but is criticized due to the welfare concerns regarding the long periods without feed. Slow digestion of starch has been suggested to increase satiety in humans, so broiler breeder pullets were fed either wheat (rapidly digested starch) or pea (slowly digested starch) based diets to study the impact of starch digestion rate on bird satiety. Ross 308 broiler breeder pullets were randomly assigned to 12 pens (16 per pen) in each of two rooms at 14 d of age. Pullets were fed a starter ration on an ad-libitum basis for three wk before being randomly assigned to either a wheat- or pea-based grower diet fed on an every-other-day basis to maintain target body weights from 3-13 wk of age. Satiety was assessed using behavioral and physiological parameters. Behavior was recorded for 48 h in two independent pens per treatment at both 9 and 10 wk of age, thereby resulting in four replicates. At 89 d of age, serum samples (four birds/treatment/time) were collected 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding and assayed for non-esterified fatty acids (NEFA) and β-hydroxybutyrate. Behavior data were analysed as a 2×2 factorial design with dietary treatment and day (on-feed, 0-24 h; and off-feed, 24-48h) as main effects. Physiological data (NEFA and β-hydroxybutyrate) were analysed as repeated measures using a compound symmetry covariance structure. The level of significance was fixed at $P \leq 0.05$. Birds fed a wheat-based diet demonstrated increased drinking and reduced pecking over the 48 h period. Interactions between diet and day showed that pullets fed a pea-based diet expressed increased comfort and resting behavior, and reduced foraging and walking behavior during the off-feed day (24-48 h). Feeding a pea-based diet resulted in reduced levels of serum NEFA and β-hydroxybutyrate starting at 26

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to 28 h post-feeding. Overall, both physiological and behavioral indicators suggest increased

satiety in feed restricted broiler breeder pullets when fed a pea- compared to a wheat-based diet.

5.2 Introduction

Improved performance of modern day broilers is attributed to extensive genetic selection for

growth, feed efficiency as well as a variety of other characteristics. Selection for faster growth

results in simultaneous increase in growth and appetite in broiler breeders to an extent that

feeding on an ad-libitum basis results in negative impact on health status and reproductive

capacity. More specifically, ad-libitum feeding broiler breeders results in increased mortality due

to metabolic and skeletal problems, decreased productivity during the laying phase, disrupted

follicular hierarchies (multiple ovulations) and reduced fertility (Robinson et al., 1991; Mench,

2002; Renema and Robinson, 2004). In order to prevent the negative consequences related to ad-

libitum feeding, feed restriction is applied to maintain the body weight targets of broiler breeders.

The degree of feed restriction varies with age with a maximum occurring during rearing phase

(de Beer and Coon, 2007). Furthermore, feed restriction can be achieved by daily feeding, but

often is applied on an every-other-day (EOD) basis during the periods of most severe feed

restriction with an objective to improve flock uniformity (Savory and Kostal, 1996; de Jong et al.,

2002; de Beer and Coon, 2007). Beneficial effects of feed restriction include reduced losses due

to metabolic and skeletal problems, and improved productivity and reproductive capacity

(Mench, 2002). Improved health and production achieved with feed restriction is mainly

attributed to reduced body weight and accumulation of fat. For an example, ovarian dysfunction

in ad-libitum fed broiler breeders might be explained on the basis of toxic effects of excessive

body lipids in non-adipocyte sites such as ovarian tissue (Chen et al., 2006). In addition to its

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positive impact, feed restriction also results in long periods without feed, which are associated with hunger.

Hunger is defined as natural motivational state that results in search and consumption of feed (Webster 1995). Assessment of hunger in feed restricted broiler breeders cannot be achieved by monitoring a single parameter, but collective use of behavioural and physiological indices acts as an important tool to indirectly measure the degree of hunger (Tolkamp and D'Eath, 2016). Behavioural indices include expression of redirected oral behaviours (drinking, foraging and pecking), and resting and comfort behaviours. Expression of redirected oral behaviors reflects frustration related to feeding motivation, thereby indirectly reflecting degree of hunger in poultry species (Baumeister et al., 1964; Duncan and Wood-Gush 1972, Hughes and Wood-Gush 1973; Savory et al., 1993). Additionally, Duncan and Mench (1993) categorized comfort behaviours as behaviours performed after fulfillment of basic needs and when birds are free from suffering. As a consequence, the decreased expression of comfort behaviours may indicate reduced welfare associated with a particular environment such as feed restriction. Feed restricted broiler breeder demonstrate increased expression of drinking, foraging, spot pecking and activity behaviour in comparison to those fed on an ad-libitum basis (Savory et al., 1992; de Jong et al., 2002, 2003; Jones et al., 2004; Merlet et al., 2005; Hocking et al., 2006). In contrast, expression of comfort and resting behaviour is reduced with feed restriction as compared to breeders fed on an ad-libitum basis (de Jong et al., 2002, 2003; Jones et al., 2004; Merlet et al., 2005).

Physiological measures of hunger include serum levels of metabolites reflecting either stress (H/L ratio or corticosterone) or metabolic state (non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-HBA)) of feed restricted broiler breeders pullets (Hocking, 1993, Hocking et al., 1996; de Jong et al., 2003, Brady et al., 1978; Le Maho et al., 1981; Cherel et al., 1987). The

relationship between physiological parameters (H/L ratio, and corticosterone) and level of feed

restriction is inconsistent with studies showing variable effects (Savory et al., 1992; Hocking,

1993; Jones et al., 2004). Furthermore, the levels of these stress indicators are also affected by the

metabolic state of the body such as body weight or degree of starvation or fasting (Tolkamp and

D'Eath, 2016). In contrast, levels of NEFA and β-HBA have been shown to increase in a

consistent manner with short term fasting/starvation in birds (Brady et al., 1978; Le Maho et al.,

1981; Cherel et al., 1987). In conclusion, an approach to assess degree of hunger using multiple

indicators was used in this research.

Extensive research has been conducted to reduce the degree of negative impact of feed

restriction on welfare of broiler breeders. Previous studies have looked at various managemental

and nutritional strategies, such as the use of feeding programs that increase foraging and the use

of diluted feed (Zuidhof et al., 1995; de Jong et al., 2011; Nielson et al., 2011). The rationale for

using scattered feeding or dietary dilution includes the increase in time spent eating and improved

satiety related to increased gastrointestinal tract (gut) fill. Recently the use of dietary appetite

suppressants has also been studied to improve the satiety of feed restricted broiler breeders

(Morrissey et al., 2014a and b). The authors concluded that the use of diets containing

suppressants resulted in reduced stereotypic feather pecking, thereby signaling improved satiety.

Another nutritional alternative may be to feed a diet based on ingredients with slowly digested

starch (SDS) such as pea. Weurding et al. (2001) investigated the impact of SDS on broiler

performance and concluded that its addition to diets improves productivity. The functional

significance of SDS is mainly attributed to slow release of glucose, which subsequently alters

physiological and metabolic processes. Slowly digestible starch reduces the fluctuations in post-

prandial glucose and insulin concentration and has been shown to reduce the incidence of the

health issues including oxidative damage, metabolic syndrome, cardiovascular problems and obesity (Lehmann and Robin, 2007; Vinoy et al., 2017). Furthermore, human research has shown that SDS results in reduced hunger and increased satiety. Improved satiety with SDS is attributed to slow and prolonged starch oxidation in comparison to rapid oxidation with rapidly digested starch (RDS; Sparti et al., 2000). Another mechanism known as the ileal brake relates to the role of incretins including GLP-1. Wachters-Hagedoorn et al. (2006) demonstrated that diet SDS increased release of GLP-1, which has been related to slow gastric emptying and SI transit, and as a consequence, improved satiety and reduced feed intake (Edholm et al., 2010; Hasek et al., 2017). Therefore, sustained and slow release of nutrients by using SDS has potential to reduce the severity of negative effects related to feed restriction in broiler breeders.

The present study was designed to study the effects of feeding a pea- or wheat-based diet during rearing phase on behavioural (drinking, foraging, resting, standing, walking, comfort, feeder pecking, object pecking) and physiological parameters (NEFA and β-HBA) of hunger and satiety in feed restricted broiler breeders. Based on the functional significance of slowly digestible starch, it was hypothesized that feeding a diet based on pea will results in reduced degree of hunger in feed restricted broiler breeders pullets.

5.3 Material and Methods

This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use (Canadian Council on Animal Care, 1993, 2009).

5.3.1 Dietary Treatments

Feed was formulated based on the Aviagen recommendations for a four stage rearing program (Aviagen, 2007). For the first 3 wk, all chicks were provided with a common starter (I) and thereafter birds were fed either pea- or wheat-based diets on an EOD basis for the rest of the experimental period (21-89 d). A second starter (II) and a grower feed were provided from 21-41 d and 42-89 d, respectively. Diets were formulated using pea or wheat as main ingredients, to meet minimum recommended digestible amino acid levels, and to approximately equalize energy, protein and fat levels (Table 5.1). Ground oat hulls (3 mm slotted screen in a flail type hammer mill) were used to dilute the wheat based diets and assist in equalizing diet energy levels. Feed allocation was the same for both treatments until 35 d of age and thereafter increased for breeders fed a pea-based diet to equalise and maintain body weight targets (Table 3.3). Broiler breeders fed a wheat-based diet were heavier at 28, 35, 42, 49 and 56 d of age as compared to those fed a pea-based diet (Table 3.3).

Table 5.1. The ingredient and nutrient composition of pea- or wheat-based diets fed to broiler breeders.

Ingredients	Starter-I	Starter-II (Pea)	Starter-II (Wheat)	Grower (Pea)	Grower (Wheat)
Pea	50.64	69.40	-	64.74	-
Wheat	37.13	-	36.54	-	42.15
Barley	-	20.80	25.00	25.00	25.00
Soybean meal	5.74	2.84	22.43	-	12.96
Canola oil	2.00	2.54	2.53	2.24	2.17
Limestone	1.46	1.57	1.60	1.45	1.48
Dicalcium phosphate	1.69	1.39	1.30	1.18	1.16
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.33	0.44	0.38	0.45	0.38
DL-Methionine	0.27	0.26	0.10	0.15	0.05
Choline chloride	0.10	0.10	0.10	0.10	0.10
Oat hulls	-	0.08	9.44	4.12	13.96
Biocox[2]	0.05	0.05	0.05	0.05	0.05
Enzyme[3]	0.025	0.03	0.03	0.03	0.03
Calculated nutrients (%)					
AME (Mcal/kg)		2.700	2.700	2.600	2.600
Ca		1.00	1.00	0.90	0.90
Non phytate P		0.45	0.45	0.40	0.40
Total P		0.64	0.61	0.58	0.54
Analyzed nutrients (%)					
DM	-	89.67	89.84	89.93	90.06
CP	-	19.06	19.61	17.74	16.24
Total fat	-	3.56	3.62	3.34	3.12
Total starch	-	40.09	37.26	37.15	38.69
TDF	-	19.70	20.95	20.67	21.43

[1] Vitamin-mineral premix (units per kg of feed) contained the following: vitamin A, 11000 IU; vitamin D_3, 2200 IU; vitamin E, 30 IU; menadione, 2.0 mg; riboflavin, 6.0 mg; pantothenic acid, 10 mg; vitamin B_{12}, 0.02 mg; pyridoxine, 4.0 mg; thiamine, 1.5 mg; folic acid, 0.6 mg; niacin, 60 mg; biotin, 0.15 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.

