Impact of Oasis® Supplement and Lysozyme on Incidence of Early Mortality, Digestive System Development, Growth Performance and Behaviour of Turkey Poults with Delayed Access to Feed and Water

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
September 2012

DALHOUSIE UNIVERSITY

FACULTY OF AGRICULTURE

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Impact of Oasis® Supplement and Lysozyme on Incidence of Early Mortality, Digestive System Development, Growth Performance and Behaviour of Turkey Poults with Delayed Access to Feed and Water" by Ashley M. Gillcrist in partial fulfilment of the requirements for the degree of Master of Science.

Dated: September 7th , 2012

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DALHOUSIE UNIVERSITY

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DEPARTMENT OR SCHOOL: Faculty of Agriculture

DEGREE: MSc CONVOCATION: May YEAR: 2013

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Abstract

Dietary supplements were provided during 24 hour transport from hatchery and growth of turkeys in two trials. Female poults (768 and 825 respectively) were used in two 3 x 4 factorial analyses (transportation supplement x post placement supplement) with treatment provided during transport (no supplement, Oasis® and Oasis® + lysozyme (0.01%)) and as dietary supplements post-placement (no supplement, Bacitracin Methylene Disalicylate (BMD)(ANTI), lysozyme (LYS), BMD + lysozyme (AL)) as the main effects. Growth, incidence of mortality, gastrointestinal size, strength and histology and behavioural data was collected. Transport supplementation of poults did not improve growth or reduce mortality, but influenced early feeding and drinking behaviour at placement. Body weight and feed consumption increased and percent mortality decreased for birds fed AL. Gizzard and proventriculus weight increased when birds consumed ANTI and jejunal breaking strength was highest for birds consuming LYS. Villi morphology and bird behaviour were not affected by dietary supplementation.

List of Abbreviations Used

AL	Antibiotic + lysozyme dietary supplement
ANOVA	Analysis of variance
ANTI	Antibiotic dietary supplement
AOAC	Association of Analytical Chemists
APRC	Atlantic poultry research center
BMD	Bacitracin methylene disalicylate
CFIA	Canadian food inspection agency
CP	Crude protein
CTMA	Canadian turkey marketing agency
D	Drinking behaviour
F	Feeding behavior
Glc NAc	N-acetylglucosamine
h	Hours
HCl	Hydrochloric acid
L	Locomotion behaviour
LF	Lactoferrin
LZ	Lysozyme
LYS	Lysozyme transport supplement

ME	Metabolizable energy
Mur NAc	N-acetylmuramic acid
NO	No supplement transport supplement group
NRC	National research council
NS	No supplement dietary supplement group
OAS	Oasis® transport supplement
OL	Oasis® + lysozyme transport supplement
PES	Poult enteritis syndrome
PEMS	Poult enteritis and mortality syndrome
PG1	Program 1
PG2	Program 2
SS	Sitting still behaviour
St	Standing behaviour
TA	TA.TX texture analyser
TFC	Turkey farmers of Canada
VM	Virginiamycin

Acknowledgements

First I would like to thank my supervisor, Dr. Derek Anderson for his endless encouragement and support throughout this entire process. Thanks go to my supervisory Committee Dr. Bruce Rathgeber and Dr. Michelle Jendral for their advice and guidance. Many thanks to the staff of the APRC for all of their help in the barn on a daily basis, as well as during the long sampling days. Especially, Sarah MacPherson for answering my million questions and finding information for me long after my project left the barn. Also thanks to Mike McConkey in the feed mill for mixing all of my experimental diets. A big thank-you to Janice MacIsaac for her wealth of poultry knowledge as well as always making time to look things over or clarify them for me. I would especially like to thank all of the graduate and undergraduate students that volunteered their time during placement and sampling days.

Thanks to the Pathology lab for preparing the intestinal cross sections and special appreciation to Joan Stiles for taking the time to explain the procedures and answer any questions that I came across, also to Krista Budgell for all of her help with setting up for imaging. Appreciation goes to Cuddy Hatchery, for providing the poults and allowing us to set up the transport treatments within the hatchery.

Special thanks to Grad room 257, I couldn't ask for a better group of people to spend as much time together as we have through the last 3 years. Thanks for everything!

Last but certainly not least, I would like to thank my amazing family and friends. Thank you for your love and support through the many ups and downs over the last few years. I would not be where I am today without you!

Chapter 1: Introduction

Early poult mortality is a problem within the turkey industry. Each poult lost affects the potential income from a market age flock. Poults are stressed from the moment of hatching and disease challenge can greatly affect their survival (Carver *et al*., 2002). Poults die of identified illnesses or simply do not thrive upon placement (Jindal *et al*., 2009). Carver *et al.* (2000) found that 14-day mortality in female poults ranged from 0.98 to 2.11% with most mortality occurring within the first week of placement. Negative effects on appetite, growth performance, feed conversion, body weights, and time to reach market weight are observed in poults which survive early infection with disease (Jindal *et al*., 2009).

The absence of turkey hatcheries in Atlantic Canada has forced producers to transport poults long distances from other provinces (Agriculture and Agri-food Canada, 2007), with the closest hatcheries being in Ontario or Quebec. This lengthy transportation can result in increased rates of early poult mortality through a lack of nutrition and increased vulnerability to disease (Carver *et al*., 2002; Donaldson et al., 1995; Jackson, 2005). Birds fed on the day of hatch have a rapid immune response with improved resistance to disease challenge (Dibner *et al*., 1998). One feeding strategy post hatch is to provide the birds with a supplement such as Oasis® (Novus Inc.) prior to shipping. This supplement is known to provide nutrients and moisture to the chicks and has shown promising results for body weights and growth rate (Boersma *et al.,* 2003; Batal and Parsons, 2002).

Antibiotics have been extensively used sub-therapeutically in animal feeds since they first became popular in the 1950's (Solomons, 1978). Although antibiotics are still being used in animal feeds, the World Health Organization (1997) declared that antibiotic use

in animal feeds is a public health concern due to the development of resistant bacteria and possible transfer of resistance to humans. After this declaration, worldwide research into finding equivalent alternatives to antibiotics became a focus for the poultry industry. Lysozyme, a naturally occurring enzyme commonly used in wine and cheese production, acts to lyse bacterial cells by hydrolyzing the β (1-4) linkages between N-acetylmurimic acid and N-acetylglucosamine in the bacterial cell wall (Proctor and Cunningham, 1988). Lysozyme molecules are commonly found on the outer membrane of many living bacterial cells (Proctor and Cunningham, 1988). In a broiler study, feeding lysozyme or lactoferrin, which is a cationic protein known to exhibit bacteriostatic and bactericidal effects, resulted in a decreased feed consumption and decreased thickness of the lamina propria over the controls (Humphrey *et al.*, 2002). *In vitro,* lysozyme has been reported to destroy the cells of *Clostridium perfringens* and inhibit the production of the α-toxin which causes necrotic enteritis in broiler chickens (Zhang *et al.*, 2006).

There has been promising research with the use of Oasis® and lysozyme separately for their ability to increase growth and survivability of poultry (Boersma *et al.,* 2003; Batal and Parsons, 2002; Jackson, 2005; Humphrey *et al.*, 2002). A combination of these dietary supplements may lead to a growth response and increase survivability of poults. To date there has been no research published evaluating the effects of providing Oasis® in combination with lysozyme to post hatch poults during transport and as a dietary supplement following placement, on growth performance or intestinal development. With more research lysozyme could prove to be an effective alternative to sub-therapeutic antibiotics in feed and combined with a transportation feed supplement may influence early poult mortality.

Chapter 2: Literature Review

2.1 Canadian Turkey Industry

The Canadian turkey industry has been a supply managed system since 1974. It was established under the Canadian Turkey Marketing Agency (CTMA), now known as the Turkey Farmers of Canada (TFC) (Agriculture and Agri-Food Canada, 2007; Turkey Farmers of Canada, 2011). World turkey production in 2006 was approximately 5.8 million tonnes, with the Canadian production of 162, 000 tonnes equaling 3% of the world production (Agriculture and Agri-Food Canada, 2007). The TFC represents 548 registered turkey producers across the country with 37 from Atlantic Canada (Turkey Farmers of Canada, 2011). There are 19 federally inspected slaughter facilities for turkeys across Canada, with no current capacity in Atlantic Canada, resulting in the need for shipping live birds long distances for slaughter (Turkey Farmers of Canada, 2011). One of the most difficult and critical challenges faced by the Atlantic Canadian producers is the lack of hatcheries. There are 9 registered turkey hatcheries in Canada, with none in the Atlantic Provinces (Turkey Farmers of Canada, 2011; Agriculture and Agri-Food Canada, 2007). This potentially increases stress related to transport for Atlantic Canadian producers as the birds endure long transport times from the hatchery to the growing facility. Increased time between hatch and placement can lead to increased early mortality and reduced growth performance (Carver *et al.*, 2000). This decreases the return on investment for the producer, and is a significant concern for the industry.

2.2 Transport

Due to the lack of hatcheries in Atlantic Canada (Turkey Farmers of Canada, 2011; Agriculture and Agri-Food Canada, 2007), all poults coming into the region are transported long distances. Most commonly poults are received from Ontario or Quebec, with transport distances up to and exceeding 2000km. The time in transport is dependent on many factors including, season, traffic, and weather conditions. Newly hatched poults have difficulty regulating their body temperature and must be kept warm during transport (van der Linde, 2011). Keeping the temperature optimal is not always possible and during transport fluctuations can occur increasing the poults susceptibility to early mortality (van der Linde, 2011). A study by Carver *et al.* (2002) found that hen poults transported at the lower end of the 22.2-32.2˚C transport range had higher incidence of mortality at 7 and 14 days post placement. In tom flocks, transport between temperatures of 20-25.5˚C did not significantly affect mortality rates at 7 days and at 14 days mortality was lowered when toms were shipped at a lower temperature. Within their study, temperature had an effect on early poult mortality depending on the sex of poult.

The length of time birds spend in transport also has an effect on early mortality. As transportation time increases poults are susceptible to more fluctuating temperatures, weather conditions, poor ventilation, and a lack of feed and water in most cases (Carver *et al*., 2002, Donaldson *et al*., 1995). Carver *et al*. (2002) found hens were not negatively affected by shipping time with regard to early poult mortality, whereas for toms the incidence of mortality increased with every one hour increase in shipping time. Donaldson *et al.* (1995) concluded that poor ventilation during shipping resulting in increased CO_2 can reduce poult survivability through increased stress which decreased

glycogen reserves. Poults who expend their energy reserves on thermoregulation have displayed retarded development, decreased immunity, increased susceptibility to disease and reduced performance (van der Linde, 2011).

Intestinal development is affected by long transport times as poults are usually not fed until they arrive on farm. Intestinal tract development is increased when birds consume feed immediately after hatch (Potturi *et al.,* 2005; Dibner *et al*., 1998; and Yi *et al*., 2005).

2.3 Early Poult Mortality

2.3.1 Causes

Early poult mortality is defined as the mortality that occurs within the first 14 days post hatch (Carver *et al.*, 2000). Most producers view the first week mortality as an indicator of the quality of the poults received and a predictor of how the flock will perform (Aziz, 2001). The causes of early poult mortality are widespread. Carver *et al.* (2000) assessed 5 production factors which represented differences among flocks. These were considered possible risk factors for early poult mortality, which included season of placement (summer or winter), breeder flock age (phase of egg production cycle), strain of bird, sex, hatchery (of origin) and company (farm) in which the poults were raised.

Conventional thinking in the poultry industry is that as the breeder flock gets older the mortality of the poults is lower due to increased poult weight at hatch (Carver *et al*., 2000). McNaughton et al. (1978) found that broiler breeders at 58 weeks of age produced chicks with lower mortality rates than those chicks produced by breeders at 29 weeks of age. Also, McNaughton et al. (1978) found that birds hatched from heavier eggs (57-72g) had lower mortality rates and increased body weight to market over birds

hatched from lighter eggs (47-54g). Similar results in turkeys were reported by MacIsaac and Anderson (2008) where poults from young breeders initially weighed an average of 44g and showed significantly higher mortality than poults from older breeder hens which initially weighed 53g. Carver *et al*. (2000) found that breeder age alone did not have an effect on the mortality of hen poults at 7 or 14 days, but for tom poults breeder age did have an effect. Tom poults from young breeders had higher mortality. Breeder age in a logistic model when combined with season of placement did significantly affect mortality of both hens and toms (Carver *et al.,* 2000). In a following study Carver *et al*. (2002) found that breeder age was a significant factor in early mortality, with breeder hens in mid-lay (in lay more than 3 weeks) producing poults with the lowest odds of mortality at day 7. Hen flocks from young breeder hens (first 3 weeks of lay) showed a 2 fold increase in mortality at both 7 and 14 days. Hen flocks from older breeder hens (second cycle of lay) showed the lowest odds of mortality at 14 days (Carver *et al.,* 2002). Schaefer *et al.* (2006) found that breeder age (33 or 55wks) did not significantly affect mortality of poults but that birds from older breeder hens showed significantly higher body weights to 63d post hatch and had reduced inflammatory responses (decreased haptoglobin levels) even at 9 and 10 weeks post hatch.

Genetic traits of the birds can also impact their ability to survive. Carver *et al.* (2000) found that birds from different strains had differences in early mortality rates. Hen poults from strain A had 1.58% mortality at 14 days while poults from strain B had 2.27% (Carver *et al.,* 2000). Strains were only identified as A and B within this study.

Aziz (2001) states that the most common causes of early poult mortality are infectious disease, trauma and starve outs. During the first week, the two most common

diseases that affect poults are yolk sac and navel infections (omphalitis) and aspergillosis (Aziz, 2001). Omphalitis is characterized by infected yolk sacs, most commonly associated with increased humidity and contamination of the hatching eggs or incubator (Kahn and Line, 2005). Poults suffering with omphalitis appear normal until shortly before death, at this point they show symptoms of depression, drooping head and huddling near a heat source if possible (Kahn and Line, 2005). The navel can appear wet and inflamed and mixed infections are common due to opportunistic bacteria present (Kahn and Line, 2005). One study found that *Clostridium perfringens* was present in birds that had died due to omphalitis that involved the yolk sac (Eleazer and Harrell, 1976). Eleazer and Harrell (1976) injected *Clostridium perfringens* in doses of 0.2 or 0.1ml into the yolk sacs of day old poults and within 48 hours of injection 67 and 60% respectively of the poults in each group died. Another study found that the fungi *aspergillus* was the main cause of poult omphalitis, with the route of infection expected to be an infected navel button (Cortes *et al.*, 2005). Cortes *et al.* (2005) reported that in a collection of 425 cases of omphalitis cases 104 of them were caused by *E. coli* and other causes included s*almonella sp*., *staphylococcus* and others. These studies report that there are various forms of infectious bacteria and fungi that are involved in omphalitis which is a considerable cause of early poult mortality in the industry. Other diseases such as poult enteritis syndrome (PES) and poult enteritis and mortality syndrome (PEMS) have caused devastating losses (Jindal *et al.*, 2009). All forms of poult enteritis can be defined as a multi-factorial intestinal infectious disease characterized by lethargy, depression, diarrhea, stunting, morbidity and mortality (Jindal *et al.*, 2009; Jackson, 2005). In PES although many pathogens including viruses, bacteria and protozoa, have been identified,

the most common agents isolated are rotavirus and *salmonella*. Whereas in PEMS infected birds coronavirus and *E.coli* are commonly identified (Jindal *et al*., 2009; Edens *et al*., 1997). Mortality in excess of 1% per day for 3 or more consecutive days was observed in birds suffering from PEMS (Edens *et al.*, 1997). Survivors were severely stunted and fail to reach market weights.

Additional common causes of early poult mortality are due to trauma; starve outs, unpalatable drinking water or feed, inappropriate lighting, or crowding of birds (Aziz, 2001). Trauma usually occurs at the hatchery when processing procedures are incorrect, birds are injected too deeply during vaccination or there is contamination during spraying or injection which causes infection (Aziz, 2001). Starve outs, another cause of mortality, can be divided into 2 groups, birds which do not start eating (nonstarters) and those who start to eat and then stop (stalled) (Aziz, 2001). Enneking (2010) reported that nonstarters account for 1-5% of early mortality in the turkey industry and hypothesized that stimulating the birds to begin consuming feed was beneficial in reducing the early mortality related to starve outs. Enneking (2010) suggested that feeder placement within the pen, which would put the birds in direct contact with it as they move about the pen may improve feeding, reducing the number of starve outs. Relating starve out and mortality, Bate (1992), found that if birds fell backwards or sideways in the pen or hatcher and were unable to right themselves they were unable to get to the feed or water and ultimately died. Karrow *et al.* (1998) stressed turkey poults through a 48 hour hold within the hatcher, and found that birds held within the hatcher showed quicker responses to feed and water than the control poults indicating that appetite and thirst or recognition of feed and water was not negatively influenced by a 48 hour hold. Also the authors

found no stress differences (through heterophil-lymphocyte ratios) at 10 days. These results indicated that a 48 hour hold within the hatcher is unlikely to account for the loss of birds due to starve out (Karrow *et al.,* 1998). Little is known about birds that begin to consume feed and then stop eating, no data specific to stalled birds was found.

2.3.2 Preventative Measures

Describing methods of prevention of early poult mortality has been outlined by Poss (1998). At the breeder level, programs are in place for washing eggs, using clean nesting material, frequent egg collection and breeder flock vaccination to prevent disease transfer to poults (Poss, 1998). At the hatchery level, disinfection of equipment by physical cleaning or the use of foggers is common for work areas (Poss, 1998). Fog disinfection within the incubators has helped control microbial contaminants (Poss, 1998). Using only clean eggs and disinfecting the incubator between uses has led to reduced potential for omphalitis as long as the exterior and rooms in which the incubators reside are also thoroughly cleaned (Kahn and Line, 2005). Antibiotic injections in day old poults are sometimes used to control eggshell-transmitted diseases (Poss, 1998). Hatchery procedures such as beak trimming can influence mortality; Renner *et al.* (1989) found that birds that were immediately beak trimmed with an electronic beak trimmer after hatch at 1.0mm anterior to the nostril had higher mortality and lower body weights than birds that were beak trimmed at 11 days of age with a hot blade beak trimmer.

In brooding poults, all-in all-out is a common practice to allow for cleaning and disinfecting after each batch, and the addition of antibiotics to feed and water are used to decrease poult mortality (Poss, 1998). Proper nutrition is critical to disease resistance, as well as a vaccination programs to immunize against common diseases on farm or

occurring in the area. Biosecurity in all operations continues to be a management process which can reduce the spread of disease between farms and buildings (Poss, 1998). Preventative control of disease is common by the use of sub-therapeutic levels of various antibiotics in the feed of almost all broiler chickens and meat turkeys produced in North America (Chapman, 2009). The most common use is for the control of coccidiosis, a protozoan disease responsible for devastating losses (Chapman, 2009). Along with antibiotics, early nutrition has been shown to improve survivability, growth and marketability of turkeys (Noy and Sklan, 1999; Corless and Sell, 1999).

2.4 Poultry Digestion

2.4.1 Digestive Anatomy

The poultry digestive tract has been specialized for flight. Birds digestive tracts have adapted to flight through the minimization of weight, as well as the reduction of length and volume within the body cavity (Klasing, 1998). The digestive tract of poultry starts with the beak which leads to a toothless mouth. Birds swallow their food in gulps without chewing. Food enters the mouth and is then propelled toward the esophagus by a short, non-protrusile tongue (Klasing, 1998). The papillae face posteriorly, which helps to move food toward the esophagus (Klasing, 1998). The salivary glands secrete mucinous saliva, this aids in moistening the feed but is insufficient to allow for extensive enzymatic digestion (Klasing, 1998). The feed then travels through the pharynx which joins to the esophagus. The esophagus in birds is very different than that of mammals to accommodate for feed that has not been previously chewed. It is elastic and expands using a set of longitudinal folds, to hold large amounts of feed (Klasing, 1998). The epithelial lining of the esophagus is thick to protect the muscle from damage caused by

course feed particles (Klasing, 1998). The function of the esophagus is to pass feed from the mouth to the proventriculus by peristaltic contraction of the inner circular and outer longitudinal muscles (Klasing, 1998). Just prior to entering the thoracic cavity there is a widening of the esophagus, commonly known as the crop. Turkeys and other Galliformes have a true crop, which is defined by the controllable sphincter that regulates the entrance and exit of feed (Klasing, 1998). The crop of the bird allows for storage of feed, to slowly release nutrients during the dark period. Also the crop allows for a moist environment where feed begins to soften, which permits more efficient digestion (Klasing, 1998).

Within the thoracic cavity, the stomach of the bird has two distinct parts. The first is the proventriculus also known as the glandular stomach (Klasing, 1998). The proventriculus of the bird is where gastric digestion occurs through the secretion of hydrochloric acid (HCl) and pepsin (Klasing, 1998). The mucosa of the proventriculus has an abundance of glands of two principle types. The first are tubular glands, which are responsible for the secretion of mucous; the second are gastric glands which secrete pepsin and HCl (Klasing, 1998). The posterior end of the proventriculus is constricted and connects to the gizzard. The function of the gizzard is to mechanically grind food to reduce its size and increase its surface area. It also serves as a location for the action of the previously added pepsin and HCl. In turkeys and other grain consuming birds the gizzard is a very muscular organ (Klasing, 1998). The mucosa of the lumen contains many deep tubular glands, which secrete a protein-rich fluid that forms horny plates known as the cuticle. The cuticle acts as a grinding surface and also protects the mucosa from the HCl and pepsin (Klasing, 1998).