[2] Salinomycin Sodium; Alpharma Canada Corp, Mississauga, ON, Canada.

[3] Endofeed W; GNC Bioferm, Cobble Hill, BC, Canada.

5.3.2   Birds and Housing

Ross 308 day old broiler breeder chicks (384) were initially brooded as a group in a large litter floor pen at the Poultry Centre of the University of Saskatchewan. At 2 wk of age, all chicks were randomly placed in 24 equal size pens (16 chicks/pen) in two identical rooms (12 pens/room). From 0 to 3 wk, all chicks were provided with a starter I (Table 1) feed containing pea and wheat as base ingredients with an objective to acclimatize chicks to the primary ingredients prior to feeding experimental diets. Each of two dietary treatments was assigned in a randomized complete block design to 12 pens (6/room) at 3 wk of age. An equal amount of straw (7-10 cm) was placed in each pen. Pen size was 2.3×2.0 m, which provided a floor space of 0.27 m^2 per bird. Each pen was equipped with a drinker with 6 nipples and a trough feeder with a feeding space of 230 cm (14.4 cm/bird). Temperature was set at 31°C at d-0 and decreased gradually thereafter to 21°C by d-24. All chicks were provided with a 23 h of light (20 lux) for the first week, followed by 8 h of light and 16 h of dark (10 lux) for the remainder of the trial. Feed was provided one hour after the beginning of the light period.

5.3.3   Data Collection

The behaviour of broiler breeders was recorded by placing an infrared camera (Deep et al., 2010) over the experimental pens (16 pullets/pen) in each room. Two independent pens per treatment were selected at 9 and 10 wk of age, thereby resulting in four replicates per treatment. In each pen, behaviour was videotaped for a period of 48 h. Behavioural expression was studied using an instantaneous scan sampling using Genetec Omnicast Live Viewer 3.5 System (Deep et al., 2010). Individual pullets involved in feeding (head in feeder when feed was present), drinking (standing with head directly under the drinker line), resting (lying on the straw not performing any other behaviours), standing (standing without doing anything else), walking (walking,

running without doing anything else), foraging (pecking, scratching the litter), pecking (pecking at empty feeder, parts of the pen and other birds) and comfort (preening, stretching, wing-flapping and feather-ruffling) were counted at every 20 min interval for the 48 h observation period. A previously developed broiler breeder ethogram was used to minimize the chances of error in diagnosing a particular behavior (de Jong et al., 2003). Comfort behaviours were defined as "behaviours performed for care and grooming of body, thus providing them physical comfort" (Wood-Gush, 1971).

At 89 d of age, four birds per treatment were randomly selected at 1 h before and 1, 2, 4, 8, 12, 16, 20, 24, 26, 28, 32, 36, 40 and 44 h after feeding. From each pullet, blood samples were collected from the brachial vein using vacutainer and non-heparinized centrifuge tubes. Serum was extracted by centrifuging blood at 2,500 × g for 10 minutes and stored at -20$^{°}$C for NEFA and β-HBA analysis.

5.3.4   Chemical Analysis (Non-Esterified Fatty Acids and β-hydroxybutyrate)

Non-esterified fatty acids and β-HBA were analyzed using HR Series NEFA-HR (2; Code # 999-34691) and Autokit 3-HB (Code # 417-73501-Cyclic enzymatic method), respectively. Both kits were supplied by Wako Diagnostics, Wako Chemicals USA Inc. (Richmond, VA, USA). The selection of these kits was based on previous studies on broiler breeders and broilers (Mckee et al., 1997; de Jong et al., 2003).

5.3.5   Statistical Analysis

Behavioural indictors of hunger were analyzed as a 2×2 factorial (PROC MIXED-SAS 9.2) with dietary treatments and day (on-feed or off-feed) as main factors. The age effect (9 vs. 10 wk) was not significant and therefore not included in the statistical model. Physiological parameters

of hunger (NEFA and β-HBA) were analyzed as repeated measures with compound symmetry covariance structure using PROC MIXED of SAS (SAS Institute, 2002). Pen was considered the experimental unit. Data were checked for normality prior to analysis and percentage data were log transformed prior to analysis. Means were separated using the Least Significant Difference (LSD) test and the level of significance was fixed at $P \leq 0.05$ unless otherwise stated.

5.4   Results

5.4.1   Behavioural Expression

Feeding behaviour was not affected by diet and only occurred during the on-feed day (Table 5.2). Drinking behaviour was reduced by feeding a pea- vs wheat-based diet ($P = 0.01$) and less drinking occurred on off-feed day ($P=0.0009$). An interaction was found between diet and day ($P=0.02$) for walking with birds fed both diets walking equally during the on-feed day, but birds fed the wheat-based diets walked more than pullets fed the pea-based diet on the off-feed day. Day did not affect the percent of time spent standing, but feeding the pea-based diet reduced standing ($P=0.01$) in comparison to those fed the wheat-based diet. The percent of time birds spent foraging was also affected by the interaction between diet and day ($P=0.05$). For both days wheat-fed birds foraged more than pea-fed birds, but the expression of this behaviour increased on the off-feed day for the wheat fed pullets and not the pea fed birds. The percentage of birds pecking was higher for those fed the pea- than wheat-based diet ($P=0.0001$), and birds pecked more on the off-feed than the on-feed day ($P=0.001$). Percent of comfort behaviours (preening, stretching, wing-flapping and feather- ruffling) was also affected by the interaction between diet and day ($P=0.0005$). Birds fed a pea-based diet expressed increased comfort behaviours as compared to wheat-based diet on the off-feed day. The interaction for resting behaviour was significant ($P=0.02$), with both diet treatments resting to the same degree during the on-feed day,

but birds fed the pea-based diet resting more than those fed the wheat-based diet on the off-feed day. Based on the pattern of behavioural expression over the 48 h period, it was noted that broiler breeders were active during first scotophase (7- 23 h after feeding) expressing behaviours such as comfort, standing, pecking and foraging. During second scotophase (31-47 h after feeding), broiler breeders were not active except the period just before the start of photophase.

5.4.2 Physiological Parameters (Non Esterified Fatty Acids and β-hydroxybutyrate)

A significant interaction effect was observed for NEFA (Figure 5.1). Pullets fed a wheat-based diet demonstrated increased NEFA levels as compared to those fed pea from 26 h post-feeding until 1 h before feeding. Similarly, a significant interaction (P=0.01) between treatment and time was found for β-HBA (Figure 5.2). In general, broiler breeders fed a wheat-based diet had higher levels of β-HBA as compared to those fed the pea-based diet, but the degree of difference was higher from 28 h after feeding until 1 h before feeding.

Table 5.2. Effect of feeding a pea- or wheat-based diet, and on or off feed day on behavioral expression (% of time) of feed restricted broiler breeder pullets[1] *(n = 4)*.

	% Eating	% Drinking	% Walking	% Standing	% Foraging	% Pecking	% Comfort	% Resting
Pea	4.03	4.51[b]	6.05	7.59[b]	8.08[b]	21.21[a]	4.98[a]	43.98
Wheat	4.42	5.44[a]	6.30	9.31[a]	15.21[a]	14.82[b]	2.31[b]	42.55
P value	0.638	0.01	0.58	0.01	<.0001	<.0001	<.0001	0.17
SEM	0.587	0.265	0.327	0.474	0.420	0.500	0.227	0.734
On-feed day	8.45[a]	5.60[a]	5.48[b]	8.87	8.40[b]	16.82[b]	4.79[a]	41.99[b]
Off-feed day	0.00[b]	4.35[b]	6.86[a]	9.03	14.9[a]	19.21[a]	2.51[b]	44.54[a]
P value	<.0001	0.0009	0.003	0.21	<.0001	0.001	<.0001	0.01
SEM	0.582	0.267	0.323	0.479	0.417	0.505	0.229	0.726
On-feed day (pea)	8.05	5.11	5.89[b]	8.28	5.39[c]	19.63	6.68[a]	41.46[b]
On-feed day (wheat)	8.84	6.09	5.09[b]	9.46	11.40[b]	14.01	2.89[b]	42.52[b]
Off-feed day (pea)	0.00	3.91	6.20[b]	6.90	10.77[b]	22.79	3.28[b]	46.49[a]
Off-feed day (wheat)	0.00	4.79	7.51[a]	9.16	19.03[a]	15.62	1.73[c]	42.59[b]
P value	0.744	0.890	0.020	0.420	0.050	0.790	0.0005	0.020
SEM	0.840	0.379	0.458	0.676	0.589	0.700	0.325	1.026

[1] Behavioural observations were assessed for 48 h at 9 and 10 wk of age.

[a,b] Means within a column with different superscripts differ significantly ($P < 0.05$).