The small intestine of the turkey functions for enzymatic digestion and absorption of nutrients. It is divided into three sections known as the duodenum, jejunum and ileum. The duodenum originates from the gizzard and forms a loop around the pancreas, where the bile and pancreatic ducts enter the duodenum (Klasing, 1998). The end of the duodenum is posterior to the duodenal loop which marks the beginning of the jejunum. The jejunum extends from the end of the duodenum to a point known as Meckel's diverticulum. Meckel's diverticulum is located midway along the length of the small intestine, and is the remnant of the yolk sac (Klasing, 1998). The last part of the small intestine, known as the ileum, is found posterior to Meckel's diverticulum to the cecal junction. As digesta moves further posteriorly through the small intestine the digestive mechanism begins to shift from an enzymatic digestion to a microbial fermentation near the end of the ileum (Klasing, 1998).

The ceca are found posterior to the ileum. In turkeys there are two equally sized ceca, each with its own opening into the rectum (Klasing, 1998). The point where the ceca meet the small intestine is known as the ileocecal junction. The function of the ceca is for microbial fermentation of complex carbohydrates that have not previously been digested and absorbed in the small intestine (Klasing, 1998). Contents enter the ceca from the ileum and exit to the rectum via controlled sphincters. The rectum of the birds is also commonly known as the colon. It is a small length of intestine between the ileocecal junction and the cloaca. The digesta then moves into the cloaca, which is divided into 3 parts: The coprodeum; which receives contents from the rectum, the urodeum; which receives the ureters and reproductive system, and the proctodeum; which opens externally through the vent (Klasing, 1998).

2.4.2 Digestive Histology

From a histologic perspective the avian gastrointestinal tract is a continuous mucosal membrane from the mouth to the vent (Klasing, 1998). The membrane consists of 3 main layers; the innermost epithelial layer next is the lamina propria and finally the lamina muscularis (Klasing, 1998). These 3 layers provide protection of the tract from food abrasion and microorganisms (Klasing, 1998). The epithelial surface is specialized for the absorption of nutrients and the exclusion of non-nutrients (Klasing, 1998). In many areas of the tract there is also a submucosa in addition to the main 3 layers. The mucosa is surrounded by a muscle coat which consists of inner circular muscle and outer longitudinal muscle. This is important for movement and mixing of digesta (Klasing, 1998). The outermost layer of the digestive tract is the serosa, providing structural integrity and protecting the tract from abrasion and trauma (Klasing, 1998). The mesenteric membrane attached to the intestines provides the blood supply through numerous blood vessels (Klasing, 1998).

In the small intestine, differentiating between the duodenum, jejunum and ileum at the histological level is unreliable (Klasing, 1998). Gross anatomical landmarks are used to separate the 3 sections (Klasing, 1998). The intestinal epithelial cells have approximately 10^5 microvilli per square millimeter on the apical surface. This large number of microvilli increases the intestinal absorptive capacity by 15 fold (Klasing, 1998). The villi of the intestine contain a rich capillary bed which allows for absorbed nutrients to be transported to the portal blood vessels to the liver (Klasing, 1998). The intestinal mucosal thickness decreases gradually along the length of the intestine (Klasing, 1998). Along with this the villi become shortened and the crypts become deeper

(Klasing, 1998). Numerous goblet cells secrete thick mucous which protects the epithelium from digestive enzymes and abrasion (Klasing, 1998). At the anterior of the intestine the mucous is particularly thick; this protects the epithelium and villi from the acidity of the digesta as it exits the gizzard (Klasing, 1998). The intestinal villi cells are formed in the base of the crypt, they then migrate up the villi (Imondi and Bird, 1966). The cells have a turnover rate of 2-4 days in growing chickens and after reaching the top of the villi the cells are shed into the lumen of the intestine (Imondi and Bird, 1966). This turnover influences length of the villi and is determined by the rate of cell division within the crypt versus the rate of cells lost at the apex (Klasing, 1998). The surface area of the intestine increases at 0.72 power of body weight, which keeps it proportional to the metabolic rate of the bird (Klasing, 1998). Villi in herbivorous birds are flatter and less finger-like than carnivorous birds (Klasing, 1998). In some species of bird the posterior region of the small intestine has a large population of microbes for nutrient fermentation (Klasing, 1998). This allows for the digestion of digesta that was not broken down in the anterior portion of the digestive system (Klasing, 1998). The birds' ceca are similar histologically to the small intestine with variable villi, crypts and mucous producing goblet cells (Klasing, 1998).

2.5 Early Nutrition

The stress that newly hatched poults endure from the moment of hatching has an effect on their overall health and metabolism (Leeson and Summers, 1997). Poults and chicks are removed from the hatching incubators after the majority have hatched and commonly numerous hatchery procedures are immediately performed (Batal and Parsons, 2002). Birds are sexed, beak trimmed, toe treated, vaccinated, sometimes receive

antibiotic injections and then are transported to the growing facility (Leeson and Summers, 1997; Batal and Parsons, 2002). This can leave the birds without food or water for up to 48 hours, even longer for those who were early to hatch putting them at a higher risk for dehydration (Batal and Parsons, 2002). During this time birds are dependent on their energy reserves and can decrease in weight by a rate of 4.0g per 24 hours. This loss is mainly moisture, but is also in the utilization of the yolk sac and pectoral muscle (Noy and Sklan, 1998a). The energy reserve in the poult is found in the yolk sac, of which the contents are composed of up of 50% lipid (Leeson and Summers, 1997; Noy and Sklan, 2001). During late development *in ovo* and hatch, birds maintain metabolism by the absorption of yolk lipids within the yolk sack (Lambson, 1970). These lipids are incorporated into circulation directly through endocytosis, allowing the birds to maintain their energy needs (Lambson, 1970). Sell *et al*. (1991) found that although the small intestine of birds goes through significant development *in ovo*, functionally it is immature and has limited absorptive capacity at hatch.

Early nutrition accelerates gut development, survivability, immunity, proportion of breast muscle, marketability and growth of poults (Noy and Sklan, 1999; Corless and Sell, 1999). Noy and Sklan (2001) found that after ingestion of feed, yolk sac lipids are secreted into the small intestine for absorption instead of being transported to circulation through endocytosis. This is thought to help trigger intestinal development and absorption of nutrients (Noy and Sklan, 2001). Early transition from embryonic yolk absorption to digestion of exogenous feed appears to be critical to maximize early growth (Nitsan *et al*., 1991). Noy and Sklan (1999) found that tom poults fed solid, semi solid or liquid nutrients immediately after hatch had increased body weights and maintained this

improvement to market weight. In their first experiment at 21 days, poults receiving feed or feed and water immediately after hatch weighed ~633g whereas control poults (held for 48hrs before placement) weighed 576g (Noy and Sklan, 1999). In their second experiment birds supplemented with Oasis® had body weights of ~613g at 21 days which was similar to poults supplemented with liquid nutrients (oral gavage of 0.4mL solution nutritionally equivalent to Oasis®) that had body weights of ~624g and both were higher than body weights of control poults (558g) (Noy and Sklan, 1999). Noy and Sklan (1999) also found that poults given Oasis® or feed immediately post placement had equivalent improvements to body weight (617 and 623g respectively) over birds fed a non-nutritive substance (sawdust) (588g) or control birds (held for 48 hours) (582g) at 21 days. At market weight (140 days) poults that received Oasis® had the highest body weights (~18.0kg) and were different from control birds (17.2kg). Poults receiving Oasis® had body weights similar to birds that received the liquid gavage (~17.8kg) or that were immediately placed on feed (~17.7kg). Feed efficiency was not affected by holding birds without feed, but the breast percentage of the birds was increased with early feeding creating a higher quality marketable turkey (Noy and Sklan, 1999). Corless and Sell (1999) found that birds denied feed for 54 hours post hatch had lighter small intestine weights along with reduced lengths of the small intestine through to 5 days post hatch. They concluded that delayed access to nutrients caused a delay in the development of the digestive system, impaired nutrient absorption and reduced body weights (Corless and Sell, 1999).

Along with creating a more desirable product, development of digestive system and increased body weights, early nutrition affects the immune system. Dibner *et al.* (1998)

reported an increase in immune system maturation and improved bird performance following a disease challenge when providing the poults nutrition immediately after hatching.

2.6 Antibiotic Use and Alternatives

2.6.1 Antibiotic Use

Dietary antibiotic use in poultry was started in 1948 as a preventative measure for coccidiosis (Grumbles *et al*., 1948). Broad spectrum antibiotics which improved growth and feed efficiency at low levels also had the ability to control endemic diseases (Gustafson and Bowen, 1998). The use of antibiotics became more prevalent in poultry and other animals as the antibiotic purchase price decreased and as confinement rearing of food animals became common (Gustafson and Bowen, 1998; Solomons, 1978; Chapman, 2009). Antibiotics in animal feed serve 3 main goals: 1) to increase the growth rate and feed efficiency, 2) disease prevention, 3) treatment of disease (Solomons, 1978). Sub-therapeutic antibiotics have significant benefits in reducing production losses by controlling preventable diseases at times when the animals are the most susceptible, as well as showing improved growth performance and feed efficiency (Gustafson and Bowen, 1998).

Miles *et al.* (2006) found that broilers supplementation with bacitracin methylene disalicylate (BMD) or virginiamycin (VM) improved body weights over the control diet in broilers at 7 weeks of age, with birds weighing 2.53, 2.54 and 2.48kg respectively. Similarly, Sims *et al.* (2004) found that supplementation with BMD improved the feed conversion ratio (2.56 kg feed/kg live weight) over birds receiving a control diet (2.72 kg feed/kg live weight) in turkey toms at 15 weeks of age. This improvement in feed

conversion ratio was not observed at 6, 12 or 18 weeks of age. Body weight of the toms was improved at 18 weeks of age, with birds receiving dietary BMD weighing 12.45kg in comparison to control birds at 11.87kg. Humphrey *et al.* (2002) found that feeding chicks a combination of BMD and roxarsone did not improve body weight gain up to day 17, but differences in feed efficiency were reported. During experiment one a marginal improvement in feed efficiency was found whereas in experiment two a greater feed efficiency occurred for birds consuming BMD and roxarsone over the control diet, 0.75 and 0.72 g body weight/g feed consumed respectively. Fasina and Thanissery (2011) reported that broiler chicks from different aged breeder hens displayed differences in growth when supplemented with BMD. At 7 days post placement birds from older breeder hens (58-59 wk of age) had higher body weights and body weight gain than birds from younger breeder hens (26-27 wk of age). At 7 days birds from older breeder hens supplemented with BMD had higher body weights (136g) over unsupplemented birds (125g). This difference was not observed in birds from young breeder hens where body weights were similar. Similarly at day 14, birds from young breeder hens receiving BMD had higher body weights (306g) than control birds from young breeder hens (268g) (Fasina and Thanissery, 2011). Birds receiving BMD from old breeder hens had significantly higher body weights than any birds from young breeder hens or control birds from old breeder hens (370g and 333g respectively). Feed conversion ratio (g feed/g gain) was not affected by the age of the breeder hen, but birds supplemented with BMD had higher feed conversion ratios than those remaining unsupplemented at both 7 and 14 days (Fasina and Thanissery, 2011). Waldroup *et al.* (1993) found improved growth of turkey hens and toms fed diets containing BMD in addition to monensin. At 70 days,

hens fed BMD and monensin had body weights of 5.03kg, whereas birds receiving just monensin weighed 4.82kg. At 101 days hen body weight was again significantly higher, 7.75 and 7.51kg respectively. Also improvements were reported at both days 70 and 101 for feed conversion. Birds supplemented with BMD had feed conversions of 1.79 and 2.33 at days 70 and 101 respectively, whereas birds unsupplemented had feed conversions of 1.92 and 2.42 respectively. The number of birds found dead or culled during the trial was not significantly affected by the addition of BMD (Waldroup *et al*., 1993).

Dietary antibiotics have also caused changes in the intestinal tract of birds (Miles *et al*., 2006; Apajalahti *et al.,* 2004). The large volume of microflora typically found within the gastrointestinal tract of a bird is thought to decrease nutrient absorption through increasing the thickness of the gastrointestinal tract (Apajalahti *et al.,* 2004). This increased thickness can be attributed to the proliferation and number of leukocytes within the lamina propria, which in turn creates a thicker lamina propria (Humphrey et al., 2002). Miles et al. (2006) found a decrease in muscularis mucosae thickness as well as lamina propria from the feeding of sub therapeutic antibiotics to broilers. Also the increased volume affects the rate of feed passage; it increases the nutrient requirements through the increased turnover of the gut mucosae and also the microflora present compete with the host animal for dietary protein and energy (Apajalahti *et al.,* 2004). The addition of antibiotics to animal feeds has improved the gastrointestinal tract by reducing competition for nutrients, reducing inflammation caused by pathogenic bacteria and overall decreasing the size of the intestine (Apajalahti *et al.,* 2004). Not all antibiotics will perform the same within the gastrointestinal tract; this is influenced by

substrate preferences, growth requirements, chemical composition and structure of the digesta (Apajalahti *et al.,* 2004). This is why some dietary antibiotic additions show changes to the gastrointestinal tract while others do not (Apajalahti *et al.,* 2004). Miles *et al.* (2006) reported a decrease in the weight of the intestinal sections when birds were supplemented with antibiotics. At 1 week post hatch birds receiving VM had a lighter gastrointestinal tract than birds receiving the control or BMD diet. At 3 and 7 weeks of age both VM and BMD resulted in lowered gastrointestinal tract weights than the control diet. In a study by Sims *et al.* (2004), birds receiving BMD had the longest intestinal villi (by visual observation) at 18 weeks, which was used as an indicator of excellent gut health and high absorptive efficiency. Humphrey *et al.* (2002) also found that birds consuming BMD and roxarsone had higher duodenum villi heights (876μm) than control birds (743μm). The increase in villi height presumably increases absorptive surface area resulting in increased nutrient digestion and absorption (Humphrey *et al*., 2002). This difference in villi height was not observed in the ileal samples of the same study (Humphrey *et al.,* 2002).

There have been improvements in the health, growth performance and feed efficiency of poultry with the introduction of low levels of antibiotics in feed. Early on, in 1969 the Swann Committee in the UK recommended that the potential hazard to human and animal health posed by drug resistant bacteria was high and that antibiotics should be classified as either "feed" or "therapeutic" use and only antibiotics designated as "feed" should be available without a veterinarian's prescription (Swann, 1969). The UK adopted this policy and designated that antibiotics used in human medicine would not be used in animal feeds at sub therapeutic levels and restrictions on acceptable "feed" antibiotics

were developed (Swann, 1969). In 1997 the World Health Organization declared that there was evidence of resistant bacteria being passed from animals to humans which will make disease treatment more difficult. Exposure to antimicrobials at low-levels long-term may create more selective resistance in both target bacteria and other exposed bacteria (WHO, 1997). Bacteria originating from animals that show resistance to antibiotics in humans include *salmonella*, *campylobacter*, *enterococci*, and *Escherichia coli.* Changes to antibiotic use in animals on the regional, national and international levels are needed to prevent further bacterial resistance from developing (WHO, 1997).

2.6.2 Feed Without Antibiotics

Subtherapeutic addition of antibiotics to the feed of almost all broiler chickens and meat turkeys produced in North America has improved growth performance, feed efficiency and reduced economic loss from disease (Chapman, 2009; Solomons, 1978). Although antibiotics are still being used in animal feeds, the World Health Organization (1997) reported that antibiotic use in animal feeds is a public health concern due to the increase in resistant bacteria and possible transfer of resistance to humans. This has encouraged research into finding equivalent alternatives to subtherapeutic antibiotics in the poultry industry.

Non-antibiotic feed supplements have varied effects on intestinal development and poult growth. A totally drug-free diet has resulted in impaired livability, decreased weight gain, poor intestinal morphology and feed conversion rates compared to a diet with antibiotics (Sun *et al.*, 2005). Sun *et al.* (2005) concluded that a combination of drug free alternatives can improve these factors compared to a negative control (no supplement). Sun *et al*. (2005) fed birds one of four diets with two being non-antibiotic

feed supplements. The supplements were divided into two programs. Program 1 (PG1) included the basal diet plus Acid Pak 4-Way. This was delivered in the water at a rate of 0.5g/L of water every day for the first 5 days of life and then provided one day per week until processing. Additionally four other ingredients were added to the feed. This included, VegPro (a vegetable protein enzyme to increase digestibility of feed at 0.91kg/tonne), MTB-100 (esterfied glucomannan which can bind and detoxify mycotoxin at 0.45kg/tonne), Bio-Mos (mannanoligosaccharide at 1.81 kg/tonne in the starter, 0.91 kg/tonne in the grower, 0.45 kg/tonne in the finisher and withdrawal) and All-Lac XCL (a probiotic containing *lactobacillus*, *enterococcus*, and *pediococcus* was sprayed at the hatchery at a rate of 5g/2000 birds) (Sun *et al.,* 2005). Program 2 (PG2) included basal diet plus Bio-Mos (same rate as in PG1) and All-Lac XCL (at hatchery) (Sun *et al.,* 2005). The other 2 diets were a negative control, without growth promoter or coccidostat and also a positive control which was the basal diet plus Lincomycin (2g/tonne in starter and 4g/tonne in grower). The birds fed PG2 diet exhibited higher villi height, crypt depth and villi height/crypt depth ratio in both the ileum and the duodenum than any other diet (Sun *et al.,* 2005). Total mortality was higher for the control birds (11.98%) than any of the drug-free treatments, with PG1 birds having 4.63% mortality and PG2 at 6.78% mortality (Sun *et al.,* 2005). Humphrey *et al.* (2002) fed broiler chicks both lysozyme (LZ) and lactoferrin (LF) as a non-antibiotic feed supplement and compared them to commonly used sub-therapeutic antibiotics. Diets containing both LF and LZ were found to be as effective at improving feed efficiency as those containing antibiotics, with 0.84 and 0.82 g gain/g feed consumed respectively. Reduced thickness of the lamina propria and increased villi height in the small intestine were reported in birds fed LF and

LZ compared to the control diet (Humphrey *et al.,* 2002). Birds receiving LF and LZ had an average villi height of 882µm, whereas control birds had an average villi height of 743µm (Humphrey *et al.,* 2002). Solis de los Santos *et al*. (2007) reported positive results on body weight and intestinal morphology when the yeast extract Alphamune was provided to poults. Poults were provided a dietary supplement of Alphamune at a rate of 0.001 or 0.002%. Birds were then weighed at 7 and 21 days. Birds receiving the yeast had higher body weight at day 7 (128.7, 128.6 and 115.7g for birds receiving 0.001, 0.002% Alphamune or control respectively). Body weights at day 21 were not different when birds were supplemented with Alphamune (Solis de los Santos *et al.,* 2007). The ileum villi height, crypt depth and surface area were significantly improved by the addition of Alphamune (Solis de los Santos *et al.,* 2007). At day 7, birds receiving 0.001, or 0.002% Alphamune had a higher villi height (1475.6µm, 1512.0 µm respectively) than those receiving control (1262.1 µm) during trial 2. Solis de los Santos *et al.* (2007) found that villi surface area was higher for birds receiving the 0.001 or 0.002% Alphamune during day 7 of trial 2 (132068.8, 130116.7 μm^2 respectively) over the control (102016.1 μm^2). The crypt depth was also improved at day 7 by the addition of 0.001, 0.002% Alphamune (236.4, 283.8 µm respectively) compared to the control (168.2 µm). Although these differences were observed during day 7 they were absent for day 14, where only the crypt depth of birds fed 0.002% Alphamune was improved over the control. Also during the first trial these differences were not observed (Solis de los Santos *et al.,* 2007). Although results varied there are positive results with non-antibiotic feed supplements indicating that there are effective alternatives to conventional sub-therapeutic antibiotics in poult feed.

2.7 Oasis®

2.7.1 Composition

Oasis® is a hydrated hatchling feed supplement which has been studied for its use in improving growth rates and body weights in post hatch poultry. Oasis® is marketed as a transport supplement or top dress for hatchling poultry (Novus International, 2008). It is a 2mm pellet which is soft in texture and green in color. Food recognition in poultry is influenced by vision, with newly hatched birds having preference for food of certain colors (Ferket and Gernat, 2006). The color may be influenced by the preference of poults for feed colored green over feed colored red (Cooper, 1971). Ingredients and nutritional composition of Oasis® are listed in table 2.1 as provided by Novus International (Novus International, 2008). A detailed nutrient profile as given by Novus International (Novus International, 2011) is outlined in Appendix K.

Table 2.1: Ingredients and nutritional composition of Oasis® hatchling supplement

Oasis® Ingredients*[z]		Nutrient Composition[z]	
Soybean Meal	35-45 %	Moisture	Min 25%
Grain Products (Primarily Corn)	20-25%	Crude Protein	Min 20%
Corn Syrup	10-15%	Fiber	Max 3%
Citric Acid	1-5%	Fat/oil	Min 0.5%
Water	10-24%		

* Inclusion is in % by weight
z- All information provided by Novus International Inc. St. Louis MO, 2008.