SEM: Standard error of means.
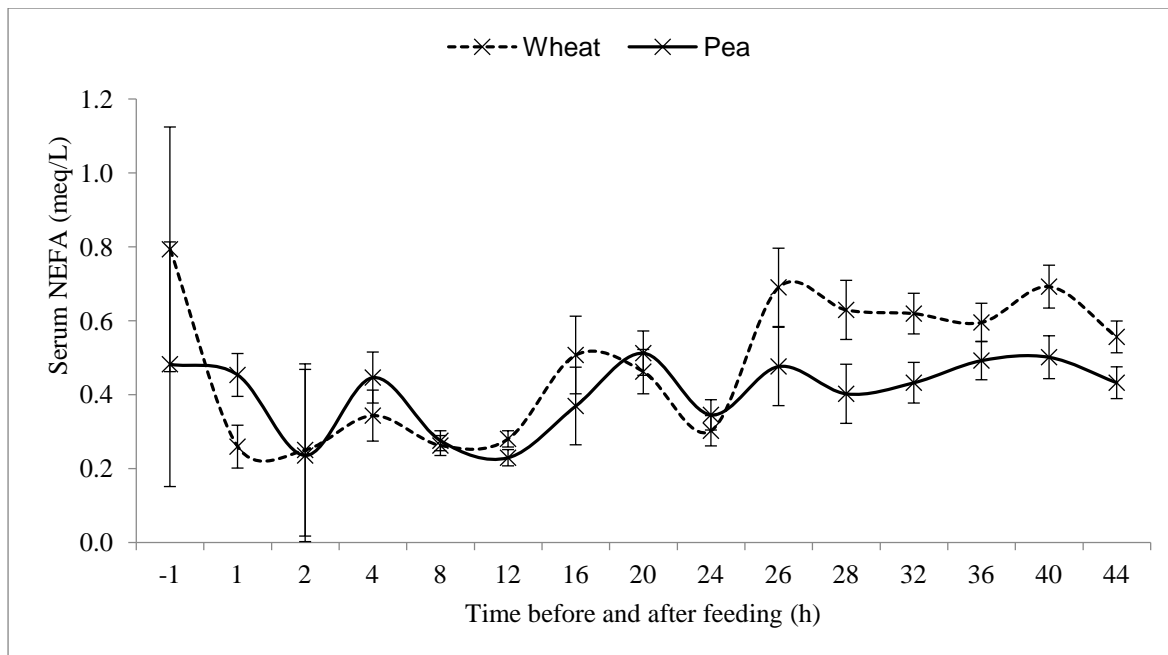
Figure 5.1. Effect of feeding a wheat- or pea diet on serum non-esterified fatty acids (NEFA; meq/L) levels of broiler breeder pullets over 48 h between feeding at 89 d of age ($n = 4$). *P* Values: Treatment = 0.03; Time = 0.0005; Treatment x Time = 0.02.
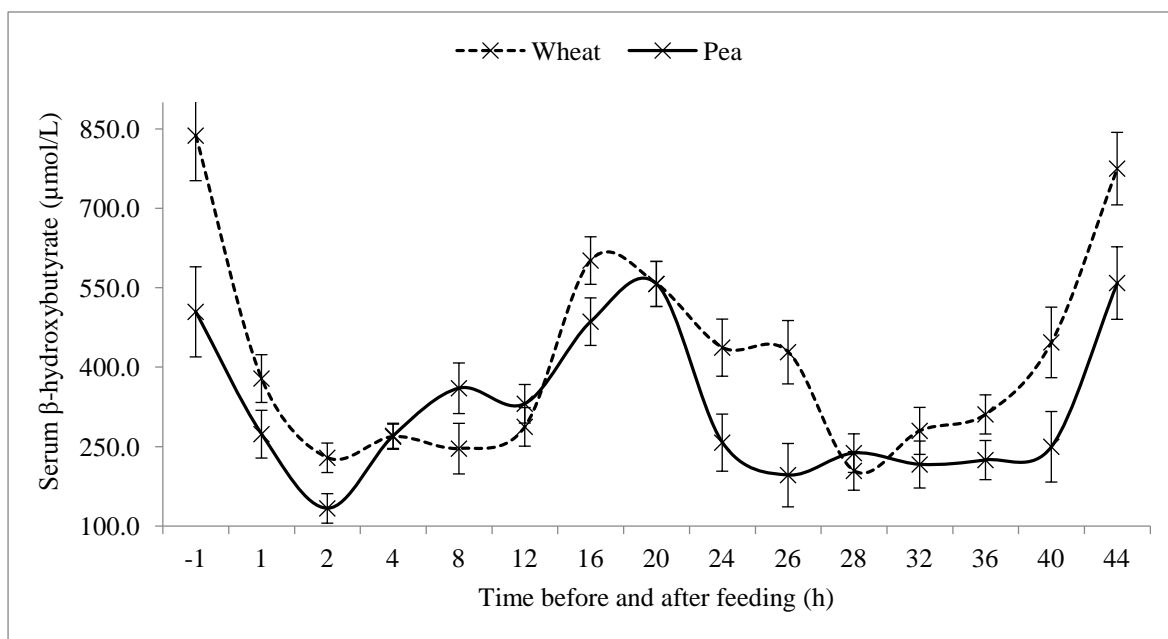
Figure 5.2. Effect of feeding a wheat- or pea-based diet on serum β-hydroxybutyrate (µmol/L) levels of broiler breeder pullets over 48 h between feeding at 89 d of age ($n = 4$). *P* values: Treatment = 0.001; Time = <0.0001; Treatment x Time = 0.01.

5.5   Discussion

Behavioural and physiological parameters to quantify hunger were studied in this research. Behavioural parameters included expression of wide range of behaviours over the period of 48 h and physiological parameters consisted of serum NEFA and β-HBA levels over the period of 48 h. The hypothesis of this research was accepted as feeding a pea based diet resulted in reduced degree of hunger as reflected by the behavioural and physiological parameters. In research that occurred simultaneously to the current research, feed was found in the small intestine for approximately 26-28 hours after feeding. Furthermore, de Beer et al. (2008) found that crop of broiler breeders fed on every-other-day basis was empty after 24 h of feeding. The presence of feed in the gut of broiler breeders may explain the expression of comfort, standing, pecking and foraging behaviours during first dark period (7- 23 h after feeding). In contrast, broiler breeders during second dark period (31-47 h after feeding) were inactive, thereby suggesting the use of darkness to conserve energy in the absence of feed. In conclusion, this information suggests that pullets would demonstrate more hunger related characteristics on the off-feed day.

It is important to discuss the diurnal rhythms in the behavioural and physiological responses of birds. Melatonin is the key hormone responsible for regulating the circadian rhythms of animals (Reiter, 1993). In chicken, house sparrows, and starlings, the main source of melatonin is pineal gland in contrast to quail and pigeons where retina acts as additional source (Pelham, 1975; Janik et al., 1992). Diurnal rhythms in melatonin are governed by the rhythmic expression of arylalkylamine-N-acetyltransferase (AA-NAT) in the pineal gland with a decreased concentration at the onset of day and peak during night (Herichova et al., 2001). Diurnal rhythms of AA-NAT activity are directly controlled by light penetrating through the eyes or skull (Morgan et al., 1995). Photoperiod and light intensity are able to impact the diurnal rhythms. The

effect of photoperiod on melatonin rhythms has been previously demonstrated in laying hens, turkeys and broilers (Lewis et al., 1989; Zawilska et al., 2007). Deep et al (2012) demonstrated that diurnal melatonin and behavioural rhythms remained unaffected by light intensity ranges from 1 to 40 lux. In the present research, all birds were provided with a 23 h of light (20 lux) for the first week, followed by 8 h of light and 16 h of dark (10 lux) for the remainder of the trial. Exposure of all birds to similar management practices ensured that all pullets demonstrated diurnal rhythms in behavioural and physiological responses, as a consequence, prevented a confounding effect on the impact of dietary treatments.

The dietary treatments affected the expression of behaviours of broiler breeders over the 48 h period after feeding. Feeding a pea based diet resulted in reduced expression of drinking and less drinking occurred on the off-feed day. Previous studies have demonstrated increased expression of drinking behaviour in feed restricted broiler breeders in comparison to *ad-libitum* fed birds (Savory et al., 1992; Savory and Maros, 1993; Hocking et al., 1996; de Jong et al., 2002). Additionally, water intake of broilers is increased during physiological stress (Puvadolpirod and Thaxton, 2000; Virden et al., 2009). Increased expression of drinking behaviour during on-feed as compared to off-feed day observed in this study might be attributed to metabolic needs after consumption of feed. The diet induced change in drinking behaviour is supportive of a reduced degree of hunger in pullets fed the pea-based diet, but changes in digestive properties can't be excluded as an alternative explanation.

The expression of walking behaviour was equal for both treatments during the on-feed day, but birds fed the wheat-based diet walked more than pullets fed the pea-based diet on the off-feed day. Day did not affect the percent of time spent standing, but feeding the pea-based diet reduced standing in comparison to those fed the wheat-based diet. Birds fed both dietary treatments rested

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to the same degree during the on-feed day, but birds fed the pea-based diet rested more than those fed the wheat-based diet on off-feed day. Similar to the present research, decreased resting has also been observed more often in feed restricted than ad-libitum fed birds (Savory et al., 1992; de Jong et al., 2002, 2003; Jones et al., 2004; Merlet et al., 2005). In a review by D'Eath et al (2009), increased activity was reported for feed restricted broiler breeders. Furthermore, the authors suggested that increased activity with feed restriction may reflect hunger and frustration related to feeding motivation (Savory and Maros, 1993; Hocking et al., 1996; Savory et al., 1996). Therefore, reduced walking and standing in broiler breeder pullets fed a pea-based diet reflects a reduced degree of hunger and improved satiety.

For both days wheat-fed birds foraged more than pea-fed birds, but the percent of this behaviour increased on the off-feed day for the wheat fed pullets as compared to birds fed pea. Previously, feed restricted broiler breeders have been shown to forage more than ad-libitum fed birds (Savory and Maros, 1993; de Jong et al., 2002; D'Eath et al., 2009). Dixon et al. (2014) compared three levels of feed restriction in broiler breeders and concluded that motivation to access an area of wood shaving for foraging was lower in birds fed twice or three times the commercially recommended feed allowance. Additionally, the authors suggest that increased foraging with increasing levels of feed restriction reflects the motivation to perform the appetitive component of feeding behaviour. Based on the relationship between feed restriction and expression of foraging behaviour, it can be concluded that reduced foraging on the off-feed day by feeding a pea based diet is indicative of improved satiety or reduced degree of hunger in feed restricted broiler breeder pullets.

Birds fed a pea-based diet had increased expression of comfort behaviours compared to wheat-based diet on off-feed day. Duncan and Mench (1993) categorized comfort behaviours as

behaviours performed after fulfillment of basic needs and when birds were free from suffering. As a consequence, the decreased expression of comfort behaviours may indicate reduced welfare associated with a particular environment. Comfort behaviours include preening, dust-bathing, foraging, wing-flapping, stretching and feather-ruffling (Wood-Gush, 1971). de Jong et al. (2002) have shown that expression of comfort behaviours increased in broiler breeders exposed to ad-libitum as compared to a restricted feeding regimen. A similar effect was observed by a number of other studies (Savory and Maros, 1993; Hocking et al., 2001, 2004; Puterflam et al., 2006). In conclusion, increased expression of comfort behaviour by feeding a pea-based diet provides evidence that these birds experience a reduced degree of hunger, thereby improving the welfare of feed restricted broiler breeders during the rearing phase.

Expression of pecking behaviour was increased as a result of feeding a pea-based diet and was higher for pullets during the off-feed day. However, the difference between on-feed and off-feed day was numerically smaller than the difference between the pea- or wheat-based diets. Based on data showing the presence of feed in the crop and small intestine of feed restricted broiler breeders (Chapter 3), it was expected that pullets would demonstrate more hunger related characteristics on the off-feed day. Based on the lack of interaction effect, it can be speculated that impact on expression of pecking behaviour might not be an accurate indicator of increased satiety or reduced hunger in this study. Previous research has shown that increased pecking was linked to the state of feed restriction. Feed restricted birds increased the expression of pecking as compared to ad-libitum fed birds (Savory et al., 1992; Savory and Maros, 1993; de Jong et al., 2002; Merlet et al., 2005). Similarly, Morrissey et al. (2014a) found reduced feather and object pecking in broiler breeder pullets fed high fibre diets in combination with appetite suppressants. Both of the above examples suggest that increased hunger stimulates pecking behaviour.

However, there may be an alternative explanation for the results of the current research. Dopamine agonists have been shown to increase the incidence of oral stereotypic behaviours in chickens, pigeons and pigs, thereby signalling the role of dopamine in the activation of oral stereotypic behaviour such as pecking. In humans, release of dopamine has been shown to be positively correlated with the degree of pleasure or reward received after eating a meal (Small et al., 2003). Based on this concept, it can be speculated that feeding a pea-based diet might be more rewarding and therefore have resulted in increased release of dopamine. In turn, the increased release of dopamine might be attributed to the increased expression of pecking in broiler breeder pullets fed the pea-based diet. No direct relationship between dopamine and feather pecking can be established in the current research and therefore the above hypothesis remains speculative until further research establishes its validity.

After a meal, available glucose is either used directly to meet immediate needs or stored as glycogen or triglycerides for future use during absence of a digestive tract nutrient supply. Triglycerides are transported from liver to adipose tissue via VLDL for storage (Engleking, 2011). In the absence of a dietary energy supply, organisms must derive energy from body tissue such as glycogen and fat. Two of the products of lipolysis are, NEFA and β-HBA. Starvation or fasting is mainly divided into three phases. The early phase is characterised by hepatic glycogenolysis and adipose tissue lipolysis as energy sources (Engleking, 2011). The intermediate phase is best characterised by the carbohydrate and nitrogen sparing impact of fat. During early to intermediate phases, the levels of free fatty acids and ketone bodies tend to increase. The late phase of fasting is characterised by the loss of body protein to maintain the blood glucose levels (Engleking, 2011). The fasting observed by broiler breeders in this experiment can be best categorised as early phase.