2.7.2 Potential in Turkey Poults

Noy and Sklan (1999) found that early feeding Oasis® for both chicks and poults resulted in improved growth rates through to marketing and that the proportion of breast meat in turkeys and broilers was improved with early access to feed. A study by Boersma *et al*. (2003) agreed with previous studies and found that Oasis® was beneficial for

increasing body weight during the first few weeks post hatch in the female broiler chick. Initially, at hatch Boersma *et al.* (2003) reported that control birds had higher body weight (38.00g) than birds in the Oasis® group (37.18g), but by the end of the first week the Oasis® birds had higher body weights (70.16g) over the controls (67.22g). This improvement continued until week 4 where birds that received Oasis® had body weights of 318.40g and controls had a body weight of 306.56g. This improvement was not observed from weeks 8-18, where birds had similar body weights (Boersma *et al.,* 2003). Batal and Parsons (2002) found Oasis® positively improved growth performance and metabolizable energy (ME) in male Plymouth Rock chicks fed a corn-soybean meal diet when chicks Oasis® were supplemented for 24 or 48 hours post hatch. Initially the 48 hour Oasis® fed birds had the same weight loss as the birds which were fasted for 48-hours (-7 and -6g respectively), but the 24 and 48 hour Oasis® fed (51 and 37g respectively) and feed fed (51g) birds were able to compensate and had higher body weight gain over fasted birds (20g weight gain) at the end of the first week (Batal and Parsons, 2002). Although there was improvements in ME with Oasis® supplementation, Batal and Parsons (2002) found there was no difference between feeding Oasis® and fasting on amino acid digestibility up to 21 days. Similar to previous studies, Yi *et al*. (2005) found that post-hatch chicks supplemented with Oasis® or placed immediately on feed displayed higher livability, body weight, cumulative weight gains, and lowered mortality rates than those who were fasted for 48 hours, even when exposed to a challenge of *Eimeria maxima*. Examination of the small intestine revealed that birds that were fasted had reduced growth in the intestinal villi and disrupted intestinal integrity (Yi *et al*., 2005). Birds fed Oasis® had lower scores of mid small intestinal lesions after a

challenge of *Eimeria maxima,* indicating improved gastrointestinal tract development and immune system maturation with early feeding. Jackson (2005) found that Oasis® did not consistently improve body weights or feed conversion in poults. It was found that livability was not affected by supplementation of Oasis® in this first trial, but during the second trial and third trials livability was improved with 95% of birds receiving Oasis® surviving compared to 91.7% for the controls. In trial 3 the rates were 92.0 and 86.3% respectively. In the third trial birds were exposed to Poult Enteritis and Mortality Syndrome (PEMS) through exposure to PEMS infected poults. Those birds who were exposed but had received Oasis® prior to exposure, had unchanged intestinal morphology, indicating that gut repair was enhanced with Oasis®. Additionally Oasis® fed birds had a shortened period of infection with PEMS. PEMS infected birds fed Oasis® had improved feed conversion at 21 days, improved livability, and higher lipase activity within the duodenum. Intestinal stimulus such as increased lipase activity is thought to improve the ability to grow in the face of stressors (Noy and Sklan, 1999) and disease (Yi *et al.*, 2005).

2.8 Lysozyme

2.8.1 Structure

Lysozyme is an antimicrobial enzyme derived from chicken egg white, which is certified to contain 15.3% protein and 8.6% moisture (Neova Technologies Inc., 2009). Lysozyme is defined as 1, 4-β-N-acetlmuramidase which hydrolyzes the glycosidic bond between the C-1 of N-acetylmuramic acid (MurNAc) and the C-4 of N-acetylglucosamine (GlcNAc) in the bacterial peptidoglycan (Proctor and Cunningham, 1988). This damages the bacterial cell wall rendering the cell incapable of normal

function. Phillips (1967) described the first detailed mechanism of lysozyme by detailing the action for the hen egg lysozyme.

2.8.2 Function

Lysozyme has a direct bacteriolytic action (Biggar and Sturgess, 1977). Lysozyme is present in many tissues of the body as well in inflammation sites where both Gram positive and Gram negative bacteria are present. Through simple digestion or the splitting of products (from peptidoglycans) the accumulation of similar compounds with a higher molecular weight can occur. These compounds (lysozyme included) belong to a group of natural compounds which are immunostimulating, thus allowing stimulation of production of antibodies against bacteria and other antigens (Jollès, 1976). Immunostimulation has been theorized as a possible physiological function of lysozyme, which could be due to an indirect effect during the course of its action on bacteria which destroys the cell wall (Jollès, 1976). By increasing antibody response the body is able to fight off bacteria quicker.

2.8.3 Potential in Turkey Poults

Zhang *et al*. (2006) found that *in vitro* lysozyme destroyed the vegetative cells of *Clostridium perfringens* at a level of 156 μg ml^{-1}, but at lower levels of 50 μg ml^{-1} could inhibit the production of α-toxin. Since *Clostridium perfringens* is a known cause of necrotic enteritis in poultry with the main cause of gross lesions being α-toxin, these findings could prove to be very useful against necrotic enteritis once tested *in vivo* (Zhang *et al.* 2006). Moore and Owen (1966) stated that although hens have their own immunological system, the egg and developing embryo do not produce immunoglobulins until approximately 7 days before hatching. Therefore, it is possible that the egg has a

high lysozyme content to protect the embryo from disease (Moore and Owen, 1966). Humphrey *et al.* (2002) who fed lysozyme (10% of the diet) in addition to lactoferrin (5% of the diet) to broiler chicks determined that a combination of the two enzymes improved feed efficiency and histological indices of intestinal health (thinner lamina propria, higher villi height, and decreased lamina propria leukocytes). Although the combination of both enzymes produced the best results, separately lysozyme had a better effect than lactoferrin on the birds. Birds consuming solely lysozyme showed decreased feed consumption, increased villi height in the duodenum, and decreased leukocytes resulting in a thinner lamina propria. Overall the experiment indicated that lysozyme and lactoferrin displayed antibacterial properties in the presence of pathogenic microflora and could be a useful alternative to antibiotics.

2.9 Early Poult Behaviour

There is very little research documented on the post placement behaviour of poults, especially those who have endured a long transport before placement. In an attempt to reduce starve outs and early mortality Bate (1992) found that sound stimuli improved poult feeding behaviour post hatch starting at day 3 and continuing to day 21. Hearing feeding calls or broody calls and feeding calls coming from the feeder increased ingestive behavior, resulting in an 8-15% increase in body weight at 21 days. This study suggests that stimulating the poults before hatch with broody vocalizations may have a better effect than just stimulating post hatch with feeding calls. A significant difference was reported at days 18 and 21 where birds stimulated with broody and feeding calls had an 8% increase in body weight over those stimulated solely by feeding calls. Nielsen (2004)

reviewed various aspects of feeding behaviour of broiler chickens. The author indicated that there appears to be a subtle effect of feed type, environmental condition and the use of social grouping on the feeding behaviour of broilers and that short term feeding observation of birds would be beneficial to trials concerned with nutrition, growth, genetics and production of broilers. In group housing, synchronized behaviours such as feeding and resting are common and can be affected by available feeding or resting space (Nielsen, 2004). Picard *et al.* (1992) found that under group housing, feeding sessions are reduced to approximately three or four per hour, but these sessions are longer in duration than when birds are individually housed and had increased intake that was associated with enough trough space for all birds at once. Although the synchronized behaviour is said to be similar between chickens and turkeys it is unknown how much similarity there is between the species. Aziz (2001) linked management practices to a reduction of early poult mortality where producers know the typical behaviour of the birds. This implies that by following the reactions and behaviours expressed by the birds we can easily see if changes to environment can be altered to improve survivability (Aziz, 2001).

2.10 Summary

Current research has shown that stress imposed on the newly hatched poults ranging from hatchery practices, diseases and transport conditions, have influenced early poult mortality and subsequent growth (Jindal *et al.*, 2009; Carver *et al.*, 2002). Preventative measures such as providing Oasis® during transport and sub-therapeutic antibiotics in feeds have been proven to have positive effects on survivability and growth of poults (Dibner *et al.*, 1998; Boersma *et al.,* 2003; Solomons, 1978). The development of the intestinal tract is critical to the growth and performance of the poult. Early nutrition

increases the absorptive surface of the intestinal tract which leads to increased growth rate and performance (Dibner *et al*., 1998, Potturi *et al.,* 2005). Due to increasing consumer demand for antibiotic free poultry there is more and more research into the use of enzymes to reduce pathogens. Lysozyme has been studied for its protective function in the egg but research is limited regarding its use within the diet to prevent disease and increase survivability of poults (Biggar and Sturgess, 1977). Research on feeding, drinking and locomotive behaviour of poults is also very limited (Aziz, 2001). More knowledge in these areas could help reduce current levels of early poult mortality.

2.11 Objectives

To determine the effect of providing Oasis® alone or combined with lysozyme during transport and dietary lysozyme after transport on growth performance, mortality rates, gastrointestinal tract growth and behavior of turkey poults.

2.12 Hypotheses

Due to the reported improvement of growth and intestinal characteristics from early feeding, it is hypothesized that birds receiving Oasis® or the combination of Oasis® and lysozyme will have improved growth performance and gastrointestinal tract sampling measures with decreased overall mortality. The early uptake of nutrients will allow the birds more energy making them more active in their behaviours after placement. Birds that do not receive any transport supplementation are hypothesized to be more lethargic upon placement. After transport lysozyme's antimicrobial properties are hypothesized to provide an improved resistance to pathogenic bacteria allowing for equal or improved growth to birds receiving an antibiotic supplemented diet.

Chapter 3: The Effect of Oasis® and Lysozyme during Transport and Lysozyme Supplementation Post-transport on Growth Performance and Mortality of Turkey Poults

3.1 Objectives

To determine the effect of supplementing Oasis® and lysozyme during long transport and lysozyme after transport on the growth performance and mortality of newly hatched turkey poults.

3.2 Hypotheses

It is hypothesized that feeding Oasis® or Oasis® plus lysozyme during long transport will improve growth performance parameters of poults. Early feeding during transport will allow the birds to have increased body weights and improved feed conversion. The mortality rate for birds provided Oasis® and Oasis® plus lysozyme are hypothesized to be lower.

The addition of dietary lysozyme, with its antimicrobial properties, is hypothesized to improve the birds' resistance to pathogens allowing for increased growth performance and decreased mortality. After transport, birds provided with lysozyme are hypothesized to have similar or improved performance to birds receiving an antibiotic diet.

3.3 Materials and Methods

This research was conducted using two separate trials. Trial 2 was a repeat of trial 1 with minor modifications to data collection. Any deviations are explained. Each trial was a 3x4 factorial analysis with pre-transport treatment and post-transport treatment as the main factors.

3.3.1 *Preparation for Transport*

Seven hundred and eighty three female Hybrid poults were hatched at Cuddy Hatchery in Strathroy, Ontario (June 29th, 2009 for trial 1 and December 14, 2009 for trial 2). The poults were hatched using standard hatchery procedures but were not vaccinated, microwave toe treated or beak trimmed. The transport boxes measured 61cm long, 50cm wide and 19cm high and were separated into quadrants with a cardboard partition. Each quadrant contained a poult box pad (Midlantic Poultry Products Inc., Greensboro NC, USA). Poults were randomly placed into transport boxes with 100 poults per box equally distributed in four quadrants (Figure 3.1) per treatment group. In the last box of each treatment group there were 20 birds in the first two quadrants, 21 in the third quadrant, and the fourth quadrant was left empty.

Birds were randomly placed in three transport treatments which were initiated at the hatchery, provided during transport and terminated at the destination. The first treatment was birds transported without provision of a supplement (NO), this group was used as the control. The second transport supplement treatment was provided Oasis® (OAS), a dietary hatchling supplement provided by Novus International. The third supplement treatment was fed Oasis® + 0.01% lysozyme (Inovapure™ 213) (OL) (Neova Technologies Inc., 2009). There were a total of 11 quadrants of birds within each treatment (2 full boxes and one box ¾ full). The transport supplements were prepared 2 hours (h) before arriving at the hatchery (~0400h June 29th, 2009 and December 14th, 2009 respectively). Novus International recommended adding up to 20% water to the

Figure 3.1: Assignment of newly hatched turkey poults in quadrants of the transport box.

Oasis® supplement to increase moisture 1-2 hours before providing it to the birds (Novus® International, Inc., 2008). 200mL of water was added to 1.0Kg of Oasis®. For the OL treatment the lysozyme was added at 0.01%, which equaled 0.12 g of lysozyme added to, 1.0 Kg Oasis® and 200 mL of water. The supplements were thoroughly mixed and left for 2 hours to allow absorption of the water. Upon arrival at the hatchery, the mixed supplements were provided on a per bird basis at approximately 2.5g/bird, at a rate of 76.0 g of the wetted supplement per quadrant. Novus recommends 200-250 g per 100 birds when un-hydrated. The supplements were provided in one plastic trough attached to the side of the transport boxes (figure 3.2) in each quadrant. Each quadrant within a box of the same treatment was used as the experimental unit.

Figure 3.2: Hydrated Oasis® pellets in clear plastic troughs attached to the sides of each quadrant of the transport boxes.

Transport occurred in environmentally controlled trucks. During transport temperature was measured at loading and unloading. Transport time was ~24 hours; distance was 1907km and terminating at the Atlantic Poultry Research Center (APRC) in Truro, Nova Scotia on June 30th 2009 and December 15, 2009. Birds were immediately placed in floor pens upon arrival.

3.3.1.1 Trial 2 Modifications

In trial 2, at the hatchery before being placed in the transport boxes poults were individually weighed using a top pan balance. There were a total of 825 birds in the trial, resulting in 275 poults per treatment group. The birds were placed in 2 full transport boxes, and the third transport box for each treatment group had 25 poults per quadrants in 3 quadrants. The birds arrived at the APRC at ~1200h December 15, 2009 and were immediately placed in floor pens.

3.3.2 Treatment Conditions

Before arrival of the birds, 48 pens were prepared with litter at a depth of ~4cm made of clean pine shavings, bell drinkers, and tube feeders. There were 4 separate rooms, each containing 12 pens measuring 2.13m x 1.40m (area 2.98m²). Supplemental feed was also provided in a cardboard feed box, measuring 53.3 x 43.2 x 5.1cm during the first 3 days after placement. The rooms were preheated to a brooding temperature of 35°C. Temperature and lighting was consistent across the rooms and was adjusted as the poults matured (table 3.1). Temperatures in the rooms were measured electronically using a data logger within each room recording every fifteen minutes, as well as twice daily during health checks throughout the trial, using a Raytek Mini temp gun (Appendix G, Appendix H).

After arrival of the birds, the feed trays within the transport box quadrants were reweighed and feed disappearance recorded. Poults from within each transport group were group weighed and randomly picked from all quadrants, then allocated to one of 4 post transport treatments. The birds were then batch weighed and placed into floor pens with 16 birds per pen and a total of 4 pens per treatment. All birds were placed at the back of the pen near the drinker. Each poult had its beak dipped in water in the bell drinker to ensure they recognized where the water was.

3.3.2.1 Trial 2 Modifications

In trial 2, birds were individually weighed upon arrival to the APRC. Birds were removed from the transport box, individually weighed and returned to the same quadrant. Weights were recorded so weight loss or gain during transport could be calculated. Also 19 birds from each transport treatment were killed by cervical dislocation and intestinal

sections for histology were taken. During dissection it was noted that all birds receiving the transport supplements had green digesta, indicating that they had all consumed the supplements provided. Following this added step the same procedures as from trial 1 were followed for placement.

Table 3.1: Temperature (ºC) and Lighting Schedule for Turkey Poults at the Atlantic Poultry Research Center

Day	Temperature (ºC)	Hours Light/ Hours Dark
0	35	24/0
3	34	23/1
5	33	16/8
10	31	16/8
12	30	16/8
15	29	16/8
17	28	16/8
19	27	16/8
21	26	16/8
24	25	16/8
26	24	16/8
29	23	17/7
33	22	18/6
35	21	18/6
57	21	19/5
70	21	19/5

3.3.3 Experimental Diets

All diets throughout the trial were isonitrogenous and isocaloric within phases. The starter mash diets were formulated to contain 29% crude protein and 2850 kcal ME/kg and were fed from placement to 14 days (Table 3.2), one of four treatments. The first was an un-supplemented treatment (NS). These birds were fed the breeder recommendations with no dietary supplements used. The second treatment was a positive

control and was supplemented with 0.004% of the antibiotic Bacitracin Methylene Disalicylate (BMD) antibiotic. This treatment was referred to as the ANTI group. The third treatment was supplemented with 0.01% lysozyme and was referred to as the LYS group. The fourth treatment was provided with BMD and lysozyme at levels used independently. This treatment was referred to as AL.

Table 3.2: Diet Formulations for Poults Receiving an Antibiotic and Lysozyme Diet during the Starter Period from Day 0-14 (g/100g of diet)

	No Supplement	Antibiotic & Lysozyme	Antibiotic	Lysozyme
Ingredient				
Soybean Meal	42.2	42.2	42.2	42.3
Corn	33.8	33.8	33.8	33.8
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.6	1.6	1.6	1.6
Poultry Fat	1.1	1.2	1.1	1.1
Mineral and Vitamin Premix[z]	1.0	1.0	1.0	1.0
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.3	0.3	0.3	0.3
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	-----	0.01
Total	99.9	100.0	99.9	100.0

Calculated Nutrient Content: Metabolizable Energy 2850 Kcal/Kg, Crude Protein 29.0%, Linoleic Acid 1.4%, Crude Fiber 2.5%, Calcium 1.4%, Total Phosphorus 0.9%, Potassium 1.1%, Magnesium 0.2%, Lysine 2.0%, Manganese 89.6 mg/Kg, Selenium 0.4 mg/Kg, Thiamin 5.6 mg/Kg, Arginine 2.0%, Histidine 0.7%, Methionine 0.6%, Methionine and Cystine 1.1%, Sodium 0.2%, Dry Matter 90%.

[z] Supplied per kg starter diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 2871 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 3045 mg; ethoxyquin, 50 mg; wheat middlings, 1049 mg; ground limestone, 500 mg.

[y] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x] BMD- Bacitracin Methylene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne^{-1} mixed feed).

[w] Lysozyme, Neova Technologies Inc. (Providing 10.0mg tonne^{-1} mixed feed).

At 15 days of age birds were fed the next diet phase until day 28. These diets were in the same 4 treatment groups (NS, ANTI, LYS, AL) and were fed as a mash. These diets were formulated to contain 26.5%CP, 3000 kcal ME kg^{-1} (Table 3.3).

Table 3.3: Diet Formulations for Poults Receiving an Antibiotic and Lysozyme Diet during the Grower Period from Day 15-28 (g/100g of diet)

	No Supplement	Antibiotic and Lysozyme	Antibiotic	Lysozyme
Ingredient				
Soybean Meal	38.3	38.3	38.3	38.3
Corn	37.1	37.0	37.0	37.0
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.1	1.1	1.1	1.1
Poultry Fat	2.9	2.9	2.9	2.9
Mineral and vitamin premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	------	0.01
Total	100.0	99.9	99.9	99.9

Calculated Nutrient Content: Metabolizable Energy 3000 Kcal/Kg, Crude Protein 26.5%, Linoleic Acid 1.8%, Crude Fiber 2.5%, Calcium 1.3%, Total Phosphorus 0.8%, Potassium 1.1%, Magnesium 0.2%, Lysine 1.6%, Manganese 88.6 mg/Kg, Selenium 0.4 mg/Kg, Thiamin 5.5 mg/Kg, Arginine 1.9%, Histidine 0.7%, Methionine 0.5%, Methionine and Cystine 1.0%, Sodium 0.2%, Dry Matter 90%.

[z]Supplied per kg grower diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 1079 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 29.7 mg; ethoxyquin, 50 mg; wheat middlings, 905 mg; ground limestone, 500 mg.

[y]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x] BMD- Bacitracin Methylene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

[w] Lysozyme, Neova Technologies Inc. (Providing 10.0mg $tonne^{-1}$ mixed feed).

From 29 days onward the number of dietary treatments was reduced to two experimental diets. Due to Canadian Food Inspection Agency (CFIA) regulations preventing the continued use of lysozyme in diets for birds intended for human consumption, lysozyme was removed from all diets at 28 days. This removal was requested through correspondence with Manisha Mehrotra, Human Safety Division CFIA February 25th, 2009. This was also the period chosen as it was assumed the greatest influence of lysozyme was likely expressed during early growth of the birds. Birds previously on the ANTI or AL diet were fed the grower 2 antibiotic diet. This diet was supplemented with 0.004% BMD. Birds which were previously on the LYS or NS diet were fed the grower 2 no supplement diet (Figure 3.3). Grower 2 diets were fed from 29 to 56 days of age as pelleted diets formulated to contain 23% CP and 3200 kcal ME/kg (Table 3.4). At 56 days birds were placed on the final diet phase. Pelleted finisher diets were formulated to contain 19% CP and 3250 kcal ME/kg. The birds remained on the same treatments as the grower 2 phase until the termination of the trial at 70 days of age (Table 3.4).

All diets throughout the trial met or exceeded the National Research Council (NRC, 1994) requirements for turkey growth. For all phases, feed was weighed in as needed and feed weigh backs occurred at the end of each dietary phase, or as mortality occurred. All mortalities throughout the trial were recorded and sent to a veterinary pathologist for necropsy (Appendix I, Appendix J).

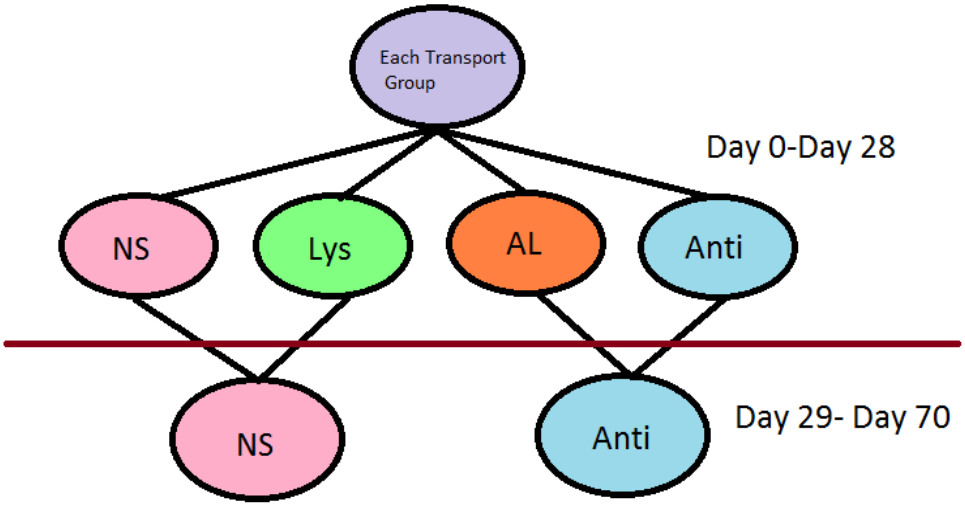

Figure 3.3: Schematic of the Treatment Group Changes Occurring At Day 29 of the Trial.

3.3.4 Data Collection

During the starter period there were 16 birds per pen, with three being sampled at the end of the starter period on day 14. During grower 1 there were 13 birds remaining in the pen. On day 28, three birds per pen were sampled and 10 birds per pen remained for the grower 2 period. At the end of the grower 2 period, day 56, three birds per pen were sampled leaving a total of seven birds per pen for the finisher period. Three of the remaining seven birds were sampled at the end of the trial on day 70.

One day previous to each sampling day the birds were weighed by pen (Day 13, 27, 55, 69). Up to 27 days birds were batched weighed using a balance equipped with live weigh capability (Mettler PM 34-K Delta Range, Mississauga ON). To batch weigh, the weighing containers were zeroed on the balance. The birds were placed into the weighing container (number of birds was determined by the number of the birds that can stand on the bottom of the weighing container) and container was placed on the balance. Weight

of the birds and number of birds were recorded. Birds were then returned to the pen and weight of batches continued until all birds had been weighed. At days 55 and 69 birds were individually weighed using a balance equipped with shackles. The balance was placed on a platform where a bracket attached the balance to a shackle. Birds were individually caught and were placed with their legs into the shackles. The bird weight was recorded, and the bird was removed from the shackles and returned to the pen. Immediately following weighing the birds, the feeders were weighed back and any remaining feed was discarded. During weighing any stunted birds (those weighing 50% below average of pen) were culled and sent for necropsy.

3.3.5 Feed Analysis

Feed samples were collected during each dietary phase. These samples were stored at -18ºC until analyzed. Feed samples were analyzed in duplicate. Dry matter was determined by oven-drying a 2g sample for 24h at 50ºC by method 935.29 (Association of Official Analytical Chemists (AOAC), 2005). Crude protein (Nx6.25) was determined by method 990.03 using a LECO FP-528 Combustion Nitrogen Analyzer (Leco Corporation, St. Joseph, MI) (AOAC, 2005). For mineral analysis feed samples were dry ashed in duplicate, then Argon Plasma Spectrometry (Jarrel Ash Model 9000, Thermo Elemental, Franklin, MA) using method 968.08 (AOAC, 2005) was conducted to measure calcium, phosphorous, sodium, potassium, magnesium, manganese, copper, and zinc. The determination of crude fat content was done using the Ankom XT10 Extraction System. The process involves drying a weighed sample in a filter bag. The sample was placed in the extractor where petroleum ether was the solvent used for extraction. After extraction the samples were then placed in the drying oven, and once dry were weighed

for crude fat (ANKOM Technology, Macedon, NY). These analyses are reported in appendix A-F.

Table 3.4: Diet Formulations for Grower 2 (23%CP And 3200 Kcal ME/Kg) and Finisher (19%CP And 3250 Kcal ME/Kg) Period (g/100g of diet)

	Grower 2 (Day 29-56)		Finisher (Day 57-70)	
Ingredient	NS	Anti	NS	Anti
Soybean Meal	29.5	29.5	22.5	22.5
Corn	43.8	43.8	53.1	53.1
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	5.6	5.6
Limestone (ground)	1.6	1.6	1.7	1.7
Mono-Dicalcium Phosphate	0.8	0.8	0.6	0.6
Poultry Fat	4.8	4.8	4.8	4.8
Vitamin and Mineral Premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
Ameri-bond 2x[x]	0.5	0.5	0.5	0.5
Lysine	----	----	0.2	0.2
BMD[w]	----	0.004	----	0.004
Total	100.0	100.0	100.0	100.0

Calculated Nutrient Content Grower 2: Metabolizable Energy 3200 Kcal/Kg, Crude Protein 23.0%, Linoleic Acid 1.3%, Crude Fiber 2.4%, Calcium 1.2%, Total Phosphorus 0.7%, Potassium 0.9%, Magnesium 0.2%, Lysine 1.3%, Manganese 86.3 mg/Kg, Selenium 0.5 mg/Kg, Thiamin 5.3 mg/Kg, Arginine 1.6%, Histidine 0.6%, Methionine 0.5%, Methionine and Cystine 0.9%, Sodium 0.2%, Dry Matter 90%.

Calculated Nutrient Content Finisher: Metabolizable Energy 3250 Kcal/Kg, Crude Protein 19.0%, Linoleic Acid 1.4%, Crude Fiber 2.4%, Calcium 1.1%, Total Phosphorus 0.6%, Potassium 0.8%, Magnesium 0.2%, Lysine 1.2%, Manganese 84.3 mg/Kg, Selenium 0.5 mg/Kg, Thiamin 5.2 mg/Kg, Arginine 1.3%, Histidine 0.5%, Methionine 0.5%, Methionine and Cystine 0.8%, Sodium 0.2%, Dry Matter 90%.

[z]Supplied in grower 2 diet per kg diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 29.7 mg; ethoxyquin, 50 mg; wheat middlings, 530 mg; ground limestone, 500 mg.

[z]Supplied per kg diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.03 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.3 mg; pyridoxine, 4.9 mg; thiamine, 2.9 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1296 mg; ground limestone, 500 mg.

[y] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]Ameri-bond 2x, Ligno Tech, Rothschild, WS, USA (providing 6.25mg $tonne^{-1}$ mixed feed).

[w] BMD- Bacitracin Methylene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

3.3.6 Statistical Analysis

This experiment is described as completely randomized with treatments arranged in a factorial block design. Growth performance and mortality data were subjected to analysis of variance (ANOVA) using the Proc Mixed procedure of SAS (Littell *et al.*, 1996). The model was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k+} \alpha\gamma_{ik} + \varepsilon_{ijkl}$$

Where Y_{ijkl}= Response, μ= Population Mean, α= Factor 1 or transport supplement (i=1-3), β= blocking factor or room (j=1-4),γ=factor 2 or post transport supplement (k=1-4), ε= 1, 2, 3…error Effect, i=Levels of factor 1 (OAS, OL, NO), j=levels of blocking factor (151, 152, 153, 156) k=Levels of factor 2 (NS, ANTI, LYS, AL), l= number of replicates (12).

Repeated measures were performed on growth performance data, when the factor of age was included in the original model. The new model used was:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\gamma_{ik} + \delta_l + \alpha\delta_{il} + \gamma\delta_{kl} + \alpha\gamma\delta_{ikl} + \varepsilon_{ijklm}$$

Where Y_{ijklm}= Response, μ= Population Mean, α= Factor 1 or transport supplement, i=Levels of factor 1 (OAS, OL, NO), β= blocking factor or Room, j=Levels of blocking factor (room 151, 152, 153, 156),γ= Factor 2 or dietary supplement, k=Levels of factor 2 (NS, ANTI, LYS, AL), δ=Factor 3 or Age, l = levels of factor 3 (14, 28, 56, 70),ε = 1, 2, 3…Error Effect, m= number of replicates (4).

If significant main effects or interactions were found in the ANOVA, the Tukey's option was used to compare differences among the least-square means ($P \leq 0.05$).

3.4 Results and Discussion

3.4.1 Experimental Diets

All diets were analyzed in duplicate for dry matter, crude protein, crude fat, and mineral content. Tabular analysis of all diets for both trials can be found in Appendices A-F. Analyzed diets were similar to the calculated values for treatments in a period as well as across both trials. Oasis® composition was not analyzed during feed anlaysis. Compositions given by Novus Int. were assumed to be correct.

3.4.2 Growth Performance

The two trials were subject to different conditions involving the breeder flock and the time of year. Therefore, all analyses were performed on each trial separately.

Body weight of the birds was similar ($P>0.05$) regardless of transport supplements at each weigh day during trial 1 (Table 3.5). This is not the first study evaluating the growth of turkeys provided Oasis®. Some have found Oasis® improved bird growth (Noy and Sklan, 1999; Boersma *et al.,* 2003), where others have found growth improvements to be inconsistent (Jackson, 2005; Batal and Parsons, 2002; Yi *et al.,* 2005).

The body weights of poults at 13 days are slightly higher than those in a study by Jackson (2005) who reported weights of 326±3.3g at day 14. Jackson (2005) also found that Oasis® pre-fed birds had lower average body weight at 14 days, 257.8±3.1g. A decrease in weight after feeding Oasis® was not found in this experiment. Bird weights at placement in this experiment averaged 55.7g, while Jackson (2005) had placement weights ranging from 51-54g. The increased weight at placement of birds in this

Table 3.5: Effect of Transport Supplement on the Body Weight of Heavy Hen Turkeys (Trial 1)

Body weight (g·bird^{-1})					
	Age (days)				
Transport[z]	**13**	**27**	**55**	**69**	**Mean**
OAS	344 ±44.1[†]	946 ±44.1	3393 ±44.1	5033 ±44.1	1954 ±25.4
OL	341 ±44.8	961 ±44.8	3471 ±44.8	5097 ±44.8	1985 ±25.9
NO	337 ±44.1	933 ±44.1	3367 ±44.1	5054 ±44.1	1949 ±25.4
Age Mean	*341d ±25.6*	*947c ±25.6*	*3410b ±25.6*	*5061a ±25.6*	

ANOVA	**P-value**
Transport (T)	0.58
Age (A)	<0.0001
T x A	0.94

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[†] Mean ± Standard Error
a-d – Means within a row with different letters differ significantly ($P \leq 0.05$).

experiment are likely responsible for the higher body weights at 13 days. In the current experiment Oasis® was provided at 2.5g per bird, whereas Jackson (2005) offered Oasis® at 5.0g/bird. Noy and Sklan (1999) found an increase in body weight through to 21 days in poults that had early access to feed. This included liquid feed, solid feed, or Oasis®. These birds were held for 48 hours, whereas birds in this trial were only held for 24 hours during transport. The greatest benefit from Oasis® may occur when birds are without feed and water for longer term. This longer period prior to placement, could have resulted in the increase in body weight for birds fed Oasis®. Corless and Sell (1999) reported mixed results; in one trial they found that birds held for 30 hours before placement had a lower body weight at 14 days of age, 241.1g, than those that were placed within 6 hours of hatch, at 260.2g. In another trial birds placed after a 30 hour hold, had

similar body weights as those that were placed after hatch, which is similar to this experiment. At 28 days of age Corless and Sell (1999) found that a 30 hour hold did not affect body weight of poults, whereas a 54 hour hold continued to have a negative effect.

There was a 2 way interaction of age and supplement ($P \leq 0.05$) in regards to the body weight of the birds after transport (Table 3.6). At day 13, birds receiving all supplements had similar body weights with a mean of 341g/bird, and at day 27 with a mean of 946 g/bird. The body weight of birds was different ($P \leq 0.05$) among treatments at days 55 and 69. At day 55 poults receiving the AL treatment weighed more (3588g) than those fed the ANTI treatment (3228g). At day 69 birds receiving the AL or LYS treatments (5238 and 5159g respectively) had higher body weights than those receiving the ANTI treatment (4856g). Birds receiving NS had body weights similar to those receiving all other treatments at both ages.

The increase in body weight at days 55 and 69 for birds consuming AL indicated that there may be a synergistic relationship between BMD and lysozyme. Synergistic activity has been documented for combinations of antibiotics since 1950. Jawetz *et al.* (1950) reported an *in vitro* synergism between penicillin and streptomycin in the killing of *enterococci*. Research into antibiotic and non-antibiotic synergism has been described by Sims *et al.* (2004) who found synergistic activity between BMD and mannan oligosaccharide in poult toms at 105 days where the combination produced a higher body weight than birds fed the control diet. Birds supplemented with just LYS had improved weights over birds provided ANTI at day 69. This contradicts research by MacIsaac and Anderson (2008) who found no difference between birds supplemented with dietary BMD and those supplemented with 0.04% lysozyme at day 69.

Table 3.6: Effect of Dietary Supplement and Age on the Body Weight of Heavy Hen Turkeys (Trial 1)

Body weight (g·bird^{-1})					
	Age (days)				
Supplement[z]	**13**	**27**	**55**	**69**	**Mean**
NS	334*f* ±50.9[†]	925*e* ±50.9	3341*cd* ±50.9	4992*ab* ±50.9	1930±29.4
ANTI	346*f* ±51.1	871*e* ±51.1	3238*d* ±51.1	4886*b* ±51.1	1881±29.6
LYS	339*f* ±53.8	994*e* ±53.8	3478*cd* ±53.8	5159*a* ±53.8	2003±31.1
AL	343*f* ±49.2	997*e* ±49.2	3585*c* ±49.2	5208*a* ±49.2	2038±28.4
Age mean	*341 ±25.6*	*947 ±25.6*	*3410 ±25.6*	*5061 ±25.6*	

ANOVA	**P-value**
Supplement (S)	0.003
Age (A)	<0.0001
S x A	0.002

[z] NS- No supplement added, ANTI-Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS - Lysozyme (Inovapure™213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada), AL – Antibiotic + Lysozyme

[†] Mean ± Standard Error

a-f Means with different letters differ significantly ($P \leq 0.05$).

MacIsaac and Anderson (2008) found birds supplemented with 0.04% lysozyme alone or in combination with BMD had improved body weights ($P \leq 0.05$) at day 55 over birds that remained un-supplemented or received only BMD. Body weights at days 55 and 69 were comparable between this trial and MacIsaac and Anderson (2008)

Body weight of the birds from the second trial indicates a three way interaction ($P \leq 0.05$) between age, transport supplement and post transport supplement (Table 3.7). Within weigh days the differences can be observed. During day 0 and 13 birds across transport and post transport supplements are not different in body weight ($P > 0.05$). Birds during this time had similar ($P > 0.05$) body weights regardless of the transport and post transport supplement combinations. Body weights of birds at placement (day 0) were

similar to those reported by Jackson (2005). Birds in this trial ranged from 52-54g and Jackson (2005) recorded bird weights of 51-54g. Uni *et al*. (1999), reported day 0 body weights of ~60g which is 10% higher than the body weights of birds in this trial. The birds used by Uni *et al*. (1999) may be larger due to the egg size or breeder flock used (Fasina and Thanissery, 2011, Carver *et al.,* 2002). Day 27 had significantly higher ($P \leq 0.05$) body weights than day 0 and day 13, as expected, but there were no differences ($P > 0.05$) between transport and post transport supplementation on the body weight of the birds. This result is similar to results found in trial 1 where there was no effect of transport or post transport supplementation on birds during days 13 and 27. At day 55 all birds were significantly heavier ($P \leq 0.05$) than birds at days 0, 13, or 27, but no differences ($P > 0.05$) appear among treatments. This was different than results found in trial 1, or results found by MacIsaac and Anderson (2008) where there was a difference reported between post transport supplementation on body weight at day 55. Day 69 resulted in significantly higher ($P \leq 0.05$) body weights than days 0, 13, 27 and 55 regardless of treatment, but birds receiving OL/LYS, OL/ANTI, OAS/AL, OAS/ANTI, OAS/NS or NO/AL have a significantly heavier ($P \leq 0.05$) body weight than birds fed the NO/LYS supplement combination. Birds receiving NO/NS, NO/ANTI, OAS/LYS, OL/NS, or OL/AL had similar body weights to birds that received all other treatment combinations. These results, similar to trial 1, contradict those found by MacIsaac and Anderson (2008) who found no significant differences when supplementing diets with lysozyme or in combination with BMD. Between trials 1 and 2 the birds were placed with similar body weights and this similarity continued as the birds aged.

Table 3.7: Effect of Transport, Dietary Supplement and Age on the Body Weight of Heavy Hen Turkeys (Trial 2)

Bird Weight (g·bird^{-1})												
No supplement					***Oasis®[z]***				***Oasis®[z] + Lysozyme[y]***			
Day	**NS[x]**	**ANTI**	**AL**	**LYS**	**NS**	**ANTI**	**AL**	**LYS**	**NS**	**ANTI**	**AL**	**LYS**
0	53e[*†]	54e	55e	52e	53e	53e	53e	54e	54e	54e	52e	54e
13	309e	321e	315e	316e	332e	337e	323e	318e	337e	322e	321e	328e
27	901d	893d	910d	922d	929d	907d	941d	920d	943d	889d	935d	967d
55	3445c	3549c	3556c	3565c	3757c	3556c	3620c	3554c	3673c	3419c	3705c	3753c
69	5117ab	5298ab	5418a	4943b	5503a	5419a	5413a	5175ab	5330ab	5119ab	5253ab	5527a

ANOVA	**P-value**
Transport (T)	0.11
Supplement (S)	0.58
Age (A)	<0.0001
T x S	0.08
T x A	0.15
S x A	0.51
T x S x A	0.01

[z] Oasis® - Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA.
[y] Lysozyme - Entergard® (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada.
[x] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), AL – Antibiotic + Lysozyme, LYS – Lysozyme
[*]Standard Error for O x S x A = 73.2 for all treatments.
[†] Mean ± Standard Error
a-e Means with different letters differ significantly ($P \leq 0.05$)

The daily body weight gain of the birds was not changed by the transport or dietary supplements (Table 3.8) in trial 1. All birds had similar ($P>0.05$) body weight gain throughout the periods regardless of the transport or dietary supplement assigned. There is a significant ($P\leq0.05$) age difference which was expected. The highest body weight gain per day occurred during the 57-69 day (finisher) period with an average gain of 118g·bird^{-1} day^{-1}.

The body weight gain of the birds (g·day^{-1}) was not changed ($P>0.05$) by the transport supplements in trial 2 (Table 3.9). All birds displayed similar body weight gain throughout the periods. There was an age difference with ($P\leq0.05$) the highest body weight gain (g·bird^{-1} day^{-1}) during the 56-69 day (finisher) period, with birds during that time gaining an average of 121g per day. The weight gain (g·day^{-1}) of the birds was not changed by the dietary supplements provided ($P>0.05$), with all birds having similar body weight gain within each period.

The weight gain of birds in trial 2 is similar to trial 1 in all age periods except during days 14-27 where weight gains during trial 2 averaged 64 g·bird^{-1} day^{-1}, which was higher than the average of 43 g·bird^{-1} day^{-1} reported in trial 1. This did not seem to affect the body weight of the birds, with overall body weights being similar between trials. Both trials show similar body weight gain for each period in comparison to MacIsaac and Anderson (2008). MacIsaac and Anderson found birds had similar body weight gain regardless of supplementation of lysozyme up to day 29, but found a difference in body weight gain from day 29-56. Birds supplemented with BMD and Lysozyme (0.02%) showed significantly higher body weight gain than control birds or

Table 3.8: Effect of Transport and Dietary Supplementation on the Body Weight Gain of Heavy Hen Turkeys during Each Age Period (Trial 1)

Body Weight Gain (g·bird^{-1} day^{-1})					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	21 ±2.6[†]	43 ±2.6	87 ±2.6	117 ±2.6	67 ±1.4
OL	20 ±2.7	44 ±2.7	90 ±2.7	116 ±2.7	68 ±1.4
NO	20 ±2.7	43 ±2.7	87 ±2.7	121 ±2.7	68 ±1.4
Supplement[y]					
NS	20 ±3.0	42 ±3.0	86 ±3.0	118 ±3.0	67 ±1.6
ANTI	21 ±3.0	37 ±3.0	85 ±3.0	118 ±3.0	65 ±1.6
LYS	20 ±3.2	47 ±3.2	89 ±3.2	120 ±3.2	69 ±1.7
AL	21 ±2.9	47 ±2.9	92 ±2.9	116 ±2.9	69 ±1.5
Age mean	*20d* ±1.5	*43c* ±1.5	*88b* ±1.5	*118a* ±1.5	

ANOVA	**P-value**
Transport (T)	0.95
Supplement (S)	0.22
Age (A)	<0.0001
T x S	0.88
T x A	0.89
S x A	0.68
T x S x A	0.73

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), AL – Antibiotic + Lysozyme, LYS – Lysozyme

[†] Mean ± Standard Error

a-d Means within a row with different letters differ significantly ($P \leq 0.05$).