Based on the intermediary metabolism during fasting, it can be speculated that increased level of serum NEFA and β-HBA during the off-feed day reflects the state when animals are deprived of exogenous nutrients and need to utilize fat reserves as a source of energy. Broiler breeder pullets fed a wheat-based diet had higher levels of serum NEFA compared to those fed the pea-based diet over the period of 48 h of data collection. However, the degree of difference was particularly marked from 26 h after feeding to one h before feeding. A similar effect was noted for serum β-HBA levels with the biggest difference between dietary treatments occurring from 28 h after feeding and one h before feeding. Cheryl et al. (1988) demonstrated that levels of NEFA and β-HBA increased during the early phase, remained at high levels during intermediate phase, and decreased during late phase of fasting in geese. Previous studies have concluded that short term fasting in birds also results in increased levels of NEFA and β-HBA (Brady et al. 1978; Le Maho et al. 1981). In the present research, reduced levels of serum NEFA and β-HBA in broiler breeders fed a pea-based diet indicate that food was available for longer period of time, thereby reducing the need to utilize fat reserve as an energy source during the off-feed day. This effect was attributed to slowly digestible nature of pea starch.

In conclusion, feeding a pea-based diet resulted in reduced expression of drinking and standing behaviour and increased expression of pecking as compared to pullets fed a wheat-based diet. Interactions between diet and day showed that pullets fed a pea-based diet expressed increased comfort and resting behavior, and reduced foraging and walking behavior during 24-48 h after feeding. Feeding a pea-based diet reduced levels of serum NEFA and β-HBA, particularly between 26 to 28 h after feeding and one h before feeding. Both physiological and behavioral indicators suggest reduced hunger in feed restricted broiler breeder pullets when fed a pea- compared to a wheat-based diet.

6 EFFECTS OF STARCH SOURCE AND FEEDING FREQUENCY ON PERFORMANCE OF FEED RESTRICTED BROILER BREEDERS DURING EARLY LAYING PHASE

Preface

The objective of this chapter was to study the effects of feeding a wheat- or pea-based diet on a once-a-day (OAD) or twice-a-day (TAD) basis on the reproductive performance and chick yield of feed restricted broiler breeders during the laying period, as well as the early post-natal growth of their offspring.

Author Contributions

Aman Deep (Department of Animal and Poultry Science, University of Saskatchewan) planned, designed and conducted the experiment, analyzed the data, and drafted the manuscript.

Henry Classen (Department of Animal and Poultry Science, University of Saskatchewan) provided guidance and scientific input, reviewed and edited the manuscript, and provided funding for the research.

6.1 Abstract

A total of 160 broiler breeder pullets (Ross 308) were randomly housed in individual cages and fed either a pea- or wheat-based diet once (08:00; OAD) or twice (08:00, 15:00; TAD) a day as a 2×2 factorial design. Five birds served as a replicate and each treatment was replicated eight times. Data collection included body weight, body weight uniformity, egg production, egg weight and composition, ovarian structure, fertility, embryonic mortality, and the hatch weight and growth of offspring. Data were analyzed using the mixed model of SAS and the level of significance was fixed at $P \leq 0.05$. Weekly body weights were maintained at target levels by adjusting feed intake and were similar among treatments. Feed clean up time was longer for a pea- and OAD feeding as compared to wheat-based diet and TAD feeding regimen, respectively. When fed once-a-day, the coefficient of variation was significantly lower for birds fed the pea- than the wheat-based diet. When the diets were fed twice-a-day, diet did not affect uniformity. Feeding the wheat-based diet on a TAD basis improved hen-day egg production as compared to TAD feeding program, but feeding frequency did not affect production of hens fed the pea-based diet. Hens fed the pea-based diet once-a-day had a lower average egg weight than other diet by feeding frequency subclasses. A similar effect was observed for chick weight at hatch. Feeding a pea-based diet resulted in a lower (0.73 versus 1.23) percentage of double yolked eggs. Feeding a wheat-based diet and the TAD feeding program resulted in increased wet shell weight. TAD feeding resulted in improved specific gravity as compared to OAD feeding regimen with wheat-based diet. No impact of feeding frequency was noted by feeding a pea-based diet. Ovarian structure was unaffected by diet or feeding frequency. Feeding a pea-based diet reduced total embryonic mortality. Growth of broiler offspring was unaffected by broiler breeder diet or

feeding frequency. Overall, feeding a pea-based diet improved the performance of feed restricted broiler breeders during early laying phase.

6.2   Introduction

Broilers have been heavily selected for decades for rapid growth and improved feed efficiency, thereby increasing their feed intake capacity and making bodyweight control in broiler breeders more difficult. Ad-libitum feeding of broiler breeders results in excessive weight gain, which subsequently has a negative impact on production, health and welfare of broiler breeders. More specifically, previous studies have shown higher mortality (skeletal and metabolic), disrupted follicular hierarchies, and reduced reproductive performance (Robinson et al., 1991; Mench, 2002; Renema and Robinson, 2004) during the laying phase as a result of ad libitum feeding. As a consequence, body weight is controlled by applying feed restriction during the rearing and laying phases. Feed restriction has both positive and negative impacts on broiler breeder production and welfare. Positive effects include reduced mortality and improved production and reproductive performance. In contrast, the negative impact includes reduced welfare attributed to chronic hunger and stress (Mench, 2002). Different strategies have been investigated to lessen the negative impact of feed restriction such as extending bird feeding with the use of scattered feeding or diet dilution (de Jong and Van Krimpen, 2011). Feeding slowly digestible ingredients (pea) or changing the frequency of feeding may also be viable alternatives.

Previous research showing the impact of slowly digestible starch (SDS) on performance and health of poultry species is limited. Weurding et al. (2003) demonstrated that addition of some SDS to diets improved broiler performance, in particular feed efficiency. The authors suggested that improved efficiency with feeding SDS might be attributed to improved nutrient synchronization (energy, protein). Based on the physiological and metabolic effects in humans

(Lehmann and Robin, 2007), incorporation of SDS into diets has the potential to improve productivity of feed restricted broiler breeders by reducing the degree of post-prandial and post-absorptive nutrient storage and mobilization, thereby improving efficiency. To our knowledge, no research has been conducted to investigate the impact of rate of starch digestion on performance of feed restricted broiler breeders during the early laying phase.

Broiler breeders have traditionally been fed every 48 h during the rearing phase and every 24 h during the laying period. de Beer et al. (2007, 2008) and de Beer and Coon (2007, 2009) investigated the impact of every-other-day (EOD) and every-day (ED) feeding programs on production and physiological parameters of feed restricted broiler breeder pullets. The authors concluded that hepatic lipid metabolism, and plasma hormones and metabolite profiles were affected by feeding program between meals, with a much greater degree of change with the EOD program. During the laying phase, the authors concluded that the rearing phase EOD feeding regimen resulted in larger, but fewer total and settable eggs. Improved performance and efficiency with ED feeding programs was attributed to reduced degree of nutrient storage and mobilization. Furthermore, Spradley et al. (2008) demonstrated that feeding twice-a-day (TAD) resulted in improved egg production and higher egg weights as compared to once-a-day (OAD), but research focusing on its impact on hatchability, chick yield and post-natal growth is lacking. Overall, frequent feeding programs (ED or TAD) resulted in improved performance and efficiency in feed restricted broiler breeders.

It can be speculated that the positive impact of feeding SDS and increased feeding frequency on performance is based on the reduced degree of post-prandial and post absorptive nutrient storage and re-mobilization. Therefore, this study was designed to study the effects of feeding a wheat- or pea-based diet on a OAD or TAD basis on the reproductive performance and chick

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yield of feed restricted broiler breeders during the laying period, as well as the early post-natal growth of their offspring. It was hypothesized that feeding a pea-based diet and twice-a-day feeding would result in improved laying performance and reproductive capacity by reducing the degree of nutrient storage and re-mobilization during post-feeding phase.

6.3 Material and Methods

Experiments were approved by the Animal Research Ethics Board of the University of Saskatchewan and were performed in accordance with recommendations of the Canadian Council on Animal Care (1993, 2009).

6.3.1 Dietary Treatments

Feed was formulated based on the Aviagen recommendations for a four stage rearing program (Aviagen, 2016). Pre-breeder and breeder feeds were provided for 21-23 wk and 24-35 wk, respectively. Hens were provided with either wheat- or pea-based diets in a mash form, on a OAD (8:00 AM) or TAD (equal amounts at 8:00 AM and 3:30 PM) basis. The resulting experimental design was a 2×2 factorial arrangement with two diets and two feeding frequencies. The composition of diets (Table 6.1) was adjusted to maximize differences in starch source, while maintaining equal levels of dietary energy, protein and fat. Amino acids were formulated on a digestible basis and to meet recommended minimum values. Oat hulls were used as a diluent as required to equalize diet energy. The rate of starch digestibility of diets was tested using an in-vitro assay as described in Chapter 3 of this thesis. The results confirmed the slowly digested rate of starch digestion of pea in comparison to wheat starch.

Table 6.1. The ingredient and nutrient composition of wheat- or pea-based diets fed to broiler breeders.

Ingredients	Pre-breeder* (Pea)	Pre-breeder* (Wheat)	Breeder** (Pea)	Breeder** (Wheat)
Pea	60.50	-	57.3	-
Wheat	-	39.34	-	36.8
Barley	25.00	25.00	25.0	25.0
Soybean meal	1.96	14.12	2.68	14.7
Canola oil	5.03	4.96	5.71	5.66
Limestone	2.38	2.41	7.11	7.14
Dicalcium phosphate	0.92	0.90	0.94	0.92
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50
Sodium chloride	0.45	0.39	0.44	0.39
DL-Methionine	0.19	0.10	0.21	0.11
Choline chloride	0.10	0.10	0.10	0.10
Oat hulls	2.91	12.09	-	8.62
Biocox[2]	0.05	0.05	-	
Enzyme[3]	0.02	0.02	0.03	0.03
Calculated nutrients (%)				
AME (Mcal/kg)	2.800	2.800	2.800	2.800
Ca	1.20	1.20	3.00	3.00
Available phosphorus	0.35	0.35	0.35	0.35
Analyzed nutrients (%)				
DM	90.01	90.07	89.83	89.77
CP	17.61	16.31	16.93	15.84
Total fat	6.12	5.58	6.62	5.68
Total starch	38.31	38.43	34.08	35.55
Total dietary fiber	21.03	22.01	19.63	19.72

[1] Vitamin-mineral premix (units per kg of feed) contained the following: vitamin A, 11000 IU; vitamin D_3, 2200 IU; vitamin E, 30 IU; menadione, 2.0 mg; riboflavin, 6.0 mg; pantothenic acid, 10 mg; vitamin B_{12}, 0.02 mg; pyridoxine, 4.0 mg; thiamine, 1.5 mg; folic acid, 0.6 mg; niacin, 60 mg; biotin, 0.15 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[2] Alpharma Canada Corp 2000, Mississauga, ON, Canada.
[3] Endofeed W (GNC Bioferm, Cobble Hill, BC, Canada).
*Pre-breeder diet was fed for 21-23 wk of age.
** Breeder diet was fed for 23-35 wk of age.