Table 3.9: Effect of Transport and Dietary Supplementation on the Body Weight Gain of Heavy Hen Turkeys during Each Age Period (Trial 2)

Body Weight Gain (g·bird^{-1} day^{-1})					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	19 ±2.2[†]	65 ±2.2	96 ±2.2	125 ±2.2	76 ±1.1
OL	19 ±2.2	65 ±2.2	96 ±2.2	119 ±2.2	75 ±1.1
NO	19 ±2.2	63 ±2.2	94 ±2.2	119 ±2.2	74 ±1.1
Supplement[y]					
NS	19 ±2.5	64 ±2.5	96 ±2.5	121 ±2.5	75 ±1.3
ANTI	19 ±2.5	63 ±2.5	93 ±2.5	126 ±2.5	75 ±1.3
LYS	19 ±2.5	65 ±2.5	96 ±2.5	114 ±2.5	73 ±1.3
AL	19 ±2.5	65 ±2.5	96 ±2.5	124 ±2.5	76 ±1.3
Age mean	*19d* ±1.2	*64c* ±1.2	*95b* ±1.2	*121a* ±1.2	
	ANOVA		**P-value**		
	Transport (T)		0.22		
	Supplement (S)		0.57		
	Age (A)		<0.0001		
	T x S		0.10		
	T x A		0.67		
	S x A		0.10		
	T x S x A		0.07		

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), AL – Antibiotic + Lysozyme, LYS – Lysozyme
[†] Mean ± Standard Error
a-d Means within a row with different letters differ significantly ($P \leq 0.05$).

those receiving just BMD (MacIsaac and Anderson, 2008). This result was not seen in the body weight gains of birds from days 56-69 or seen in either of these trials.

The feed consumption of the birds was not affected ($P>0.05$) by the transport supplements provided (Table 3.10). Birds had similar feed consumption within each period, regardless of what transport supplement they received. Feed consumption was

Table 3.10: Effect of Transport and Dietary Supplementation on the Feed Consumption of Heavy Hen Turkeys during Each Age Period (Trial 1)

Feed Consumption (g·bird^{-1} day^{-1})					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	27 ±5.0[†]	62 ±5.0	155 ±5.2	208 ±5.2	113 ±2.9
OL	27 ±5.1	65 ±5.1	147 ±5.1	215 ±5.1	113 ±2.9
NO	27 ±5.0	62 ±5.0	155 ±5.0	219 ±5.0	116 ±2.8
Supplement[y]					
NS	26 ±5.8	62 ±5.8	151 ±5.8	217 ±5.8	114*ab* ±3.3
ANTI	27 ±5.8	57 ±5.8	146 ±5.8	198 ±5.8	107*b* ±3.3
LYS	27 ±6.1	66 ±6.1	147 ±6.4	216 ±6.4	114*ab* ±3.5
AL	27 ±5.6	66 ±5.6	166 ±5.6	225 ±5.6	121*a* ±3.2
Age mean	*27d* ±2.9	*63c* ±2.9	*152b* ±2.9	*214a* ±2.9	

ANOVA	P-value
Transport (T)	0.71
Supplement (S)	0.05
Age (A)	<0.0001
T x S	0.10
T x A	0.63
S x A	0.36
T x S x A	0.23

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-d Means within a main effect in the same row or column with different letters differ significantly ($P \leq 0.05$).

affected by dietary supplements ($P \leq 0.05$). Birds receiving the AL supplement had significantly higher feed consumption than birds receiving the ANTI supplement. The birds ate more as they aged ($P \leq 0.05$).

The increased feed consumption of birds provided with AL differs from MacIsaac and Anderson (2008) who found that feed consumption was not significantly affected by dietary supplementation of BMD and lysozyme independently or in combination. The level of BMD used is comparable but there are differences related to the level of lysozyme used or the duration of lysozyme inclusion. MacIsaac and Anderson (2008) used a higher level of inclusion of lysozyme (0.04%) and also fed dietary lysozyme until the termination of the trial. It appears there may be an effect of lysozyme when in combination with BMD that had residual effects on the feed consumption of the birds over the entirety of the trial. The increased feed consumption in birds consuming AL did not have an effect on body weight gain, but birds consuming AL had higher body weights at day 55 and 69 over birds consuming ANTI.

The feed consumption of the birds in trial 2 was not significantly ($P>0.05$) affected by transport or dietary supplement (Table 3.11). All birds displayed similar feed consumption. There was a difference ($P\leq0.05$) observed as the birds aged, which was expected.

When comparing the 2 trials it appears trial 2 has significantly higher feed consumption than trial 1 for all periods except days 0-13 where trial 1 appeared to have a higher feed consumption. MacIsaac and Anderson (2008) found no differences in feed consumption when supplementing dietary lysozyme to turkey poults. Feed consumption during trial one was similar from day 15-27. In this trial birds consumed 63 $g \cdot bird^{-1}$ day^{-1} which was similar to the feed consumption of 62.5 $g \cdot bird^{-1}$ day^{-1} reported by MacIsaac and Anderson (2008). Contradicting both trials, Corless and Sell (1999) found a significant difference in the feed consumption of birds that were held for 30 hours before

Table 3.11: Effect of Transport and Dietary Supplementation on the Feed Consumption of Heavy Hen Turkeys during Each Age Period (Trial 2)

Feed Consumption (g·bird^{-1} day^{-1})					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	25 ±5.3[†]	79 ±5.3	177 ±5.3	306 ±5.3	147 ±3.0
OL	24 ±5.5	78 ±5.7	175 ±5.3	285 ±5.3	140 ±3.0
NO	24 ±5.3	78 ±5.3	169 ±5.3	283 ±5.3	138 ±3.0
Supplement[y]					
NS	25 ±6.2	76 ±6.4	177 ±6.2	295 ±6.2	143 ±3.4
ANTI	24 ±6.5	76 ±6.5	169 ±6.2	293 ±6.2	141 ±3.5
LYS	24 ±6.2	82 ±6.2	175 ±6.2	284 ±6.2	141 ±3.4
AL	24 ±6.2	79 ±6.2	174 ±6.2	293 ±6.2	142 ±3.4
Age mean	*24d* ±3.1	*78c* ±3.1	*174b* ±3.2	*291a* ±3.1	

ANOVA	**P-value**
Transport (T)	0.14
Supplement (S)	0.95
Age (A)	<0.0001
T x S	0.44
T x A	0.28
S x A	0.96
T x S x A	0.61

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-d Means within a row with different letters differ significantly ($P \leq 0.05$).

placement. At day 14 birds consumed less feed ($P \leq 0.05$) than those placed within 6 hours, but at 28 days this difference disappeared, with birds having similar feed consumptions to what was reported in trials 1 and 2. In a second experiment Corless and Sell (1999) found a feed consumption difference at 7 days but by day 14 this difference was no longer evident. Consistently birds held for 54 hours had lower feed consumption throughout the trials (Corless and Sell, 1999). This may imply that the longer the hold time, the more effective supplementation may be to improving feed consumption of the birds.

The feed conversion of the birds was not affected (P>0.05) by the transport and dietary supplements provided in trial 1 (Table 3.12). Birds showed similar feed conversion within each period, regardless of what transport and dietary supplement they received. Birds during days 28-55, and 56-70 had significantly (P≤0.05) higher feed conversion than those during days 14-27. Those during the grower 1 period (15-28) had higher feed conversion (P≤0.05) than birds from 0-13 days.

The feed conversion of the birds was not significantly (P>0.05) affected by the transport and dietary supplements provided in trial 2 (Table 3.13). Birds had similar feed conversion within each period. Birds during days 56-69 had significantly (P≤0.05) poorer feed conversion than any other periods. Feed conversion was significantly (P≤0.05) higher during days 28-55 than days 14-27 and 1-14. Similar to these trials, MacIsaac and Anderson (2008) did not find any significant differences in feed conversion when supplementing dietary lysozyme to turkey poults.

Trial 2 days 56-70 had a slightly higher feed conversion than in trial 1, but was similar for all other age periods between trials. Similarly to both trials, Noy and Sklan (1999) found that birds fed Oasis®, liquid nutrients, or feed had similar feed efficiency to birds that remained un-supplemented. Similar results were found by Corless and Sell (1999) where feed efficiency of birds was not affected by withholding feed for up to 54 hours.

Table 3.12: Effect of Transport and Dietary Supplementation on the Feed Conversion of Heavy Hen Turkeys during Each Age Period (Trial 1)

Feed Conversion					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	1.32 ±0.056[†]	1.44 ±0.056	1.78 ±0.059	1.76 ±0.059	1.57 ±0.022
OL	1.31 ±0.057	1.46 ±0.057	1.64 ±0.057	1.90 ±0.057	1.58 ±0.022
NO	1.33 ±0.057	1.46 ±0.057	1.79 ±0.057	1.87 ±0.057	1.61 ±0.022
Supplement[y]					
NS	1.32 ±0.065	1.48 ±0.065	1.75 ±0.065	1.85 ±0.065	1.6 ±0.025
ANTI	1.30 ±0.065	1.53 ±0.065	1.72 ±0.065	1.76 ±0.065	1.58 ±0.025
LYS	1.34 ±0.069	1.42 ±0.069	1.68 ±0.072	1.82 ±0.072	1.57 ±0.027
AL	1.33 ±0.063	1.40 ±0.063	1.82 ±0.063	1.97 ±0.063	1.63 ±0.024
Age mean	*1.32c ±0.033*	*1.45b ±0.033*	*1.74a ±0.033*	*1.84a ±0.033*	

ANOVA	**P-value**
Transport (T)	0.39
Supplement (S)	0.31
Age (A)	<0.0001
T x S	0.53
T x A	0.54
S x A	0.55
T x S x A	0.39

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-c Means within the same row with different letters differ significantly ($P \leq 0.05$).

Table 3.13: Effect of Transport and Dietary Supplementation on the Feed Conversion of Heavy Hen Turkeys during Each Age Period (Trial 2)

Feed Conversion					
	Age (Days)				
Transport[z]	**0-13**	**14-27**	**28-55**	**56-69**	**Mean**
OAS	1.27 ±0.064[†]	1.22 ±0.064	1.84 ±0.064	2.45 ±0.064	1.70 ±0.031
OL	1.27 ±0.066	1.20 ±0.069	1.81 ±0.064	2.44 ±0.064	1.68 ±0.032
NO	1.28 ±0.064	1.22 ±0.064	1.80 ±0.064	2.42 ±0.064	1.68 ±0.031
Supplement[y]					
NS	1.28 ±0.074	1.17 ±0.078	1.84 ±0.074	2.45 ±0.074	1.69 ±0.036
ANTI	1.26 ±0.078	1.21 ±0.078	1.80 ±0.074	2.33 ±0.074	1.66 ±0.037
LYS	1.26 ±0.074	1.24 ±0.074	1.82 ±0.074	2.57 ±0.074	1.72 ±0.036
AL	1.29 ±0.074	1.21 ±0.074	1.80 ±0.074	2.43 ±0.074	1.68 ±0.036
Age mean	*1.27c* ±0.037	*1.21c* ±0.038	*1.82b* ±0.037	*2.45a* ±0.037	

ANOVA	P-value
Transport (T)	0.85
Supplement (S)	0.66
Age (A)	<0.0001
T x S	0.12
T x A	0.10
S x A	0.89
T x S x A	0.25

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error
a-c Means within the same row with different letters differ significantly ($P \leq 0.05$).

Similarly to both trial 1 and trial 2, MacIsaac and Anderson (2008) found no significant differences in feed conversion when supplementing with BMD and lysozyme independently or in combination. During the grower 2 period, MacIsaac and Anderson (2008) found similar feed conversion (1.67) for poults supplemented with lysozyme to those birds supplemented with lysozyme in trial 1, 1.66. Trial 2 showed similar feed conversion 1.27 in the starter (day 0-13) and 1.21 in the grower 1 (days 14-27) as those reported by MacIsaac and Anderson (2008), 1.26 in the starter and 1.24 in the grower 1. In contrast, Sims *et al.* (2004) found that tom poults had a significantly better feed

conversion when supplemented with mannan oligosaccharide or BMD at 105 days, compared to the control birds. This difference was not noticeable at 42 days where the birds had a similar feed conversion regardless of supplement. At day 42 the feed conversion ranged between 1.52 and 1.62 which appears slightly better than the feed conversions found in both trial 1 (1.74) and trial 2 (1.82) during the grower 2 period (days 29-56). Comparison between current study and Sims *et al.* (2004) revealed that the current study had similar diet compositions at day 42, both used Hybrid poults, but Sims *et al.* (2004) used toms poults where hens were used in the current study which possibly affects feed conversion differences.

3.4.3 Percent Mortality

Percent mortality was not significantly ($P>0.05$) affected by the transport supplements provided (Table 3.14). All birds displayed similar mortality rates within the transport supplements with an average of 0.7%. In trial 2, there were six mortalities during transport. These mortalities included 2 birds receiving OAS and 4 birds that received NO. There were no transport mortalities for birds consuming OL during transport. The post transport supplements had a significant ($P\leq0.05$) difference on mortality rates, with birds receiving ANTI having higher mortality rates than birds fed AL or NS. Although the differences are significant from the post mortem exams it does not appear that the mortality increase is treatment related. Mortality rates decreased as the birds got older ($P\leq0.05$). The starter period had the highest mortality, which was expected. During grower 1, mortality was higher than the grower 2 and finisher periods. During trial 1, two birds (OAS/NS (264g at 29d) and OAS/ANTI (501g at 33d) were culled due to body weights being 50% lower than the average.

Percent mortality during trial 2 was not significantly affected by the transport and dietary supplement, with an average mortality of 0.5% (Table 3.15). There was a difference among the ages of the birds. The starter period showed the highest mortality at 1.4%. The grower 1 period was significantly higher than the grower 2 and finisher period with mortality of 0.6%. The grower 2 and finisher periods were not significantly different from one another. During trial 2 four birds were culled at day 28 as their body weight was 50% or less than the average. These birds ranged in weight from 263-469g.

Table 3.14: Effect of Transport Supplement, Dietary Supplement, and Age on the Percent Mortality of Heavy Hen Turkeys (Trial 1)

Transport	**Percent Mortality**	**Mean**	**P-value**
Oasis®[z]	0.3 ±0.27[†]		
Oasis®[z] & Lysozyme[y]	1.2 ±0.27	0.7	0.14
No supplement	0.6 ±0.27		
Supplement			
Antibiotic[x]	1.4a ±0.31		
Antibiotic[x]& Lysozyme[y]	0.2b ±0.30	0.7	0.03
Lysozyme[y]	0.9ab ±0.33		
No supplement	0.3b ±0.31		
Age Period			
Starter	1.7a ±0.32		
Grower 1	1.0b ±0.32	0.7	<0.0001
Grower 2	>0.01c ±0.32		
Finisher	>0.01c ±0.32		

[z] Oasis® - Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA.
[y] Lysozyme - Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada.
[x] Antibiotic - BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada.
[†] Mean ± Standard Error
a-c Means with different letters within the same section differ significantly ($P \leq 0.05$).

Table 3.15: Effect of Transport Supplement, Dietary Supplement, and Age on the Percent Mortality of Heavy Hen Turkeys (Trial 2)

Transport	Percent Mortality	Mean	P-value
Oasis®[z]	0.4 ±0.26[†]		
Oasis®[z] & Lysozyme[y]	0.5 ±0.26	0.5	0.73
No supplement	0.7 ±0.26		
Supplement			
Antibiotic[x]	0.4 ±0.30		
Antibiotic[x]& Lysozyme[y]	0.7 ±0.30	0.5	0.86
Lysozyme[y]	0.4 ±0.30		
No supplement	0.7 ±0.30		
Age Period			
Starter	1.4a ±0.31		
Grower 1	0.7b ±0.31	0.5	0.004
Grower 2	>0.01c ±0.31		
Finisher	>0.01c ±0.31		

[z] Oasis® - Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA.
[y] Lysozyme - Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada.
[x] Antibiotic - BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada.
[†] Mean ± Standard Error
a-c Ls means with different letters within the same section differ significantly ($P \leq 0.05$).

Similar to both current trials, Noy and Sklan (1999) found no difference in mortality between treatments when male poults were supplemented and held for 48 hours, or held for 48 hours without supplementation. Noy and Sklan (1999) reported poult mortality below 2% for their 3 experiments which is similar to the mortality observed within both current trials. Sims *et al.* (2004) found low mortality rates (2.3%) during the first 12 weeks of their trial with no differences among treatment groups. Jackson (2005) found a significant difference between mortality during the first 7 days, with a pooled mortality of 8% for non-fed, and a lower 5.2% for prefed. This pooled mortality consisted of 3 experiments, in the first there was no significant difference between prefed and un-supplemented on mortality rates, but in the second and third

experiments there was a drop in mortality rates when birds were supplemented. MacIsaac and Anderson (2008) found different results presenting no significant effect when supplementing BMD and lysozyme independently or in combination in their first trial, but in their second trial when poults arrived at a lower initial body weight, supplementation of dietary BMD and lysozyme significantly improved survivability of poults during the first 14 days over birds that received no supplementation.

3.4.4.Weight Loss from Hatchery to Placement

Percent weight loss (trial 2) did not differ (P>0.05) among the transport treatments (Table 3.16). Birds showed similar percent weight loss with a mean loss of 8.7%.

Table 3.16: Effect of Transport Supplementation on the Weight Loss (%) From Hatchery to Placement (Trial 2)

	Weight Loss (%)	Mean
Transport		
Oasis®[z]	9.4 ± 0.70[†]	
Oasis®[z]& Lysozyme[y]	9.2 ± 0.70	8.7
No supplement	7.6 ± 0.70	
P-value	0.15	

[z] Oasis® - Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA.
[y] Lysozyme – Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada.
[x] Antibiotic - BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada.
[†] Mean ± Standard Error

Regardless of supplement provided, all birds lost weight during the transport period. Jackson (2005) found that all birds lost weight, but in both trials birds that were un-supplemented during 24 hour transport had more weight loss (5.85%, 6.90%) than birds that were prefed (3.95%, 5.50%). These weight losses are lower than those found in

this trial, perhaps due to a higher feeding rate of Oasis®. Birds in this trial received 2.5g/bird, while birds in Jackson (2005) were prefed 5.0g/bird. The birds used by Jackson (2005) were toms; the difference in sex could have an effect on ability to retain weight while transported. Boersma *et al*. (2003) found that prefeeding broiler chicks Oasis® did not prevent weight loss after hatch for all treatments and hypothesized that Oasis® stimulates the gastrointestinal tract which results in excretion. Contrasting this study, Noy and Sklan (1999b) reported that control birds lost weight during a 48h hold, whereas birds placed immediately post hatch on feed increased body weight. Also yolk weight decreased exponentially and the decrease was greater in birds that were fed immediately (Noy and Sklan, 1999b). Fat and protein content of the yolk was also decreased and fed chicks utilized fat and protein more quickly than birds that were held (Noy and Sklan, 1999b). There is no clear explanation for the transport weight loss in this study since all of the supplements provided (OAS and OL) were consumed and day 0 gut analysis in trial 2 (Appendix R, Appendix S and Appendix T) indicated that birds had eaten the supplements.

Carver *et al.* (2002) found differences relating to poult mortality involving many factors. Carver *et al*. (2002) reported that season of shipping and strain had effect on mortality, with hen flocks from strain A having lower mortality at days 7 and 14 when shipped in the winter than those shipped in the summer. Strain B reported lower 7 day mortality in the winter, but equivalent mortality at 14 days for both seasons. Breeder age has been associated with poult mortality, Carver *et al.* (2002) found that mid-cycle breeder hens had the lowest odds of mortality, whereas young hens (first 3 weeks of lay) produced smaller eggs and had a twofold increase in odds of mortality. Fasina and

Thenissery (2011) found no difference in mortality rates when chicks from old and young breeder hens were evaluated. Temperature of shipping has an effect on mortality, Carver *et al*. (2002) found that hens shipped in trucks ranging from 22.2-32.2°C had different mortality, but that shipping time did not increase incidence of mortality. Hens shipped on the lower end of this range showed a higher incidence of mortality at days 7 and 14. This effect was not observed in tom poults, where 14 day mortality rates were improved by lower temperatures. This may indicate that tom poults may be less sensitive to colder trucking temperatures (Carver *et al.,* 2002). Toe trimming was performed on hen poults by Carver *et al*. (2002) and did not affect incidence of mortality. All of these factors that have an influence on poult mortality rates may also affect the bird's ability to retain weight during shipping, resulting in the differences between the current trials, and Jackson (2005).