6.3.2 Birds and Housing

Pullets for the current research were derived from a previous experiment comparing the impact of feeding pea- and wheat-based diets during the rearing period. The details about rearing phase are included in Chapter 3. Pullets were fed the same grain during the laying period as they were fed during the rearing phase. At 147 d of age, pullets were randomly housed in individual cages (30 cm × 45 cm) and assigned to one of four treatments until 244 d of age. A group of five birds were fed together and served as a replicate; each treatment was replicated 8 times (total 40 birds per treatment). Each cage was equipped with a cup drinker. Birds were provided with 14 h of daylength with a light intensity of 10 lx (incandescent bulbs). The ambient temperature was maintained at 20-22°C throughout the experiment. In order to assess reproductive capacity, 25 males were raised to provide semen for artificial insemination. All roosters were individually cage housed (male cages), fed a wheat-based diet and managed according to Aviagen recommendations (Aviagen, 2016).

Chicks hatched from eggs collected during wk 30 were used to assess the impact of maternal nutrition on early post-natal growth. Newly hatched chicks (576) were randomly assigned to 96 battery cages (50 cm width, 85 cm length, and 25 cm high) with wire mesh floors. In each cage, 3 male and 3 female chicks were housed, with 24 replications per broiler breeder treatment. The cages were equipped with a trough feeder and two cup drinkers. The temperature of the experimental room was initially 35°C at d 0 and gradually decreased at the rate of 2.8°C per week throughout the experimental period. The lighting program was fixed at 23 h of light and 1 h of dark, throughout the experimental period. All birds were fed a commercially manufactured (Federated Co-operatives Ltd., Saskatoon, Saskatchewan, Canada) corn and soybean meal based broiler starter ration on an ad-libitum basis for 20 d.

6.3.3   Data Collection

Hen body weight was monitored on a weekly basis by randomly weighing one bird/replicate (32 in total) and feed allocation was adjusted by treatment to achieve target weights. Individual body weight of all hens was recorded at 21, 27, 29, 31, 33 and 34 wk of age. Feed clean-up time was measured by monitoring time taken to consume feed by the group of five pullets in each replicate. Results are reported as time taken to consume 100 g of feed by group of five birds.

Weight of first egg was measured for all pullets. Egg production was recorded on a daily basis from 24-34 wk of age. Furthermore, the number of double yolk, cracked or broken and soft shell eggs were noted. All eggs from one day per wk, starting at 24 wk of age, were weighed and tested for shell quality using specific gravity. At 28 and 33 wk of age, eggs laid within a day were collected and composition was monitored by weighing albumen, yolk and wet shell.

Reproductive capacity of pullets was assessed at 30, 31 and 32 wk of age. Semen was collected from same age males using the abdominal massage technique (Burrows and Quinn (1937). No diluent was added and fresh semen was pooled and used for artificial insemination within an hour of collection. All pullets were artificially inseminated twice a week, starting from a week (29 wk) prior to egg collection for incubation to ensure the difference between the day of insemination and the last egg collection day didn't exceed 5 d. For each set of incubation, eggs laid for a week were collected and stored at 18°C with a relative humidity of 60%. Any abnormal eggs were removed and the remainder of the eggs were weighed and placed in an incubator (Robbins IH-B). At d 14 of incubation, candling was performed to determine fertility and embryonic mortality; infertile eggs and eggs showing embryonic death were removed and their status confirmed by macroscopic examination of egg content. Age of embryonic death was also estimated using macroscopic visual examination of dead embryos and classified approximately as

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early (0-7 d), middle (8-14) or late dead (15-21). Prior to transfer to hatcher (Robbins H-C) at d 18, eggs were weighed to determine the moisture loss during incubation. Temperature and relative humidity (RH) for incubation and hatching was maintained at 37.5°C and 65-75% and 36.4°C and 60%, respectively. On the day of hatch (21 d), chick weight and yolk free body mass (YFBM; body weight - un-retained yolk) were recorded. All un-hatched eggs were opened to determine the time of death. Data for reproductive performance represents the average of three sets of incubation and hatching. For post-natal growth, body weight, feed intake and feed efficiency were monitored at 6, 13 and 20 d of age. Mortality was checked twice-a-day and recorded.

At 244 d of age, all hens were humanely killed using cervical dislocation and ovaries were removed for more detailed evaluation. Data collection included number of large (LYF; > 10 mm) and small (SYF; 5-10 mm) yellow follicles, and weight and size of LYF. The largest LYF was considered F1 follicle, but, if there was a membranous egg in the oviduct, then the largest LYF was assigned F2 status. After recording ovarian parameters, 16 carcasses (including ovary and follicles) per treatment were randomly selected for total body composition based on the methodology described by Robinson et al. (1991). Briefly, carcasses were autoclaved (Steris, Amsco Eagle, Scientific Quality Sterilizer, Mentor, OH, USA) for 10 h, blended (Waring Commercial Blender) and a representative sample was stored at -20°C prior to analysis. Frozen samples were freeze-dried (Labconco, Kansas City, MO, USA) and analyzed for moisture (AOAC 930.15, 1990), ash (AOAC 942.05, 1990), protein (AOAC 984.13, 1990) and fat (AOAC 1920.39, 1990). Carcass composition was calculated on a dry matter basis.

6.3.4   Statistical Analysis

Data were analysed using Proc MIX of SAS (SAS Institute, 2002) as a 2×2 factorial design with diet (pea or wheat based) and feeding frequency (ED or TD) as main effects. Percentage data were log transformed prior to analysis. Means were separated using the Least Significant Difference (LSD) test and the level of significance was fixed at $P \leq 0.05$ unless otherwise stated.

6.4   Results

6.4.1   Growth

Average body weight was maintained at target levels as recommended for Ross 308 (Table 2). Feed allocation was based on weekly body weights and changed accordingly. To maintain equal body weights, hens fed the pea-based diet were allocated more feed compared to birds fed the wheat-based diet (Table 6.2). An interaction between diet and feeding frequency was shown for body weight uniformity (Table 6.3). When fed once-a-day, the coefficient of variation was significantly lower for birds fed the pea- (7.11) than the wheat-based diet (9.07). When the diets were fed twice-a-day, diet did not affect uniformity. Feed clean up time was longer for hens fed the pea- than the wheat-based diet and OAD than the TAD feeding regimen (Table 6.4); the interaction between grain type and feeding frequency was not significant. Mortality was very low and unaffected by experimental treatments, and therefore not reported.

Table 6.2. Effect of diet on weekly feed allocation (g/bird/day), and diet and feeding frequency on the body weight (kg) of broiler breeders (21-34 wk).

| | Feed allocation | | Body weight | | | | P values | | | |
| | | | Diet (D) | | Frequency (F) ** | | | | | |
Age*	Wheat	Pea	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
21	105.0	107.0	2.54	2.49	2.52	2.51	0.28	0.86	0.61	0.030
22	108.0	110.0	2.53	2.51	2.51	2.53	0.81	0.76	0.62	0.046
23	116.0	120.0	2.67	2.63	2.64	2.66	0.53	0.81	0.38	0.045
24	124.0	132.0	2.81	2.80	2.79	2.82	0.97	0.66	0.98	0.045
25	131.0	144.0	2.99	2.95	2.97	2.99	0.52	0.71	0.45	0.044
26	142.0	156.0	3.08	3.04	3.06	3.07	0.61	0.44	0.84	0.052
27	142.0	160.0	3.31	3.22	3.25	3.29	0.07	0.42	0.77	0.031
28	142.0	162.0	3.24	3.36	3.34	3.26	0.07	0.20	0.18	0.041
29	142.0	166.0	3.42	3.39	3.41	3.41	0.26	0.93	0.90	0.034
30	146.0	166.0	3.44	3.40	3.42	3.43	0.44	0.93	0.46	0.048
31	146.0	166.0	3.54	3.53	3.53	3.54	0.76	0.86	0.99	0.034
32	146.0	166.0	3.56	3.55	3.57	3.53	0.47	0.76	0.87	0.036
33	146.0	166.0	3.59	3.58	3.62	3.54	0.84	0.07	0.64	0.036
34[£]	156.0	156.0	3.68	3.67	3.69	3.66	0.70	0.69	0.91	0.035

*One bird per replicate was weighed every week to calculate average weight. At 27, 29, 31 and 33 wk, all birds were weighed to calculate the average weight.
** OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
[£] Feed intake was equalized for tissue collection.

Table 6.3. Effect of diet and feeding frequency on the uniformity of broiler breeders during the early laying phase** *(n=8)*.

	Diet (D)		Frequency (F)*		P values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
CV	8.51ᵃ	7.64ᵇ	8.09	8.06	0.02	0.94	0.005	0.259

D×F Interaction		
CV	Wheat	Pea
OAD	9.07ᴬ	7.11ᴮ
TAD	7.96ᴮ	8.16ᴬᴮ

* OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
CV – Coefficient of variation.
** Data was based on the individual body weights recorded at 27, 29, 31 and 33 wk of age.
ᵃ,ᵇ Means within a row with different superscripts differ significantly ($P < 0.05$).
ᴬ,ᴮ For interaction, means with different superscripts differ significantly ($P < 0.05$).

Table 6.4. Effect of diet and feeding frequency on time taken to consume 100 g of feed by the replication group of five broiler breeder pullets during the early laying phase *(n=8)*.

	Diet (D)		Frequency (F)		P values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
Time (min)	9.4b	11.2a	11.7a	8.9b	<0.0001	<0.0001	0.14	0.480

* Observations were completed at 24, 29 and 34 wk of age.
* OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
ᵃ,ᵇ Means within a row with different superscripts differ significantly ($P < 0.05$).

6.4.2 Laying Performance

The interaction between diet and feeding frequency was significant for hen-day egg production (Table 6.5). Feeding the wheat-based diet on TAD basis improved hen-day egg production (76.6 vs 73.5%), but feeding frequency did not affect production of hens fed the pea-based diet. Hens fed the pea-based diet laid less double yolked eggs (0.7%) than the birds fed the wheat-based diet (1.2%). The percentages of cracked and broken, and soft-shelled eggs were not

affected by the treatments or their interaction. Weight of first egg was not affected by main factors (diet and feeding program) or their interaction (data not shown).

Hens fed the pea-based diet on OAD basis had a lower average egg weight than other diet by feeding frequency subclasses and this resulted in a significant interaction between diet and feeding frequency (Table 6.6). To examine the effect of treatment on egg components, eggs were broken and the yolk, albumen and wet egg shell weighed. The weights of egg yolk and albumen were not affected by main factors, but feeding a wheat-based diet and feeding diets TAD resulted in increased wet shell weight (Table 6.7). Feeding TAD improved specific gravity as compared to OAD feeding regimen for egg from birds fed the wheat-based diet. No impact of feeding frequency was noted for birds fed the pea-based diet.

Table 6.5. Effect of diet and feeding frequency on hen-day egg production and percentage of abnormal eggs of broiler breeders (24-34 wk; $n = 8$).