3.5 Growth Performance Conclusions

Although in other studies supplementing Oasis® to birds during transport has shown improvement in growth performance parameters (Noy and Sklan, 1999; Boersma *et al.,* 2003; Batal and Parsons, 2002), supplementation of Oasis® and lysozyme during 24 hours of transport in this study did not result in consistent differences in growth performance. Trial 1 resulted in no growth performance improvement with transport supplementation. In trial 2 there was no effect on body weight gain, feed consumption, or feed conversion for birds supplemented with Oasis® and lysozyme, but body weight was affected by the combination of dietary supplementation of birds at days 55 and 69. The mortality and weight lost during 24 hour transport of poults was not affected by supplementation during transport. In future studies an increased amount of supplement

during transport or longer transport time is recommended. Jackson (2005) found improvements when feeding 5.0g/bird of Oasis® and all Oasis® provided in the transport boxes during these trials was consumed, indicating that if there was more available the birds may have consumed more and results may have been changed. Also longer transport times may allow for more significant differences between the control birds and the supplemented birds. Transport holds longer than 24 hours have shown significant effects in other studies (Corless and Sell, 1999; Pinchasov and Noy, 1993) but studies including enzymes such as lysozyme have not been explored.

Dietary supplementation of turkey poults with lysozyme in a preliminary study indicated its usefulness alone and in addition, with antibiotics (MacIsaac and Anderson, 2008). Similarly this study found increased body weight and feed consumption of birds fed a combination of lysozyme and BMD during trial 1. Possibly a synergistic response in birds was present even after the lysozyme has been removed at day 28. This possibility was not examined in previous research. Birds had similar body weight gain, feed conversion, and mortality regardless of dietary supplementation. More definitive studies into the residual effect of lysozyme supplementation in the diet, as well as the possible synergistic response present are needed to determine the effectiveness of lysozyme on growth performance parameters in poults.

Chapter 4: The Effect of Oasis® and Lysozyme during Transport and Dietary Lysozyme Supplementation Post-transport on Gastrointestinal Tract Weights, Measures and Morphology of Turkey Poults

4.1 Objectives

To determine the effect of supplementing Oasis® and lysozyme during long transport and lysozyme after transport on the gastrointestinal tract weights (proventriculus, gizzard, duodenum, jejunum and ileum) and lengths (duodenum, jejunum and ileum) of newly hatched turkey poults.

To determine the effect of supplementing Oasis® and lysozyme during long transport and lysozyme after transport on the gastrointestinal tract morphology (mucosal width, villi height, crypt depth, midwidth and villi surface area) of newly hatched turkey poults

4.2. Hypotheses

Birds supplemented with Oasis® and lysozyme during long transport are hypothesized to have decreased intestinal weights and changes in intestinal length. The addition of Oasis® or the combination of Oasis® and lysozyme is expected to increase surface area of the intestines, decrease gut microflora and reduce competition for nutrients resulting in decreased gastrointestinal tract weights and increased villi height, thinner mucosal widths, deeper crypts and increased villi area. The addition of lysozyme to the diet after transport is hypothesized to perform equal to a sub-therapeutic addition of antibiotics to the feed, resulting in a possible decrease in intestinal weights due to the diminished influence of pathogenic bacteria. The intestinal morphology of birds provided

with lysozyme after transport is hypothesized to show improvement as potential pathogenic bacteria would not impede the growth and proliferation of the villi. This difference would be seen by a thinner intestinal mucosa as well as increased villi characteristics such as height, crypt depth and area.

4.3 Materials and Methods

4.3.1 Experimental Design and Conditions

The sampling of the intestinal tract of the birds was in association with the diets and housing conditions described in Chapter 3. Sampling occurred in both trials and differences between trials were noted as they occurred.

4.3.2. Data Collection

4.3.2.1 Intestinal Sampling

At days 14, 28, 56 and 70, three birds from each pen were randomly selected and euthanized by cervical dislocation. Each bird was weighed and the proventriculus, gizzard, ileum, jejunum, and duodenum removed following the sampling procedure in figure 4.1. Procedure outline, notes and sampling image were created by Jennifer Dobson based on modifications from Klasing (1998). These sections were weighed using a top pan balance (Mettler Toledo, Thermo Fisher Scientific Inc.) and the length of the ileum, jejunum and duodenum measured using a standard metric ruler. From two of these birds representative samples of the duodenum, jejunum, and ileum were taken (figure 4.1) and stored in scintillation vials with 10% buffered neutral formalin for subsequent histological analysis.

1. Remove proventriculus, cut open and clean then weigh.
2. Remove gizzard, cut open and clean then weigh.

Duodenum

3. Cut the intestine parallel with the end of the pancreas to remove the duodenum.
4. Make a slice through the intestine just after the loop of the duodenum.
5. Remove the pancreas….CAREFUL not to switch the ends of the duodenum!
6. Weigh the duodenum… CAREFUL not to switch the ends of the duodenum!
7. Measure the total length of the duodenum (will be in two sections)
8. Cut a 1cm sample section of the duodenum from the slice after the loop. Put sample 1 in formalin.

Ileum

9. Find the end of the digestive tract and cut the intestine just above the ileum/cecal junction (below where the open ends of the ceca are attached).
10. Cut the intestine above where the blind pouch ends of the ceca are attached.
11. Remove the ceca from both sides.
12. Weigh the ileum and measure the length.
13. Cut a 1cm sample section from the intestine just above where the open ends of the ceca are attached. Put sample 2 in formalin.

Jejunum

14. Remove the rest of the remaining intestine tract, this is the jejunum.
15. Remove the mesentery, weigh the jejunum and measure the length.
16. Measure 15cm from the end of the jejunum that was closest to the duodenum.
17. Take a 1cm sample from the intestine at this point. Put sample 3 in formalin.

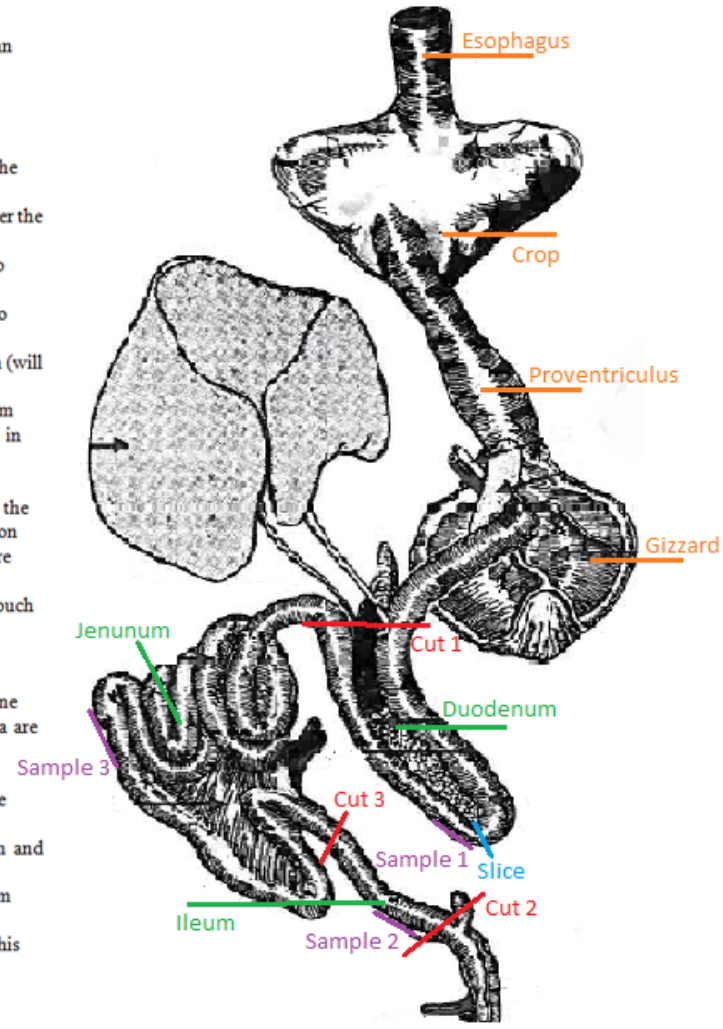

Figure 4.1: Dissection Protocol Prepared by Jennifer Dobson, modified from Klasing (1998) for Intestinal Sampling of Birds during Trial 1 and 2

At days 28 and 70 the intestinal breaking strength of the jejunum was measured using the TA.TX texture analyser (TA) (Texture Technologies Corp. and Stable Micro Systems Ltd., Scarsdale NY). Two jejunums from the dissected birds were taken after being measured and stretched to the point of breaking by the TA. The TA was programmed with a 5.0kg load cell and the clamp probe attachments (Figure 4.2). It also had a 1.0g trigger force and was set at a test speed of 1.0mm/sec. Although, anterior and

posterior ends of the jejunum were not identified prior to being placed in the clamps the measurement was taken in the middle of the jejunum section. The jejunum section from each sample bird was attached at the top and bottom in the clamps so that it was not already being stretched.

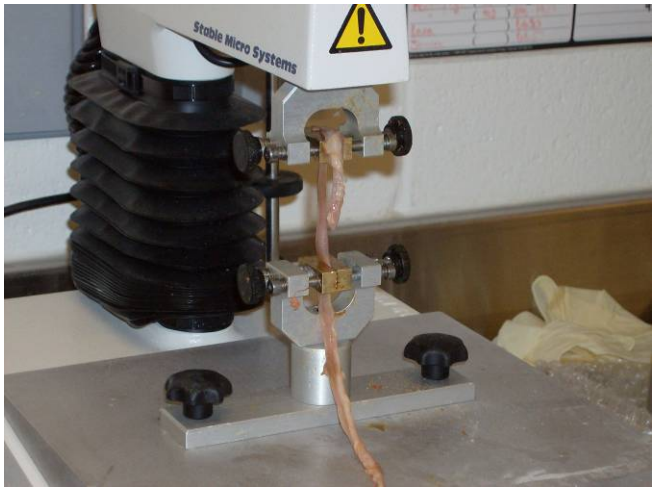

Figure 4.2: TA.TX texture analyzer with clamp probe attachments used for jejunum breaking strength.

4.3.2.2 Histology Preparation

The stored samples of ileum, jejunum, and duodenum underwent the paraffin wax tissue processing method (Drury and Wallington, 1980). Tissues were removed from the 10% buffered neutral formalin and then each sample was divided into up to 3 sections. If the sample was of small or had poor tissue integrity then fewer divisions were made. The samples were then placed in plastic cassettes (Figure 4.3).

Figure 4.3: Plastic labeled tissue cassettes with tissue samples

The cassettes were then placed in the Tissue-Tek® VIP™ (Sakura Finetek USA Inc., Torrance CA) to dehydrate in a graded series of alcohols in increasing concentration (70-100%) and infiltrated with xylene and paraffin wax in the sequence shown in figure 4.4.

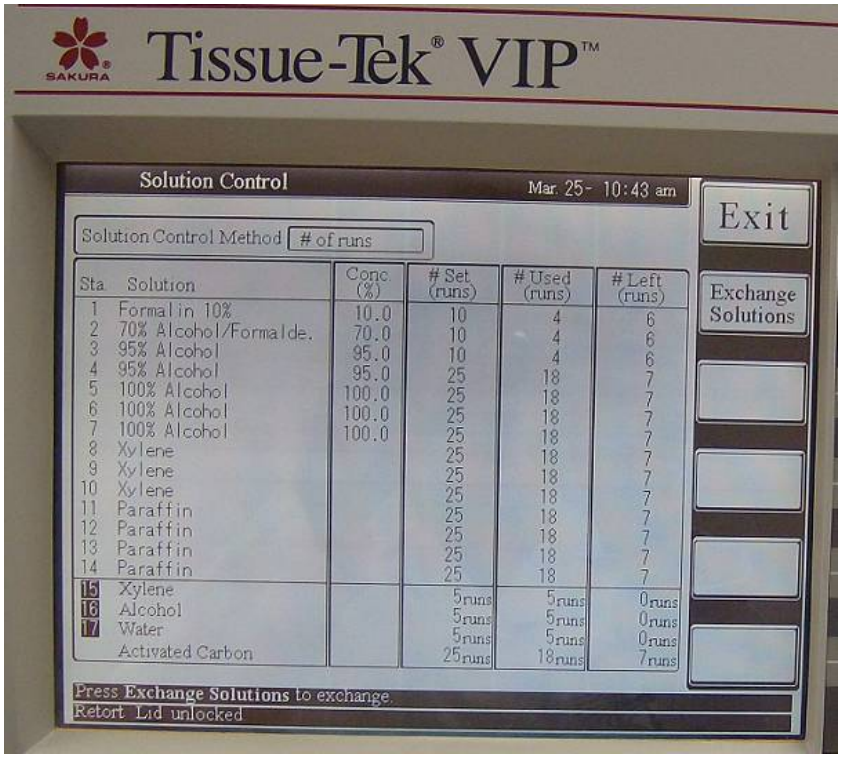

Figure 4.4: The sequence of infiltration of alcohol, xylene and paraffin into the intestinal tissues

After this process the samples were arranged and then imbedded in paraffin wax using a Tissue-Tek® TEC™ (Sakura Finetek USA Inc., Torrance CA). A microtome (Leica RM2255, Nussloch Germany) was used to cut a 0.5µm section which was then placed in a 35.5ºC water bath to be placed on a slide (figure 4.5).

Figure 4.5: Microtome (right) and water bath (left) used to cut and place intestinal sections on slides.

This section was then mounted on a slide and stained using haematoxylin and eosin staining using the procedure of Drury and Wallington (1980) and the Tissue-Tek® DRS™ (Sakura Finetek USA Inc., Torrance CA) (Figure 4.6).

Figure 4.6: Tissue-Tek® DRS™ used for haematoxylin and eosin staining intestinal slides.

After the staining process the slides were covered using an automated coverslipper (Thermoscientific Clearvue, Waltham MA) and were then ready for imaging. All slides were prepared by Joan Stiles at the Hancock Veterinary Building (Truro, NS).

4.3.3 Measurements and Calculations

After collection, the weights of the gizzard, proventriculus, duodenum, jejunum and ileum were expressed as a percentage of the bird weight at the time of dissection. These have been designated "relative" due to the relationship to bird body weight at the time of dissection. The lengths of the duodenum, jejunum and ileum were recorded in

centimeters. For intestinal weights and measures the average of the three sampled birds was used as a per pen average for statistical analysis during each sampling period.

After slide preparation, slides were scanned using a Nikon Super Coolscan 4000 ED (Nikon Inc., Japan) and images captured by Nikon Scan 4.0.2 (Nikon Inc., Japan). Measurements were made on all cross-sections using the SigmaScan Pro 5 (SPSS Inc., Chicago, IL). The imaging software was calibrated using a 1.00mm calibration slide which was also scanned into the computer using the Nikon Super Coolscan (Nikon Inc., Japan). Mucosal width was measured (μm) from the muscularlaris mucosae to the exterior edge of the mucosa (Figure 4.7). The mucosal width was measured on the duodenum, jejunum, and ileum.

For the next measurements only the ileum of each bird was used. The villus height was measured from the top of the villus to the top of the crypt (Figure 4.7). Crypt depth was measured from the top of the crypt to the muscularis mucosae (Figure 4.7). The midwidth was a measure of the villus at the midpoint (Figure 4.7). The area of the villus was measured by SigmaScan Pro 5 (SPSS Inc., Chicago, IL) as the sum of the calibrated pixel units in a defined region. For each cross-section up to ten measurements were taken for each parameter.If the cross-section displayed poor integrity of the villi then a minimum of 6 measurements were used to consider the slide readable. To quantify gut damage, or villi that were folded over and unreadable, intestinal cross sections were scored for readability. Parameters were based on those from Budgell (2008) with adjustments to the percentages and an additional section added to show those with complete unreadability of the slide (Table 4.1).

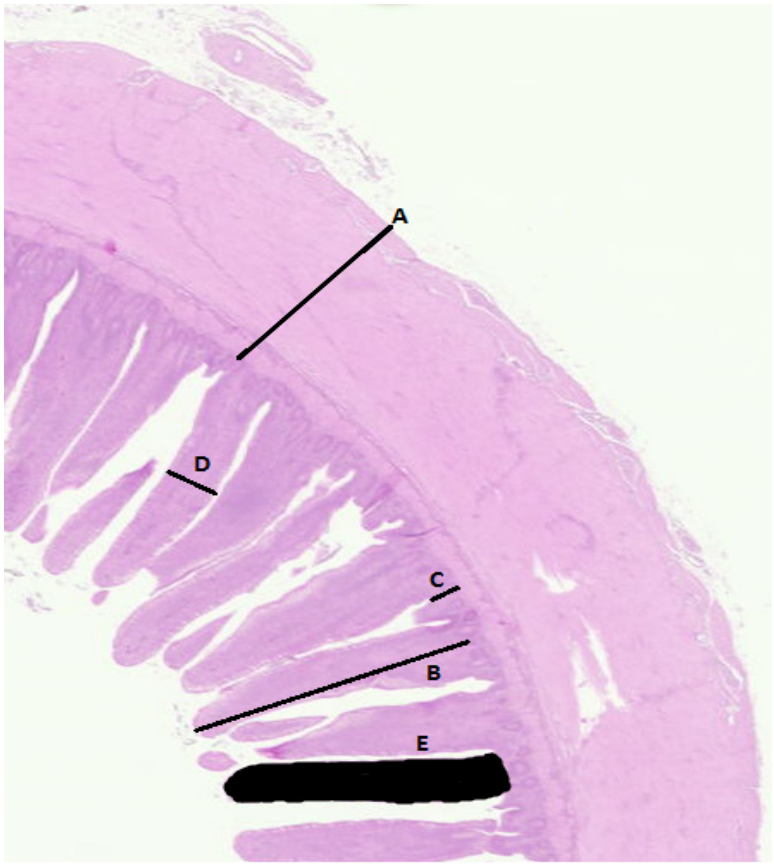

Figure 4.7: Histology Measurements of the Cross-Section A: mucosal width, B: villus height, C: crypt depth, D: villus midwidth, E: villus area

Table 4.1: Scoring of turkey poult intestinal cross-sections for readability

Villus Defects	Score
Few villi unreadable in cross section (0-25%)	1
Several villi unreadable in cross section (26-50%)	2
Numerous villi unreadable in cross section (51-75%)	3
Severe unreadability in cross section (>75%)	4
Cross section completely unreadable (100%)	5

4.3.4 Statistical Analysis

Intestinal measures and morphology data was subjected to analysis of variance (ANOVA) using the Proc Mixed procedure of SAS (Littell *et al.*, 1996). The moc

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_{k+} \alpha\gamma_{ik} + \varepsilon_{ijkl}$$

Where Y_{ijkl}= Response, μ= Population Mean, α= Factor 1 or transport supplement, i=Levels of factor 1 (O, OL, NO), β= blocking factor or Room, j= levels of blocking factor (151, 152, 153, 156),γ=factor 2 or post transport supplement, k=Levels of factor 2 (NS, ANTI, LYS, AL), ε = 1, 2, 3…Error Effect, l= number of replicates (12).

If significant main effects or interactions were found in the ANOVA, the Tukey's option was used to compare differences among the least-square means ($P \leq 0.05$).

4.4 Results and Discussion

4.4.1 Intestinal Weights and Measures

The relative gizzard weight was not affected ($P > 0.05$) by the transport supplements used in Trial 1 or 2 (Table 4.2). During day 28 there was significant difference ($P \leq 0.05$) in gizzard weight with consumption of different supplements (Table 4.2). ANTI produced a significantly higher gizzard weight at 3.2% of body weight than the other supplements which had relative gizzard weights between 2.8 and 2.9% of body weight. On days 56 and 70 birds had similar ($P > 0.05$) gizzard weights regardless of dietary supplement. In trial 2 the relative gizzard weight was not affected ($P > 0.05$) by the dietary supplements used at any sampling point.

Sell *et al.* (1991) found that the proportion of body weight constituted by the gizzard was only slightly increased after 4 days of age; while Bennett *et al.* (2002) had 4/6 treatments

result in decreased gizzard weight from day 18 to day 32. This is similar to the decrease in gizzard weight over time reported in both trial 1 and trial 2 of the current study. Rauber *et al.* (2007) found birds with a lower gizzard percentage than birds in this study with values of 2.32% at day 21 and 1.61% in 42 day old turkey poults. Bennett et al. (2002) found gizzard weights of 3.03% at day 18 and 2.60% at day 32, these weights are again lower than those found in the current study. Bennett *et al.* (2002) found no differences among treatments. In contrast to this study, Corless and Sell (1999) found that birds held for 30 hours had decreased relative gizzard weights at 2 days of age, while birds held for 54 hours without feed showed decreased gizzard weights from 2 to 4 days of age (Corless and Sell, 1999). Contrasting Corless and Sell (1999), Pinchasov and Noy (1993) found that birds held for 24 or 48 hours had increased relative gizzard weights compared to those not held prior to placement. Rougière *et al.* (2012) found a positive correlation between the motility of the gizzard and the weight of the stomach (gizzard and proventriculus) in 2 lines of chickens selected for either high digestion efficiency or low digestion efficiency.