Age (wk)	Diet (D)		Frequency (F)*		P values			SEM
	Wheat	Pea	OAD	TAD	D	F	D×F	
HDEP	74.21	74.93	74.63	75.40	0.93	0.49	0.03	0.779
% Double yolk	1.23^a	0.72^b	1.09	0.86	0.01	0.35	0.72	0.174
% Cracked/broken	1.73	1.13	1.67	1.18	0.08	0.11	0.25	0.229
% Soft shell	0.51	0.51	0.56	0.46	0.99	0.64	0.11	0.181

D×F Interaction		
HDEP	Wheat	Pea
OAD	73.48^B	75.78^{AB}
TAD	76.64^A	74.15^{AB}

* OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
HDEP – Hen-day egg production.
^{a,b} Means within a row with different superscripts differ significantly ($P < 0.05$).
^{A,B} For interaction, means with different superscripts differ significantly ($P < 0.05$).

Table 6.6. Effect of diet and feeding frequency on average egg weight* and specific gravity*** of broiler breeders (24-33 wk; *n = 8*).

	Diet (D)		Frequency (F)**		P values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
Egg weight	57.4	57.0	56.9[b]	57.6[a]	0.09	0.01	0.001	1.178
Specific Gravity	1.082	1.080	1.081	1.081	<.0001	0.39	0.04	0.0003

D×F interaction		
Egg weight	Wheat	Pea
OAD	57.5[A]	56.2[B]
TAD	57.3[A]	57.8[A]
Specific Gravity	Wheat	Pea
OAD	1.082[B]	1.081[BC]
TAD	1.083[A]	1.080[C]

*Average weight was calculated based on weighing one day's eggs weekly from 24-33 wk of age, inclusive.
*** Specific gravity was calculated using one day's eggs weekly from 24-33 wk of age, inclusive.
** OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
[a,b] Means within a row with different superscripts differ significantly (*P* < 0.05).
[A,B] For interaction, means with different superscripts differ significantly (*P* < 0.05).

Table 6.7. Effect of diet and feeding frequency on the weight (g) of broiler breeder egg constituents* *(n = 8)*.

	Diet (D)		Frequency (F)**		P values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
Egg weight	59.9	59.3	59.3	60.0	0.22	0.46	0.52	0.418
Yolk weight	16.9	17.0	16.9	17.0	0.93	0.89	0.32	0.160
Wet shell	6.5[a]	6.2[b]	6.2[b]	6.5[a]	0.0003	0.007	0.34	0.059
Albumen	36.5	36.1	36.2	36.5	0.28	0.42	0.69	0.294

* Data represent average values based on one day's eggs collected at 29 and 33 wk of age.
** OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
[a,b] Means within a row with different superscripts differ significantly (*P* < 0.05).

6.4.3   Reproductive Performance

   Fertility was very high and unaffected by dietary treatment or feeding frequency (Table 6.8). Total embryonic (early, middle and late dead) mortality was lower for eggs from hens fed a pea-based diet (6.4%) than from eggs derived from hens fed the wheat-based diet (8.9%). The percent hatchability of eggs set (P=0.08) and fertile eggs (P=0.07) tended to increase when feeding a pea-based diet. Although not significant, feeding on a TAD basis reduced percent early dead (P=0.10) and total embryonic mortality (P=0.11), and increased hatchability of eggs set (P=0.15) and fertile eggs (P=0.12). Moisture loss between set and transfer was not affected by diet, but was lower for eggs fed TAD (9.4%) in contrast to OAD (10.1%). No interactions were found between diet and feeding frequency for reproductive traits shown in Table 6.8. A significant interaction was observed for chick weight (0-d) (Table 6.9). Feeding a pea-based diet on TAD basis resulted in higher chick weight as compared to the OAD feeding regimen. Chick weight from eggs derived from hens fed the wheat-based diet were not affected by feeding frequency.

   Number and size of LYF and SYF were not affected by main factors or their interaction (data not shown).

Table 6.8. Effect of diet and feeding frequency on reproductive parameters* of broiler breeders ($n = 8$).

	Diet (D)		Frequency (F)**		*P* values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
% Fertility	99.2	99.2	99.2	99.3	0.97	0.98	0.15	0.807
% Moisture loss***	9.9	9.8	10.1[a]	9.4[b]	0.78	<.0001	0.64	0.161
% Early dead	6.1	4.5	6.1	4.6	0.10	0.10	0.99	0.968
% Middle dead	0.46	0.47	0.63	0.29	0.98	0.17	0.89	0.272
% Late dead	2.3	1.4	1.7	1.9	0.13	0.82	0.41	0.537
% Total	8.9[a]	6.4[b]	8.4	6.8	0.03	0.11	0.68	0.761
% Hatchability	88.8	91.0	89.0	90.9	0.08	0.15	0.98	2.012
% Hatchability (fertile eggs)	89.4	91.7	89.7	91.5	0.07	0.12	0.56	1.375

* Data represent average values based on three hatches at 30, 31 and 32 wk of age.
** OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
*** Percent egg moisture loss between egg setting and transfer at 18 d of incubation.
[a,b] Means within a row with different superscripts differ significantly ($P < 0.05$).

Table 6.9. Effect of broiler breeder diet and feeding frequency on the hatch day chick weight* of their progeny ($n=8$).

	Diet (D)		Frequency (F)**		*P* values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
Chick weight	40.9	40.7	40.6[b]	41.6[a]	0.15	0.0001	<.0001	0.301

	D×F interaction	
	Wheat	Pea
OAD	41.1[AB]	40.4[C]
TAD	40.8[B]	41.4[A]

* Data represent average values based on three hatches at 30, 31 and 32 wk of age.
** OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
[a,b] Means within a row with different superscripts differ significantly ($P < 0.05$).
[A,B] For interaction, means with different superscripts differ significantly ($P < 0.05$).

6.4.4 Carcass Composition

The percentage of moisture in hen carcasses ranged from 61. 5 to 62.1% and was unaffected by dietary treatment or feeding regimen (Table 6.10). On a dry matter basis, the percentage of protein and fat were also unaffected by treatment. The interaction between diet and feeding frequency was significant. Feeding a pea-based diet TAD resulted in higher ash content (% dry matter) compared to breeders fed OAD. In contrast, ash content for carcasses of hens fed wheat-based diet were unaffected by feeding frequency

Table 6.10. Effect of diet and feeding frequency on broiler breeder carcass composition (244 d of age; *n=8*).

Item	Diet (D)		Frequency (F)*		*P* values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
% Moisture	61.22	62.07	61.84	61.45	0.34	0.66	0.36	0.616
% Protein	19.47	18.92	19.42	18.97	0.22	0.31	0.72	0.309
% Fat	15.84	15.64	15.82	15.66	0.74	0.79	0.31	0.437
% Ash	2.72	2.70	2.68	2.74	0.76	0.42	0.04	0.053
D×F Interaction								
% Ash	Wheat				Pea			
OAD	2.77[AB]				2.59[B]			
TAD	2.57[B]				2.81[A]			

*OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
[A,B] For interaction, means with different superscripts differ significantly (*P* < 0.05).

6.4.5 Post Natal Growth

The interaction between diet and feeding frequency was significant for d-0 chick weight (Table 6.11). Chick weight for the pea-based diet hen treatment was higher when hens were fed TAD in contrast to OAD feeding. In contrast, feeding frequency did not affect the weight of

chicks derived from hens fed the wheat-based diet. Both main and interactive effects were insignificant for body weight gain, feed intake and feed conversion efficiency over the 0-20 d experimental period.

Table 6.11. Effect of broiler breeder diet and feeding frequency on early post-natal growth of progeny (0-20 d; *n=24*).

	Diet (D)		Frequency (F)*		P values			
	Wheat	Pea	OAD	TAD	D	F	D×F	SEM
Body weight (g; d 0)	39.70	39.65	39.62	39.72	0.89	0.74	0.03	0.225
Average gain (g; 0-20 d)	858.3	847.1	848.2	857.1	0.19	0.31	0.29	6.099
Feed intake (g; 0-20 d)	1208.1	1202.3	1204.0	1208.4	0.75	0.66	0.89	8.164
Gain to feed (0-20 d)	0.710	0.706	0.712	0.705	0.26	0.11	0.09	0.003

	D×F interaction	
Body weight (d 0)	Wheat	Pea
OAD	40.00^{AB}	39.24^{B}
TAD	39.39^{AB}	40.06^{A}

*OAD – once-a-day (8:00 AM); TAD – twice-a-day (8:00 AM, 3:00 PM).
^{A,B} For interaction, means with different superscripts differ significantly ($P < 0.05$).

6.5 Discussion

This experiment was conducted to examine the concept of improved efficiency with reduced degree of nutrient storage and mobilization in meal fed broiler breeders. As a consequence, treatments (pea-based diet and increased feeding frequency) with a potential to reduce nutrient storage and mobilization were selected. Broiler breeders used in this experiment were fed either a wheat or pea based diet during the rearing period, therefore residual effect of diets fed during rearing phase cannot be ruled out. However, pullets were selected to minimize the variation in initial body weights at the start of the laying period. Data collected included production,

reproductive performance, chick yield and early post-natal growth of feed restricted broiler breeders during the laying period. As a result of adjusting feed intake based on body weight, treatments did not affect hen weight during the experiment. However, birds fed the pea-based diet required more feed to achieve target body weights. Diets were formulated to be equal on the basis of AME values from previous research (National Research Council, 1994). However, differences in the actual AME of the ingredients used in the current experiment cannot be ruled out and might explain the differences in the feed allocation to maintain the target body weight.

Feed clean up time was longer for broiler breeders fed pea-based diet and OAD feeding regimen. The rate of starch digestion for pea-based diets used in this experiment was slower as compared to wheat (Chapter 3). In humans, slowly digestible starch results in reduced hunger and improved satiety (Anderson et al., 2010). Improved satiety with slowly digestible starch is attributed to slow and prolonged starch oxidation in comparison to rapid oxidation with rapidly digestible starch (Sparti et al., 2000). Another mechanism relates to the role of incretins including GLP-1. Wachters-Hagedoorn et al. (2006) demonstrated that slowly digestible starch resulted in increased release of GLP-1, which has been related to slow gastric emptying and SI transit, and as a consequence, improved satiety (Edholm et al., 2010). Therefore, it can be speculated that the slowly digestible nature of pea starch might be responsible for increased feed clean-up time. It was observed that broiler breeders reared on OAD feeding program were not able to finish the allocated feed in one bout and birds took breaks after eating a certain amount of feed. In contrast, birds fed on TAD basis were allocated half of the feed at each time, and therefore were able to finish it without taking breaks. This might have resulted in reduced feed clean-up time with TAD feeding program. The significance of increased feed clean-up time might be more relevant in

commercial setting where birds are housed in groups that result in more competition during feeding.

Body weight uniformity was improved when hens were fed wheat-based diet TAD as compared to OAD, but this was not the case for the hens fed the pea-based diet. This interaction reflects that both the pea-based diet and TAD feeding regimen improved uniformity. The impact of competition for feed on uniformity was ruled out as broiler breeders were housed in individual cages within a replication. Therefore, it can be speculated that feeding wheat vs pea, and OAD vs TAD, result in less efficient nutrient utilization and variation in the ability of individual birds to store and reutilize nutrients may have negatively impacted body weight uniformity. Further research is required to fully elucidate this mechanism. A similar effect was observed during the rearing phase where body weight uniformity was improved in broiler breeders fed a pea- as compared to wheat based diet (Chapter 3). Also in the present research (Chapter 4), feeding a pea-based diet resulted in reduced relative liver weight over the period of 48 h post feeding. Additionally, the degree of change of hepatic fat content over the period of 48 h was reduced by feeding a pea- in contrast to wheat-based diet, thereby demonstrating the reduced degree of nutrient storage mobilization during post-feeding period. deBeer et al. (2007, 2008) and de Beer and Coon (2007, 2009) investigated the impact of EOD and ED feeding programs on the growth and feed efficiency of feed restricted broiler breeder pullets and concluded that EOD feeding reduced efficiency, growth and performance. Improved efficiency with ED feeding was attributed to reduced need for nutrient storage and mobilization between meals.

Egg production production was measured from 24 to 34 wk of age and increased by feeding a wheat-based diet on a TAD as compared to a OAD basis. No effect of feeding frequency was noted in broiler breeders fed a pea-based diet. This interaction of diet and feeding frequency on

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egg production suggest that both feeding a pea-based diet and a TAD feeding regimen affect egg production in a similar fashion. Previous studies have demonstrated increased egg production by feeding broiler breeders twice-a-day as compared to once-a-day (Spradley et al., 2008; deBeer and Coon, 2009; Taherkhani et al., 2010). A positive impact of feeding SDS was demonstrated in broilers. Weurding et al. (2003) observed that addition of some slowly digestible starch to diets improves feed efficiency of broilers. Improved efficiency was attributed to improved nutrient synchronization (energy, protein). In contrast, Igbasan and Guenter (1997) concluded that performance of laying hens was unaffected by feeding pea (yellow, green or brown) upto 400g/kg. Similarly, inclusion of pea in the diet of laying hens did not improve the egg production (Ebsim, 2013). The increased egg production found for feeding a wheat-based diet on a TAD basis and the lack of a feeding frequency effect when feeding a pea-based diet might be attributed to improved efficiency. The reduced degree of nutrient storage and mobilization by feeding a pea-based diet and TAD feeding regimen is considered as a potential mechanism for improved efficiency.

Hens fed the pea-based diet laid fewer double yolked eggs as compared to the birds fed the wheat-based diet. The impact of feeding frequency on abnormal eggs has been previously studied. de Beer and Coon (2007) compared ED and EOD feeding regimens and did not find any effect on the percentage of double yolked eggs. Similarly, Spradley et al. (2007) found that percentage of double yolked eggs was not affected by the feeding broiler breeders OAD or TAD. Chen et al. (2006) suggested that ovarian dysfunction related to over eating in broiler breeders might be explained on the basis of toxic effects of excessive body lipids in non-adipocytes including ovarian tissue. In the present research, feeding a wheat-based diet to feed restricted broiler breeders in the rearing phase resulted in increased hepatic lipogenesis during the post-

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feeding period (Chapter 4). Although dietary treatment did not affect ovarian follicular growth, and carcass protein and fat content, the impact of reduced lipogenesis by feeding a pea-based diet can't be ruled out and might be responsible for reduced double-yolked eggs.

A significant interaction demonstrated that hens fed the pea-based diet on a OAD basis had a lower average egg weight than other diet by feeding frequency combinations. This is different than uniformity and egg production where wheat birds responded to TAD, but pea fed birds did not. The reason for this difference is not apparent, but it also shows up as the same interaction for chick weight. This is logical because of the strong relationship between egg and chick weight. de Beer and Coon (2007) found that EOD feeding broiler breeders during rearing resulted in increased relative egg weight as compared to daily feeding. They suggested that an altered metabolic response, such as increased lipogenesis or changes in body composition might be responsible for increased egg weight. Spradley et al. (2008) compared OAD and TAD feeding in broiler breeder hens and found that increased feeding frequency resulted in increased egg weight. The authors suggested that increased egg weight with TAD feeding might be related to time of the feeding (later in the day) rather than increased feeding frequency *per se*. In the present study, feeding a wheat based diet during the rearing phase resulted in increased hepatic lipogenesis as reflected by the changes in the liver fat content over the period of 48 h post-feeding (Chapter 3). Although egg yolk and albumen content, and carcass fat and protein content remained unaffected by dietary treatment or feeding frequency in the present study, reduced hepatic lipogenesis related to slowly digestible pea starch might be responsible for lower egg weight by feeding pea-based diet on OAD basis. Alternatively, numerically lower weekly body weights of birds fed a pea-based diet on OAD basis might be responsible for lower egg weights.

Feeding a wheat-based diet and TAD feeding program resulted in increased wet shell weight. Furthermore, TAD feeding resulted in improved specific gravity as compared to OAD feeding regimen for eggs from birds fed the wheat-based diet. No impact of feeding frequency was noted by feeding a pea-based diet. Farmer et al. (1983) studied the impact of time of feeding (07:00 versus 15:30) on specific gravity of broiler breeders eggs. They found that egg shell quality as measured by specific gravity was significantly improved by feeding at 15:30 in contrast 0:700. A similar effect was observed by Gholami et al. (2017). These results demonstrate the effect of providing dietary calcium closer to the time of egg shell formation. The impact of feeding pea to laying hens on egg shell quality has been studied, but the results are not consistent. Igbasan and Guenter (1997) found that egg shell quality decreased with increasing dietary levels of yellow or brown pea. However, the authors were unable to explain the impact of pea on egg shell quality. In contrast, Ebsim (2013) didn't find a significant difference in the egg shell quality of laying hens fed wheat- or pea-based diets. Further research is required to establish if feeding pea can impact shell quality.

Total embryonic mortality was reduced with feeding a pea-based diet in comparison to those fed a wheat-based diet. Similarly, the percent hatchability of eggs set (P=0.08) and fertile eggs (P=0.07) also tended to increase by feeding a pea-based diet. The chick embryo is susceptible to lipid peroxidation due to accumulation of polyunsaturated fatty acids in tissue lipids (Speake et al., 1998; Surai, 1999). Rosa et al. (2012) demonstrated that supplementation broiler breeder diets with canthaxanthin reduced embryonic mortality, and improved hatchability rate and fertility. This effect was attributed to the anti-oxidant characteristics of canthaxanthin. A positive impact of SDS to reduce oxidative stress as compared to rapidly digestible starch was reviewed by Zhang and Hamaker (2009). Based on the positive impact of cantaxanthin on the embryonic

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mortality and potential of SDS to reduce oxidative stress, it can be hypothesized that reduced embryonic mortality as a result of feeding a pea-based diet in the present study might be due to reduced embryonic oxidative stress.

Feeding a pea-based diet on TAD basis resulted in higher ash content (% dry matter) as compared to breeders fed OAD. To our knowledge, there is no other study which looked at the impact of feeding a pea- or wheat-based diet on carcass composition of broiler breeders during the laying phase. Robinson et al. (1991) didn't find an impact of feed restriction (full fed versus feed restricted) on total ash content of broiler breeder carcasses. The impact of feeding time on carcass composition of broiler breeder was studied in the past (de Avila et al., 2003). Researchers found that carcass composition was not affected by feeding regimen (feeding once, twice or three times a day). The lack of effect on carcass moisture, protein and fat levels might be attributed to restricted feeding regimen and similar nutrient composition of both dietary treatments.

Feeding a pea-based diet on a TAD basis resulted in higher chick weight as compared to the OAD feeding regimen. Previous research has demonstrated that chick weight at hatch is correlated to egg weight in broiler breeders (Pinchasov, 1991). In the present study, increased chick weight can be explained on the basis of higher egg weight for broiler breeders fed the pea-based diets on a TAD basis. Production parameters (body weight gain, feed intake, and feed conversion ratio) over 0-20 d experimental period were unaffected by hen dietary treatments and feeding regimen. In contrast, Gholami et al. (2017) demonstrated that feeding regimen affected the growth of offspring. The authors concluded that feeding broiler breeders three or four times per day resulted in improved performance of broiler offspring in comparison to birds derived from breeders fed once-a-day. Furthermore, Enting (2005) found that feeding low density diets to broiler breeders resulted in improved performance of offspring. Feeding SDS resulted in

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improved efficiency of broilers (Weurding et al., 2003). However, impact of feeding slowly digestible starch to broiler breeders on post-natal growth of chicks was not studied in the past. Extensive selection of broiler for growth might be responsible for lack of impact on performance despite of initial differences in the chick weights.

In conclusion, feeding a pea-based diet and TAD feeding regimen resulted in improved reproductive performance of feed restricted broiler breeders. Additionally, pea can be successfully added to the diet of the broiler breeders without having a negative impact on production and reproductive performance. Findings from this study serve as the baseline for future studies focused on the use of slowly digestible starch to improve the production and welfare of feed restricted broiler breeders. More research is required to further understand the impact of feeding SDS on the performance of feed restricted broiler breeders during early laying phase.

7   OVERALL DISCUSSION

Broiler breeders are feed restricted during the rearing and laying phases to maintain body weight targets and prevent the negative impact of excess weight on bird health and performance. At the same time, restricting feed intake results in the state of hunger, and as a result, potentially reduced welfare. Various management and nutritional strategies including, but not limited to, diet dilution, frequent feeding, foraging feeding and use of appetite suppressants have been used to reduce the degree of hunger in feed restricted broiler breeders. The rationale for using these strategies includes an increase in time spent eating and improved satiety related to increased gastrointestinal tract (gut) fill. However, these strategies might not be able to satisfy the metabolic aspect of hunger. Based on the physiological and functional significance of slowly digestible starch (SDS), there is a potential to use a diet containing SDS such as pea to reduce the extent of hunger. The key advantage of using a diet based on SDS is that it has potential to reduce the metabolic hunger by slowing down the digestion and absorption of exogenous nutrients.

Research was conducted to investigate the impact of feeding rearing and breeding diets based on rapidly or slowly digestible starch on post-prandial metabolism, performance, behaviour and welfare of feed restricted broiler breeders. The functional significance of SDS is well researched in human subjects and this work has demonstrated that it reduces the post-prandial glucose and insulin concentration, and reduces the incidence of chronic health issues (Ludwig, 2002; Lehmann and Robin, 2007). Furthermore, studies have shown that SDS results in reduced hunger and improved satiety, attributed to slow and prolonged starch oxidation in comparison to rapid oxidation with rapidly digestible starch (Sparti et al., 2000). Research focused on use of SDS in broiler breeders is very limited. To our knowledge, only one study investigated the impact of feeding a diet based on a SDS source, pea, on the post-prandial metabolic response (Ebsim,

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2013). The author found that feeding a pea- in contrast to a wheat-based diet reduced post-prandial glucose and also reduced the degree of liver weight change over the period of 48 h between meals in pullets fed on an every-other-day basis.

Based on the functional properties of SDS and the slowly digested nature of pea starch, research was completed to study the impact of feeding pea to broiler breeders during the rearing and laying phases. During the rearing period, where feed restriction is most severe, it was hypothesized that feeding a diet based on pea would reduce the degree of nutrient storage and mobilization during the post-prandial phase. It was further hypothesized that the slower rate of starch digestion associated with feeding a pea-based diet and its effect on pullet metabolism would reduce bird hunger and thereby improve bird welfare.

Key findings of the present research on the performance and welfare of feed restricted broiler breeders are shown in Table 7.1. Feeding a pea based diet modulated post-prandial metabolism, thereby resulting in reduced degree of nutrient storage and mobilization. The dietary impact on genes encoding for fat and glycogen synthesis and lysis further support this finding (Chapter 4). In terms of performance, feeding a pea-based diet resulted in improved uniformity during rearing and laying phases and better reproductive performance during laying phase (Chapters 3 and 6). In terms of welfare, behavioural and physiological indicators support the hypothesis that feeding a pea-based diet reduces the degree of hunger in broiler breeder pullets (Chapter 5).