Duke and Evanson (1976) found that the motility of the gizzard was affected by a 2 day fast. The frequency and amplitude of gizzard contractions were decreased and diurnal cycles were much less obvious than in birds that did not experience a fast. Successive days of fasting further decrease gizzard motility (Duke and Evanson, 1976). In contrast, Fontana *et al.* (1993) found that broilers subjected to a 4 or 7 day feed restriction beginning at 4 days of age did not have different gizzard weights at 28 days for a cage trial and 49 days of age for a floor reared trial. Differences were not found when

Table 4.2: Effect of Transport and Dietary Supplement on the Relative Gizzard Weight of Heavy Hen Turkeys

Relative Gizzard Weight (g/100g body weight)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	3.4 ±0.09[†]	3.1 ±0.08	2.2 ±0.06	1.9±0.06
OL	3.4 ±0.10	2.9 ±0.08	2.2 ±0.06	2.0±0.06
NO	3.5 ±0.09	2.9 ±0.08	2.3 ±0.06	1.9±0.06
P-value	*0.85*	*0.23*	*0.72*	*0.99*
Supplement[y]				
ANTI	3.4 ±0.11	3.2a ±0.10	2.4 ±0.07	2.0±0.07
AL	3.5 ±0.10	2.8b ±0.09	2.2 ±0.07	1.8±0.07
LYS	3.5 ±0.11	2.8b ±0.09	2.2 ±0.08	2.0±0.08
NS	3.4 ±0.11	2.9b ±0.09	2.1 ±0.07	1.9±0.06
P-value	*0.72*	*0.02*	*0.15*	*0.26*
Mean	***3.4***	***3.0***	***2.2***	***1.9***
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	3.9 ±0.10	4.0 ±0.21	2.5 ±0.06	2.1 ±0.07
OL	4.1 ±0.10	3.4 ±0.21	2.6 ±0.06	2.2 ±0.07
NO	4.1 ±0.10	3.7 ±0.21	2.7 ±0.06	2.2 ±0.07
P-value	*0.26*	*0.14*	*0.24*	*0.43*
Supplement				
ANTI	3.9 ±0.11	3.3 ±0.24	2.7 ±0.07	2.1 ±0.08
AL	4.0 ±0.11	4.0 ±0.24	2.6 ±0.07	2.1 ±0.08
LYS	4.1 ±0.11	3.8 ±0.24	2.6 ±0.07	2.1 ±0.08
NS	4.1 ±0.11	3.6 ±0.24	2.6 ±0.07	2.2 ±0.08
P-value	*0.58*	*0.26*	*0.79*	*0.82*
Mean	***4.0***	***3.6***	***2.6***	***2.1***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same column and section with different letters differ significantly ($P \leq 0.05$).

another factor was added and some birds received a reduced calorie diet for the duration of the experiment (Fontana *et al.,* 1993). Reduced gizzard weights were not found in birds fed or unfed during transport in this study. This would imply that the gizzard

motility was not affected by transport or supplements provided. It is possible that the gizzard motility and subsequent gizzard weights were different from Duke and Evanson's (1976) findings due to the age and size of the birds during the fast. Birds during this trial were newly hatched and had not yet received exogenous food, whereas birds used by Duke and Evanson (1976) were 8-12 weeks of age when fasting occurred. Although there is previous work on the effects of fasting birds and their resulting gizzard weights, the effect of BMD on the gizzard weight found in trial 1 day 28, has not been reported in previous research. This response is not reported at days 56 and 70 of the trial, and was not repeated in trial 2.

The relative proventriculus weight was similar ($P>0.05$) for all of the transport supplements in trial 1 and 2 (Table 4.3). The dietary supplements showed no effect ($P>0.05$) at day 14 where the proventriculus weights were 0.5% of the birds body weight. Similarly to relative gizzard weight, on day 28 ANTI fed birds had significantly higher ($P\leq0.05$) proventriculus weight (0.41%) compared to other treatments having relative proventriculus weights of 0.37-0.38%. During days 56 and 70 there was no effect on the dietary supplements with the proventriculus weight. Relative proventriculus weight was 0.2% of the birds body weight on both days. In trial 2 the relative proventriculus weight was not affected ($P>0.05$) by the dietary supplements used (Table 4.3).

There is little research on the growth of the proventriculus when birds are held and subjected to long transport with or without supplementation. Bennett *et al.* (2002) fed whole barley and grit to turkey toms and found relative proventriculus weights, to be 0.55% at day 18. This is similar to the 0.6% found on day 14 of trial 2. Bennett *et al.* (2002) found that the proventriculus weight was 0.37% at day 32, which is similar to the

Table 4.3: Effect of Transport and Dietary Supplement on the Relative Proventriculus Weight of Heavy Hen Turkeys

Relative Proventriculus Weight (g/100g body weight)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	0.5 ±0.01[†]	0.4 ±0.01	0.3 ±0.01	0.2 ±<0.01
OL	0.5 ±0.01	0.4 ±0.01	0.2 ±0.01	0.2 ±<0.01
NO	0.5 ±0.01	0.4 ±0.01	0.3 ±0.01	0.2 ±<0.01
P-value	*0.97*	*0.40*	*0.35*	*0.25*
Supplement[y]				
ANTI	0.5 ±0.01	0.41a ±0.01	0.3 ±0.01	0.2 ±<0.01
AL	0.5 ±0.01	0.37b ±0.01	0.3 ±0.01	0.2 ±<0.01
LYS	0.5 ±0.01	0.37b ±0.01	0.3 ±0.01	0.2 ±<0.01
NS	0.5 ±0.01	0.38b ±0.01	0.3 ±0.01	0.2 ±<0.01
P-value	*0.75*	*0.001*	*0.08*	*0.08*
Mean	***0.5***	***0.4***	***0.2***	***0.2***
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	0.6 ±0.01	0.5±0.02	0.3 ±<0.01	0.2 ±0.01
OL	0.6 ±0.01	0.4±0.02	0.3 ±<0.01	0.2 ±0.01
NO	0.6 ±0.01	0.4±0.02	0.3 ±<0.01	0.2 ±0.01
P-value	*0.06*	*0.31*	*0.59*	*0.27*
Supplement				
ANTI	0.6 ±0.01	0.4±0.03	0.3 ±0.01	0.2 ±0.01
AL	0.6 ±0.01	0.5±0.03	0.3 ±0.01	0.2 ±0.01
LYS	0.6 ±0.01	0.4±0.03	0.3 ±0.01	0.2 ±0.01
NS	0.6 ±0.01	0.4±0.03	0.3 ±0.01	0.2 ±0.01
P-value	*0.84*	*0.40*	*0.49*	*0.62*
Mean	***0.6***	***0.4***	***0.2***	***0.2***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same column and section with different letters differ significantly ($P \leq 0.05$).

proventriculus weights around day 28 in both trial 1 and trial 2. Sell *et al.* (1991) analyzed the developmental patterns of the gastrointestinal tract of turkeys and found that the proventriculus increased in relative weight from the time of hatch until day 8, where

the relative weight reached ~1.2%. This may indicate that the growth of the proventriculus displays significant changes during the early growth period that may not be evident by 14 days of age. The effect of BMD increasing the proventriculus weight at day 28 appears unclear; birds had a significantly heavier stomach (gizzard and proventriculus) than those receiving any other diet.

In both trial 1 and trial 2 the relative duodenum and weight of birds was similar ($P>0.05$) regardless of transport or dietary supplement (Table 4.4). Similarly, the relative jejunum weight was not affected ($P>0.05$) by transport or dietary supplement provided in either trial (Table 4.5). The ileum weight of the birds was similar ($P>0.05$) regardless of transport or dietary supplement in trial 1 (Table 4.6). In trial 2 the ileum as a percentage body weight was not affected ($P>0.05$) by the transport supplements, but there was a significant ($P\leq0.05$) difference in the dietary supplements. At day 56 birds fed ANTI showed a higher relative ileum weight (0.19%) than birds provided AL or NS. Both had relative ileum weights of 0.17%. Birds fed LYS had a relative ileum weight of 0.18% at 56 days, which was similar to all other treatments.

Intestinal weight of turkey poults has been significantly studied in very early growth of the poult, with little information available for intestinal weights as the birds get closer to market weight. Pinchasov and Noy (1993) found an increase in duodenum weight at 14 days when turkey poults were withheld feed for up to 48 hours. Uni *et al*. (1999) found a change in intestinal weights up to fifteen days when the post hatch

Table 4.4: Effect of Transport and Dietary Supplement on the Duodenum Weight of Heavy Hen Turkeys

Relative Duodenum Weight (g/100g body weight)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	1.3 ±0.03[†]	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
OL	1.3 ±0.03	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
NO	1.3 ±0.03	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
P-value	*0.21*	*0.23*	*0.72*	*0.08*
Supplement[y]				
ANTI	1.3 ±0.03	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
AL	1.3 ±0.03	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
LYS	1.3 ±0.03	0.9 ±0.02	0.5 ±0.02	0.4 ±0.01
NS	1.3 ±0.03	0.9 ±0.02	0.5 ±0.01	0.4 ±0.01
P-value	*0.78*	*0.84*	*0.47*	*0.58*
Mean	***1.3***	***0.9***	***0.5***	***0.4***
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	1.4 ±0.06	1.1 ±0.06	0.5 ±0.01	0.4 ±0.01
OL	1.4 ±0.06	0.9 ±0.06	0.5 ±0.01	0.4 ±0.01
NO	1.4 ±0.06	1.0 ±0.06	0.5 ±0.01	0.3 ±0.01
P-value	*0.82*	*0.17*	*0.58*	*0.44*
Supplement				
ANTI	1.4 ±0.07	0.9 ±0.07	0.5 ±0.01	0.4 ±0.01
AL	1.3 ±0.07	1.1 ±0.07	0.5 ±0.01	0.3 ±0.01
LYS	1.5 ±0.07	1.0 ±0.07	0.5 ±0.01	0.4 ±0.01
NS	1.3 ±0.07	0.9 ±0.07	0.5 ±0.01	0.4 ±0.01
P-value	*0.17*	*0.37*	*0.76*	*0.68*
Mean	***1.4***	***1.0***	***0.5***	***0.3***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

Table 4.5: Effect of Transport and Dietary Supplement on the Jejunum Weight of Heavy Hen Turkeys

Relative Jejunum Weight (g/100g body weight)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	4.0±0.08[†]	2.9 ±0.07	1.2 ±0.03	1.1 ±0.03
OL	4.0±0.08	2.7 ±0.07	1.3 ±0.03	1.0 ±0.03
NO	4.0±0.08	2.9 ±0.07	1.2 ±0.03	1.0 ±0.03
P-value	*0.99*	*0.08*	*0.29*	*0.29*
Supplement[y]				
ANTI	4.0±0.09	2.8 ±0.08	1.3 ±0.04	1.1 ±0.03
AL	4.0±0.09	3.0 ±0.07	1.2 ±0.04	1.0 ±0.03
LYS	3.9±0.10	2.7 ±0.08	1.2 ±0.04	1.0 ±0.03
NS	4.0±0.09	2.8 ±0.08	1.3 ±0.04	1.0 ±0.03
P-value	*0.60*	*0.15*	*0.19*	*0.13*
Mean	***4.0***	***2.8***	***1.2***	***1.0***
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	4.5 ±0.11	3.7 ±0.19	1.2 ±0.03	1.0 ±0.03
OL	4.5 ±0.11	3.3 ±0.19	1.2 ±0.03	1.0 ±0.03
NO	4.3 ±0.11	3.3 ±0.19	1.2 ±0.03	1.0 ±0.03
P-value	*0.42*	*0.31*	*0.69*	*0.39*
Supplement				
ANTI	4.4±0.12	3.3 ±0.22	1.2 ±0.03	1.0 ±0.03
AL	4.4±0.12	3.7 ±0.22	1.2 ±0.03	1.0 ±0.03
LYS	4.5±0.12	3.4 ±0.22	1.2 ±0.03	1.0 ±0.03
NS	4.5±0.12	3.3 ±0.22	1.1 ±0.04	1.0 ±0.03
P-value	*0.92*	*0.42*	*0.17*	*0.32*
Mean	***4.4***	***3.4***	***1.2***	***1.0***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

Table 4.6: Effect of Transport and Dietary Supplement on the Ileum Weight of Heavy Hen Turkeys

Ileum Weight (%BW)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	0.7 ±0.02[†]	0.4 ±0.01	0.2 ±0.02	0.2 ±<0.01
OL	0.6 ±0.02	0.4 ±0.01	0.2 ±0.02	0.1 ±<0.01
NO	0.7 ±0.02	0.5 ±0.01	0.2 ±0.02	0.2 ±<0.01
P-value	*0.90*	*0.20*	*0.26*	*0.62*
Supplement[y]				
ANTI	0.7 ±0.03	0.5 ±0.02	0.2 ±0.02	0.2 ±<0.01
AL	0.7 ±0.02	0.5 ±0.01	0.2 ±0.02	0.1 ±<0.01
LYS	0.6 ±0.03	0.4 ±0.02	0.2 ±0.03	0.1 ±<0.01
NS	0.6 ±0.02	0.4 ±0.02	0.2 ±0.02	0.1 ±<0.01
P-value	*0.92*	*0.06*	*0.46*	*0.24*
Mean	***0.6***	***0.4***	***0.2***	***0.1***
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	0.7 ±0.05	0.4 ±0.02	0.2 ±<0.01	0.1 ±<0.01
OL	0.7 ±0.05	0.4 ±0.02	0.2 ±<0.01	0.1 ±<0.01
NO	0.7 ±0.05	0.4 ±0.02	0.2 ±<0.01	0.1 ±<0.01
P-value	*0.89*	*0.49*	*0.80*	*0.70*
Supplement				
ANTI	0.7 ±0.05	0.4 ±0.03	0.19a ±<0.01	0.15 ±<0.01
AL	0.6 ±0.05	0.4 ±0.03	0.17b ±<0.01	0.15 ±<0.01
LYS	0.7 ±0.05	0.4 ±0.03	0.18ab ±<0.01	0.14 ±<0.01
NS	0.8 ±0.05	0.4 ±0.03	0.17b ±<0.01	0.14 ±<0.01
P-value	*0.30*	*0.68*	*0.001*	*0.03*
Mean	***0.7***	***0.4***	***0.2***	***0.1***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

development of poults was observed. The relative weight of the intestines increased to a maximum proportion of body weight at days 6-7, thereafter the relative weights decreased till the end of the trial at 15 days (Uni *et al.,* 1999). Similar to Uni *et al.* (1999),

Noy and Sklan (1998b) found that in the immediate post hatch period the intestines increase in weight more rapidly than body weight. And that the rapid growth of the gastrointestinal tract reaches a maximum between 6-8 days post hatch in the poult. The jejunum made up the largest proportion of the body weight, followed by the duodenum and ileum respectively (Uni *et al.,* 1999). This relationship was also reported in our trials.

The increase in weight is due to increased muscular and mucosal development in the jejunum (Uni *et al.,* 1999). Sell *et al.* (1991) found relative small intestine weights increased steeply from hatch to day 6. Corless and Sell (1999) found that birds held for 30 hours had small intestine weights for the first 2 days compared to birds that were placed within 6 hours of hatch. When birds were held for 54 hours a decrease in intestinal weights persisted up to 5 days of age. Weights of the intestinal tract increased within each placement group when the birds had access to feed and water. The duodenum and jejunum sections of birds held for 30 hours increased in weight by 57 and 73% within 24 hours of access to feed and water. When the hold was increased to 54 hours the increase was 135 and 100% respectively. A 30 hour hold caused decreased jejunual weights at 2 days of age in experiment one, whereas in experiment 2 reduction in relative weights in the duodenum at day 4 and the ileum at days 3 and 4. A 54 hour hold resulted in more severe and constant decreases, where in both experiments decreases occurred at days 3 and 4 in all sections of the small intestine. Corless and Sell (1999) found results suggestive of compensatory growth of the small intestine occurring to account for the hold of 30 or 54 hours. Similarly to the current study, Noy and Sklan (1999) found that relative weight of the small intestine was not affected by early feeding or a 48 hour hold. The small intestine of birds increased from hatch to 5-7 days. Birds

that were held had increases in small intestine weight, although more slowly than in fed birds. Jackson (2005) found the highest weight in the duodenum, jejunum and ileum at 2 days when turkey tom poults were supplemented with Oasis® fed concurrently with starter feed, compared to birds supplemented with Solka Floc® (non-nutritive cellulose fiber) concurrently with starter feed, normal starter diet, or a 24 hour fast. At 9 and 16 days post hatch birds fed the Oasis® treatment had similar duodenum, jejunum and ileum weights to those birds that were not supplemented or fed Solka Floc®. This previous research presented that differences in intestinal weights appear more often during the early growth phases, which may indicate that differences in intestinal weights due to transport or dietary supplementation may not remain by the time birds reach 14 days of age.

Bennett *et al.* (2002) reported decreasing relative weights of the duodenum, jejunum and ileum as the birds aged, this decrease was at a similar rate to that found in the current trial. Sampling was performed at different ages, but the decrease in relative weight was between 7-8% between 18 and 32 days of age for each intestinal segment (Bennett *et al.,* 2002). Similarly, in the current trial the decrease in relative intestinal weight between 14 and 28 days was at the rate of decrease was 7%.

Birds had increased ileum weights at day 56 when receiving ANTI over birds receiving AL or NS. This effect did not appear in trial 1 or in other sampling days during trial 2. This effect of BMD on the ileum weight does not affect growth performance of the birds as there was no effect of BMD on the growth performance parameters measured in this trial. The increased weight in birds supplemented with BMD is in contrast to findings by Apajalahti *et al.* (2004) and Miles *et al*., 2006 who found that

supplementation of broiler chicks with BMD (50g/tonne of feed) resulted in decreased intestinal weights, while increasing absorptive capacity and body weight.

The duodenum length of the poults was similar (P>0.05) on each sampling day regardless of the transport or dietary supplement provided. The duodenal length increased from 18.1- 32.5cm in trial 1 and 16.9 -32.8cm in trial 2 (Figure 4.8). The jejunum length of the poults was similar (P>0.05) on each sampling day regardless of the transport or dietary supplement provided. The jejunum length increased from 69.3-139.6cm trial 1, and 67.0-143.0cm in trial 2 (Figure 4.8). The ileum length of the poults was similar (P>0.05) on each sampling day regardless of the transport or dietary supplement provided. The ileum length increased from 14.4-27.8cm in trial1 and 13.2-27.0cm in trial 2 (Figure 4.8). Measurements of the duodenum, jejunum and ileum length in tabular form are reported in Appendix N, O and P respectively. There were no significant effects (P>0.05) due to treatment.

In both trials the most rapid increase in the jejunum length appears between days 28 and 56. This could be due to differences in day represented in the sampling intervals. The interval between sampling on days 28 and 56 was the largest, including 28 days instead of the 14 days between sampling on days 14 and 28 and also between days 56 and 70. It is expected that if the sampling interval was the same for the entirety of the trial than the increasing lengths would appear more linear (Figure 4.9). Uni *et al.* (1999) reported smaller jejunum lengths and higher ileum lengths at day 14; this could be due to

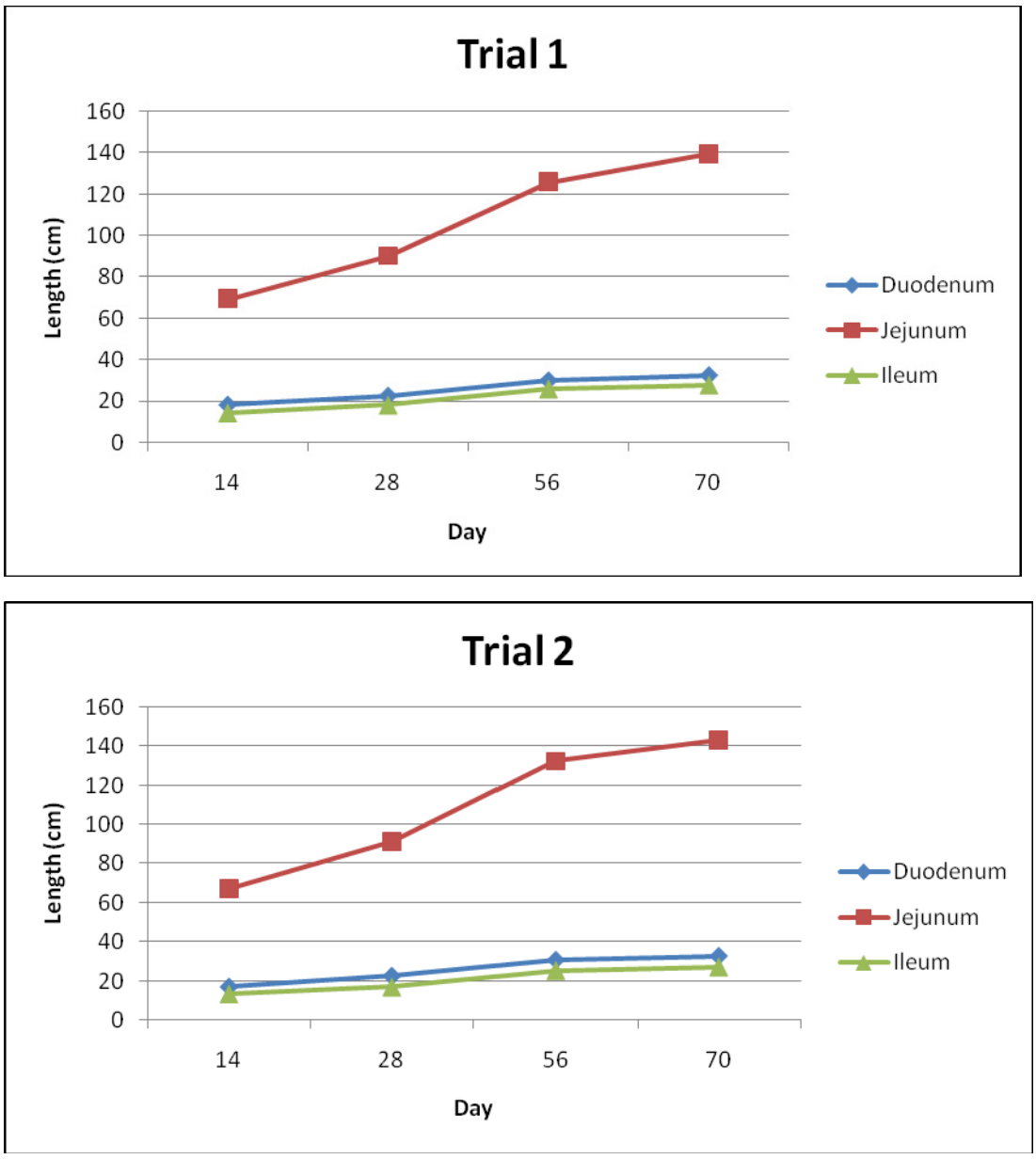

Figure 4.8: Mean length of intestinal segments (duodenum, jejunum and ileum) during Trial 1 (top) and Trial 2 (bottom) on each sampling day regardless of transport of dietary supplement provided.