Table 7.1. Impact of feeding pea- or wheat based diet on feed restricted broiler breeders.

Evidence that feeding a pea-based diet modulated post-prandial metabolism in comparison to feeding a wheat-based diet during the rearing period.

- ➢ Reduced post-prandial glucose peak
- ➢ Reduced nutrient storage and mobilization between meals
  - o Reduced liver weight gain between meals
    - ▪ Reduced increase in liver fat content
  - o Lower expression of key lipogenic enzyme genes between meals
    - ▪ Acetyl CoA carboxylase,
    - ▪ Malic enzyme
  - o Reduced expression of gene responsible for production of lipid transport protein between meals
    - ▪ VLDL-apolipoprotein

Evidence that feeding a pea-based diet improved broiler breeder satiety during the rearing period.

- ➢ Behavioural indicators of satiety
  - o Reduced expression of drinking behaviour
  - o Reduced expression of foraging and walking behaviour
  - o Increased expression of comfort and resting behaviour
- ➢ Metabolic indicators of satiety
  - o Reduced levels of non-esterified fatty acids and β-hydroxybutyrate starting at 26 to 28 h after a meal

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Evidence that feeding a pea-based diet during the rearing and laying phases maintained or improved performance.

- ➢ Target body weights were met during both phases when pea was fed
- ➢ Body weight uniformity was improved during both phases
- ➢ No effect of egg production
- ➢ Reduced total embryonic mortality during incubation of incubation and hatching

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Evidence not supporting the hypothesis

- ➢ Feeder pecking was increased in breeders fed a pea based diet during rearing phase
- ➢ Egg weights were reduced in breeders fed a pea based diet during early laying phase

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The impact of feeding a pea-based diet on post-prandial metabolic response is demonstrated by changes in glucose level, relative liver weight, hepatic fat content and expression of genes encoding for key enzymes involved in liver fat synthesis and lysis. Overall, these metabolic changes reflect the decreased need of nutrient storage and mobilization over the period of 48 h between feedings. Previous work in our lab also demonstrated the reduced degree of change of liver weight in feed restricted broiler breeders over the period of 48 h (Ebsim, 2013). Based on the differences in starch structure and composition, pea are categorised as slowly digestible as compared to rapidly digestible wheat (Weurding et al., 2001). The metabolic impact of the pea-based diet as observed in this study might be explained on the basis that feeding pea resulted in the slow release and absorption of nutrients, and the consequential reduced need for nutrient storage and mobilization in comparison to the rapid release of glucose in birds fed the wheat-based diet.

In the present study, feeding a pea based diet resulted in improved uniformity and reduced total embryonic mortality in feed restricted broiler breeders. Body weight uniformity plays a vital role in optimizing the reproductive capacity of broiler breeders. Uniformity of a flock can be affected by different management and nutrition practices including stocking density, feeder and drinker space, environmental variables (temperature, ventilation and non-uniform light intensity), quantity and quality of food, egg size and genetic variability in the parent stock (Costa, 1981; Robinson and Robinson, 1991). In the present research, uniformity during the early rearing phase and laying phase was improved by feeding a pea-based diet in comparison to wheat. Improved uniformity with a diet based on pea might be related to improved satiety, subsequently increasing the feed clean up time, thereby reducing the degree of competition between small and large size pullets. Improved uniformity with every-other-day feeding in contrast to every-day was explained

on a similar basis (de Beer and Coon, 2007). Another explanation for improved uniformity by feeding pea may be based on the reduced degree of nutrient storage and mobilization (Deep et al., 2012), attributed to the slower digestion and absorption of nutrients, which may in turn reduce associated metabolic stress. Nutrient storage and mobilization is an inefficient process as suggested by de Beer et al. (2007). A less efficient process in a highly feed restrictive state, superimposed on variation in individual bird efficiency, might affect broiler breeder body weight uniformity. More research is required to accurately determine this effect.

Total embryonic mortality was reduced with feeding a pea-based diet in comparison to those fed a wheat-based diet. Accumulation of polyunsaturated fatty acids in tissue lipids makes embryo susceptible to lipid peroxidation (Speake et al., 1998; Surai, 1999). Rosa et al. (2012) demonstrated that supplementation broiler breeder diets with canthaxanthin reduced embryonic mortality, and improved hatchability rate and fertility. This effect was attributed to the anti-oxidant characteristics of canthaxanthin. A positive impact of SDS to reduce oxidative stress as compared to rapidly digestible starch was reviewed by Zhang and Hamaker (2009). Based on the positive impact of cantaxanthin on the embryonic mortality and potential of SDS to reduce oxidative stress, it can be speculated that reduced embryonic mortality by feeding a pea-based diet in the present study might be due to reduced embryonic oxidative stress.

The results of this research suggest that feeding a pea-based diet resulted in improved welfare by reducing the degree of hunger. The quantification of hunger is a complex issue in broiler breeders and previous findings suggest that hunger can be best assessed by using multiple indicators. As a consequence, behavioral and physiological parameters were used in this study to assess the degree of hunger over the period of 48 h post feeding. Evidence for satiety was derived from examination of bird behaviour and physiological parameters (serum non-esterified fatty

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acids (NEFA) and β-hydroxybutyrate (β-HBA) levels) over the 48 h period between meals of broiler breeder pullets during the rearing period. In the present study, feed was found in the small intestine for approximately 26-28 hours after feeding for both wheat and pea fed birds. This indicates that birds are hungrier on off-feed day as compared to on-feed day. Therefore, the relative occurrence of behavioural and physiological parameters reflecting hunger is more on the off-feed day. Feeding a pea-based diet resulted in reduced expression of drinking on off-feed day. Previous studies have demonstrated increased expression of drinking behaviour in feed restricted broiler breeders in comparison to *ad-libitum* fed birds (Savory et al., 1992; Savory and Maros, 1993; Hocking et al., 1996; de Jong et al., 2002). Additionally, water intake of broilers is increased during physiological stress (Puvadolpirod and Thaxton, 2000; Virden et al., 2009). Increased expression of drinking behaviour by birds fed the wheat based diet may be attributed to the higher motivation to increase gut fill to satisfy hunger and increased stress related to feed restriction. The expression of walking behaviour was equal for both treatments during on-feed day, but birds fed the wheat-based diet walked more than pullets fed the pea-based diet on off-feed day. Birds fed both dietary treatments rested to the same degree during on-feed day, but birds fed the pea-based diet rested more than those fed the wheat-based diet on off-feed day. Similar to the present research, decreased resting has also been observed more often in feed restricted than ad-libitum fed birds (Savory et al., 1992; de Jong et al., 2002, 2003; Jones et al., 2004; Merlet et al., 2005). D'Eath et al. (2009) reviewed the literature and observed that activity increased in feed restricted broiler breeders. They suggested that increased activity with feed restriction may reflect hunger and frustration related to feeding motivation (Savory and Maros, 1993; Hocking et al., 1996; Savory et al., 1996). Therefore, reduced walking and standing in broiler breeder pullets fed a pea-based diet are indicative of reduced hunger and improved satiety.

The percent of time birds spent foraging was also affected by the interaction between diet and day. For both days, wheat-fed birds foraged more than pea-fed birds, but the percent of this behaviour increased on off-feed day for the wheat fed pullets as compared to pea. Previously, feed restricted broiler breeders have been shown to forage more than ad-libitum fed birds (Savory and Maros, 1993; de Jong et al., 2002; D'Eath et al., 2009). Dixon et al. (2014) compared three levels of feed restriction in broiler breeders and concluded that motivation to access an area of wood shaving for foraging was lower in birds fed twice or three times the commercially recommended feed allowance. Percent of comfort behaviours (preening, stretching, wing-flapping and feather- ruffling) was also affected by the interaction between diet and day. Pullets fed a pea-based diet expressed more comfort behaviours on the off-feed day as compared to birds fed the wheat-based diet. Duncan and Mench (1993) categorized comfort behaviours as behaviours performed after fulfillment of basic needs and when birds are free from suffering. As a consequence, the decreased expression of comfort behaviours may indicate reduced welfare associated with a particular environment. Comfort behaviours include preening, dust-bathing, foraging, wing-flapping, stretching and feather-ruffling (Wood-Gush, 1971). de Jong et al. (2002) have shown that expression of comfort behaviours increased in broiler breeders exposed to ad-libitum as compared to a restricted feeding regimen. A similar effect was observed by a number of other studies (Savory and Maros, 1993; Hocking et al., 2001, 2004; Puterflam et al., 2006).

Based on intermediary metabolism during fasting, it can be speculated that the increased level of serum NEFA and β-HBA during off-feed day reflects the state when animals are deprived of exogenous nutrients and need to utilize fat reserves as a source of energy. Serum NEFA levels were higher in broiler breeder pullets fed a wheat-based diet than those fed the pea-based diet over the period of 48 h of data collection. However, the degree of difference was particularly

marked from 26 h after feeding to one h before feeding. A similar effect was noted for serum β-HBA levels with the biggest difference between dietary treatments occurring from 28 h after feeding. Armstrong et al. (1993) observed an increased concentration of NEFA with feed restriction in heifers. Similarly, Cheryl et al. (1988) demonstrated that levels of NEFA and β-HBA increased during the early phase, remained at high levels during intermediate phase, and decreased during late phase of fasting in geese. Previous studies have concluded that short term fasting in birds also results in increased levels of NEFA and β-HBA (Brady et al. 1978; Le Maho et al. 1981). In the present research, reduced levels of serum NEFA and β-HBA suggest that feed nutrients were available for a longer period of time, thereby reducing the need to utilize fat reserve as energy source during off-feed day in broiler breeders fed a pea-based diet. This effect was attributed to slowly digestible nature of pea starch.

It is important to find alternative sources of slowly digestible starch or ways to reduce the rate of starch digestion by using feed processing technologies in situations when pea is not available for feeding broiler breeders. Furthermore, use of multiple strategies might be more efficient to reduce degree of hunger in broiler breeders. In the present study, it was demonstrated that use of twice-a-day feeding resulted in similar impact on the performance of broiler breeders during the early laying phase as feeding a pea based diet. More research is required to study the impact of more frequent feeding of slowly digestible starch on hunger and satiety of feed restricted broiler breeders. Similarly, it can be speculated that use of slowly digestible starch with other nutritional and metabolic strategies such as diet dilution, forage feeding and use of diet suppressants can be more beneficial in improving the welfare of broiler breeders by reducing the degree of hunger.

In conclusion, findings of this research demonstrated that feeding a pea-based diet resulted in improved performance and welfare of feed restricted broiler breeders. The positive impact on

performance and welfare of broiler breeders is mainly attributed to the slowly digestible nature of pea starch, thereby resulting in slow and sustained release of exogenous nutrients during post-feeding phase. It is acknowledged that due to severity of feed restriction, it is not possible to alleviate all negative impact of hunger. Findings of this study serve as the baseline for future studies focused on the use of slowly digestible starch to improve the production and welfare of feed restricted broiler breeders. More research is required to investigate the combination of use of slowly digestible starch and other management and nutritional methods to reduce the hunger of feed restricted broiler breeders.

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