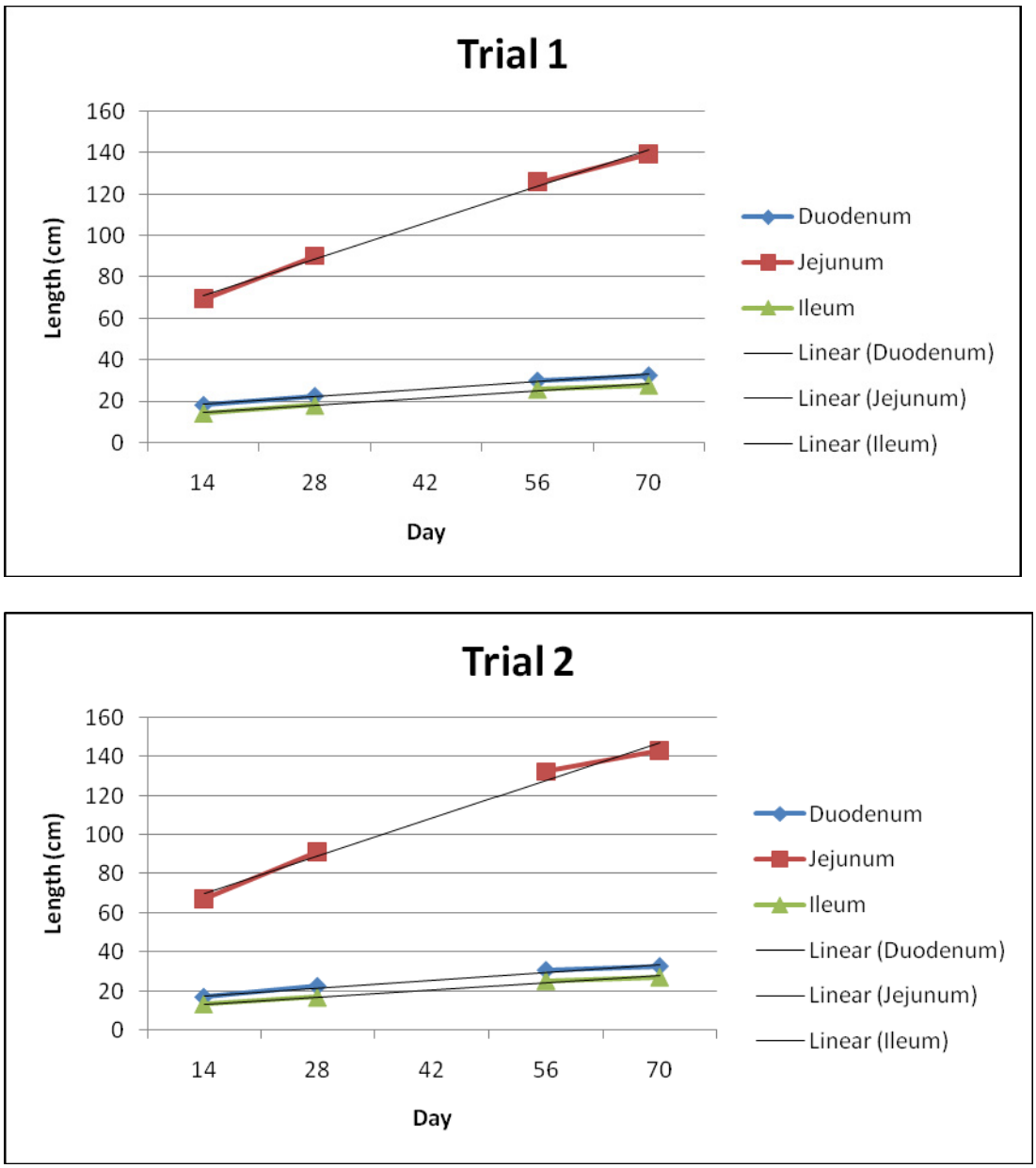

Figure 4.9: Mean Length of Intestinal Segments (Duodenum, Jejunum and Ileum) During Trial 1 (Top) and Trial 2 (Bottom) on Each Sampling Day Regardless of Transport of Dietary Supplement Provided Using Trend Lines to Show Linear Increase

a different sampling procedure for differentiation between the jejunum and ileum. Similar to these trials, Uni *et al.* (1999) showed linear increases in the length of intestinal segments as the birds aged. Uni *et al.* (1999) found a 2-4 fold increase in the length of

intestinal sections over the first 15 days post hatch. Current trials had between 1.8 and 2.0 fold increase in the length of the intestinal segments for the 70 day duration of the trial. Sell *et al.* (1991) found a 3.3-3.8 fold increase in the jejunum and small intestine length from 6 days before hatch to 8 days after hatch, and from hatch to 8 days the intestine and jejunum the increase was between 2.2-2.7 fold. Corless and Sell (1999) found that intestine length was effected by restriction of feed and water. Birds subjected to a 30 hour hold had reduced lengths of the duodenum at 5 days and the jejunum at 2 days. In a second experiment the same hold time of 30 hours decreased duodenal and ileal lengths at 3 days post hatch and reduced jejunal length between 1 and 3 days. Poults subjected to an even longer hold time of 54 hours displayed decreased intestinal lengths between 2-5 days and at 10 days of age. Previous research indicates that the most prominent growth of the intestine length occurs within the first 15 days post hatch and after which the length increases at a constant rate. Differences in intestinal length that may have been present due to transport or dietary supplementation could have been missed with sampling beginning at 14 days or the birds had already undergone compensatory growth.

4.4.2 Jejunum Breaking Strength

In trial 1 the jejunum breaking strength of the birds was similar ($P>0.05$) regardless of transport supplement at both days 28 and 70 (Table 4.7). The dietary supplements attributed to a significant ($P\leq0.05$) difference at day 28 indicating that the LYS supplements had jejunal breaking strengths of 0.30 kg force that was significantly higher than the AL supplement with a jejunum breaking strength of 0.26kg force. The NS and ANTI group both had a breaking strength of 0.29kg force and were similar to all

Table 4.7: Effect of Post-Transport Supplement on the Jejunum Breaking Strength of Heavy Hen Turkeys (Trial 1)

Jejunum Breaking Strength (Kg force)		
	Age (days)	
Transport	**28**	**70**
Oasis®[z]	0.30 ±0.009[†]	0.46 ±0.020
Oasis®[z]& Lysozyme[y]	0.28 ±0.009	0.50 ±0.020
No supplement	0.27 ±0.009	0.46 ±0.020
P-value	*0.19*	*0.25*
Supplement		
Antibiotic[x]	0.29ab ±0.011	0.48 ±0.023
Antibiotic[x]& Lysozyme[y]	0.26b ±0.011	0.48 ±0.022
Lysozyme[y]	0.30a ±0.011	0.47 ±0.024
No Supplement	0.29ab ±0.011	0.48 ±0.023
P-value	*0.04*	*0.99*
Mean	***0.28***	***0.47***

[z] Oasis® - Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA.
[y] Lysozyme - Inovapure™ 213(active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC., Canada.
[x] Antibiotic - BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada.
[†] Mean ± Standard Error
a-b Means within the column and section with different letters differ significantly ($P \leq 0.05$).

other dietary supplements. At day 70 all dietary supplements had similar (P>0.05) jejunum breaking strengths with an average breaking strength of 0.47kg force.

In trial 2 the jejunum breaking strength of the birds was affected (P≤0.05) by a transport and dietary supplement interaction (Table 4.8). Birds fed OAS during transport and NS after transport had the highest breaking strength (0.59kg force), which was significantly higher than all other treatment combinations except OAS/ANTI and NS/LYS. On day 70 the jejunum breaking strength was similar (P>0.05) for birds consuming all treatment combinations.

There is no published research on the strength of the intestine in poults and its relation to feeding Oasis® and lysozyme during transport or subsequent dietary

Table 4.8: Effect of Transport and Dietary Supplement on the Jejunum Breaking Strength of Heavy Hen Turkeys (Trial 2)

Jejunum Breaking Strength (kg force)				
	Transport Supplement[z]			
	Day 28			
Supplement[y]	**OAS**	**OL**	**NO**	**Mean**
ANTI	0.50ab ±0.035[†]	0.48bc ±0.035	0.48bc ±0.035	0.49 ±0.020
AL	0.48bc ±0.035	0.47bc ±0.035	0.48bc ±0.035	0.48 ±0.020
LYS	0.40c ±0.035	0.48bc ±0.035	0.50abc ±0.035	0.46 ±0.020
NS	0.59a ±0.035	0.40c ±0.035	0.45bc ±0.035	0.48 ±0.020
Transport Mean	*0.50 ±0.017*	*0.46 ±0.017*	*0.48 ±0.017*	
	Day 70			
ANTI	0.21 ±0.025	0.21 ±0.025	0.11 ±0.025	0.18 ±0.014
AL	0.19 ±0.025	0.19 ±0.025	0.20 ±0.025	0.19 ±0.014
LYS	0.19 ±0.025	0.21 ±0.025	0.21 ±0.025	0.20 ±0.014
NS	0.18 ±0.025	0.23 ±0.025	0.16 ±0.025	0.19 ±0.014
Transport Mean	*0.19 ±0.012*	*0.21 ±0.012*	*0.17 ±0.012*	

ANOVA	Day 28	Day 70
T	0.32	0.09
S	0.83	0.12
TxS	0.02	0.60

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error
a-c Ls means within the section with different letters differ significantly ($P \leq 0.05$).

supplementation with lysozyme in turkey poults. In trial 1 the addition of transportation supplements did not affect the overall strength of the jejunum, but the post transport dietary supplements did have an effect on the jejunum strength at day 28. At this point birds supplemented with LYS had a significant increase in the breaking strength compared to birds supplemented with AL. By day 70 this difference was no longer observed. This may indicate that during growth birds supplemented with solitary lysozyme have a higher intestinal breaking strength, but that the increase is not observed after the removal of lysozyme resulting in no differences at day 70. The jejunal breaking

strength was greater at 70 days than at 28 days, implying that the birds intestinal strength increases as the bird grows. This finding agrees with work on broilers by Bilgili and Hess (1997) who found that the breaking strength of the intestine was increased as the birds aged. Broilers had an increase in breaking strength at days 42 and 49 compared to birds sampled at 21 days of age (Bilgili and Hess, 1997).

In trial 2 there was a transport by dietary supplement effect observed. At day 28 birds provided with OAS/NS had the highest jejunal breaking strength, which was only similar to birds receiving OAS/ANTI and NO/LYS. Birds receiving OAS/ANTI had significantly higher breaking strength than birds fed OAS/LYS or OL/NS. These birds show an improved intestinal breaking strength at day 28, but the differences were no longer present at 70 days of age. Similar to trial 1, this may indicate that the effect of the transport and dietary supplement interaction is not influential at 70 days and changes in breaking strength occurring due to transport or dietary supplements may only be during earlier growth. In contrast to trial 1 and the results of Bilgili and Hess (1997), the breaking strengths at day 28 are much higher than those at day 70. This indicates that there may be differences regarding intestinal strength within flocks of birds, or there is some other influence on the intestinal breaking strength of the birds. Research conducted by Warren and Hamilton (1980) found that birds infected with ochratoxin, had a lowered intestinal breaking strength and a lowered collagen content in the large intestine. Within these current studies the differences observed between the two trials was unclear although conditions were different with trial 1 taking place during the summer and trial 2 taking place during the winter. Birds did not appear to have decreased absorption due to lowered

intestinal breaking strength as feed conversions and body weights appeared similar between trials in this study.

4.4.3 Intestinal Morphology

Duodenum mucosal width was similar (P>0.05) for birds regardless of transport or dietary supplements on day 14 (Table 4.9). On day 28 mucosal widths are similar (P>0.05) regardless of dietary or transport treatments (Table 4.9). During this day perhaps there was a marginal significance (P=0.07) with birds consuming OAS having the largest mucosal width with a value of 419.4mm. The OL supplement displayed the lowest mucosal width with a value of 362.3mm, with NO in the middle with a value of 411.4mm. At day 56 this difference is significant (P≤0.05) and indicates that the OAS and NO groups showed significantly higher duodenum mucosal values (514.9, and 495.7mm) than the OL group which had a duodenum mucosal width of 433.0mm (Table 4.9). At day 70 there is an interaction (P≤0.05) between the transport and supplement groups. Birds fed NO/AL had a duodenum mucosal width of 656.2mm which is significantly higher than all other treatments except those fed OAS/LYS (Table 4.9). Birds fed OAS/LYS had significantly higher mucosal width, 606.1mm compared to those fed OAS/ANTI, OAS/NS, OL/ANTI, OL/LYS, OL/NS and NO/LYS which ranged from 455.0-499.8mm.

The duodenum mucosal width was not affected (P>0.05) by the transport or dietary supplements during trial 2 (Table 4.10). The mucosal width initially was 151.3 μm and increased to 604.7 μm by day 70.

Table 4.9: Interaction of Transport and Post-Placement Supplements on the Duodenal Mucosal Width of Heavy Hen Turkeys (Trial 1)

Duodenum Mucosal Width (µm)				
	Transport Treatment[z]			
	Day 14			
Supplement[y]	**OAS**	**OL**	**NO**	***Mean***
ANTI	247.4 ±28.58[†]	268.7 ±28.58	290.5 ±28.58	268.9 ±16.50
AL	267.7 ±28.58	295.7 ±28.58	245.6 ±28.58	269.7 ±16.50
LYS	283.8 ±28.58	235.3 ±28.58	210.6 ±28.58	243.2 ±16.50
NS	273.3 ±28.58	323.9 ±28.58	286.2 ±28.58	294.5 ±16.50
Transport Mean	*268.0* ±14.29	*280.9* ±14.29	*258.2* ±14.29	
	Day 28			
ANTI	466.4 ±36.84	367.7 ±36.84	359.5 ±36.84	397.9 ±21.27
AL	396.3 ±36.84	365.5 ±36.84	398.0 ±36.84	386.6 ±21.27
LYS	394.9 ±36.84	340.1 ±36.84	438.7 ±36.84	391.2 ±21.27
NS	420.2 ±36.84	376.0 ±36.84	449.4 ±36.84	415.2 ±21.27
Transport Mean	*419.4*±18.42	*362.3* ±18.42	*411.4* ±18.42	
	Day 56			
ANTI	520.3 ±40.42	429.0 ±40.42	503.6 ±40.42	484.3 ±23.33
AL	497.0 ±40.42	406.8 ±40.42	536.3 ±40.42	480.1 ±23.33
LYS	547.5 ±40.42	426.9 ±40.42	441.6 ±40.42	472.0 ±23.33
NS	494.8 ±40.42	469.4 ±40.42	501.1 ±40.42	488.4 ±23.33
Transport Mean	*514.9a* ±20.21	*433.0b* ±20.21	*495.7a* ±20.21	
	Day 70			
ANTI	460.6c ±33.55	455.0c ±33.55	509.1bc ±33.55	474.9 ±19.37
AL	509.3bc ±33.55	527.9bc ±33.55	656.2a ±33.55	564.5 ±19.37
LYS	606.1ab ±33.55	505.0c ±33.55	483.5c ±33.55	531.5 ±19.37
NS	484.1c ±33.55	499.8c ±33.55	520.9bc ±33.55	501.6 ±19.37
Transport Mean	*515.0* ±16.78	*497.0* ±16.78	*542.4* ±16.78	
ANOVA	**Day 14**	**Day 28**	**Day 56**	**Day 70**
T	0.54	0.07	0.02	0.17
S	0.21	0.79	0.96	0.02
TxS	0.39	0.47	0.56	0.02

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-c Means within the same section or main effects within the same row with different letters differ significantly ($P \leq 0.05$).

Table 4.10: Effect of Transport and Dietary Supplement on the Duodenum Mucosal Width of Heavy Hen Turkeys (Trial 2)

Duodenum Mucosal Width (µm)					
	Age (days)				
Transport[z]	**0**	**14**	**28**	**56**	**70**
OAS	153.4 ±6.19[†]	282.0 ±8.90	287.4±9.60	481.2 ±18.77	626.5±18.77
OL	151.0 ±5.86	284.2 ±8.90	297.6±9.60	516.7 ±19.49	632.0±18.77
NO	149.6 ±6.59	280.6 ±9.29	294.9±9.60	553.2 ±17.97	555.7±18.77
P-value	*0.91*	*0.91*	*0.74*	*0.03*	*0.08*
Supplement[y]					
ANTI		266.7±10.27	303.7±11.08	514.3 ±21.97	649.7±18.77
AL		289.6±10.27	286.8±11.08	495.5 ±20.75	576.0±18.77
LYS		273.1±10.90	282.1±11.08	531.2 ±21.97	619.8±18.77
NS		299.7±10.27	300.6±11.08	527.0 ±21.97	573.5±18.77
P-value		*0.10*	*0.46*	*0.64*	*0.23*
Mean	***151.3***	***282.3***	***293.3***	***516.6***	***604.7***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL- Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

Little is known about the effect of transport or dietary supplementation on the mucosal width of the sections of small intestine. Jackson (2005) found that after prefeeding birds with Oasis® the mucosa of the duodenum was thicker at day 2. This difference was not observed for sampling at days 14 or 21 in their trial. Supplementing birds with OAS during transport has an effect on the mucosa width appearing around day 28 and continuing till the end of the trial. Birds receiving OAS had higher mucosal widths than birds receiving OL. At day 70 a 3-way interaction indicates that birds receiving NO/AL had increased mucosal width, compared to all other treatments except OAS/LYS. Miles et al. (2006) found a decrease in muscularis mucosae thickness in the duodenum as well as lamina propria from the feeding of sub therapeutic antibiotics to broilers. Humphrey et al. (2002) found that supplementation with lysozyme and

lactoferrin resulted in thinner lamina propria, which is used as an indicator of inflammation and gut health.

The intestinal breakage was recorded using a breakage score range of 1-5, with 1 indicating little damage to the villi and 5 being total destruction of the villi (Figure 4.10). This scoring was used on the duodenum, jejunum and ileum sections of the intestines. Examples of typical scores are presented in figure 4.10.

In trial 1, the duodenum breakage score was similar among treatments at day 14. Birds did not show any difference ($P>0.05$) in breakage score regardless of transport or dietary supplement (Table 4.11). On day 28, there was a difference ($P\leq0.05$) among the transport treatments where OL treatment had a lower breakage score than birds fed OAS or NO (Table 4.11). The dietary supplements at day 28 exhibited no difference ($P>0.05$) with similar breakage scores. At days 56 and 70 breakage scores were similar ($P>0.05$) regardless of transport or dietary supplement provided. In trial 2 the duodenum breakage score was not affected ($P>0.05$) by the transport or dietary supplements at any sampling date (Table 4.11). The breakage score ranged from 4.8-5.0 throughout the trial.

The duodenum readability scoring was very high regardless of the sampling date or trials. This high level of unreadable villi is the most extensive during trial 2 at day 56. At this sampling time every duodenum sample was unreadable regardless of transport or dietary supplement. This resulted in SAS (Littell *et al.*, 1996) not producing an ANOVA results table as all treatments showed the same value. This high level unreadability throughout the duodenum samples is presumed to be caused by the sample collection procedure. The procedure included removing the intestinal contents

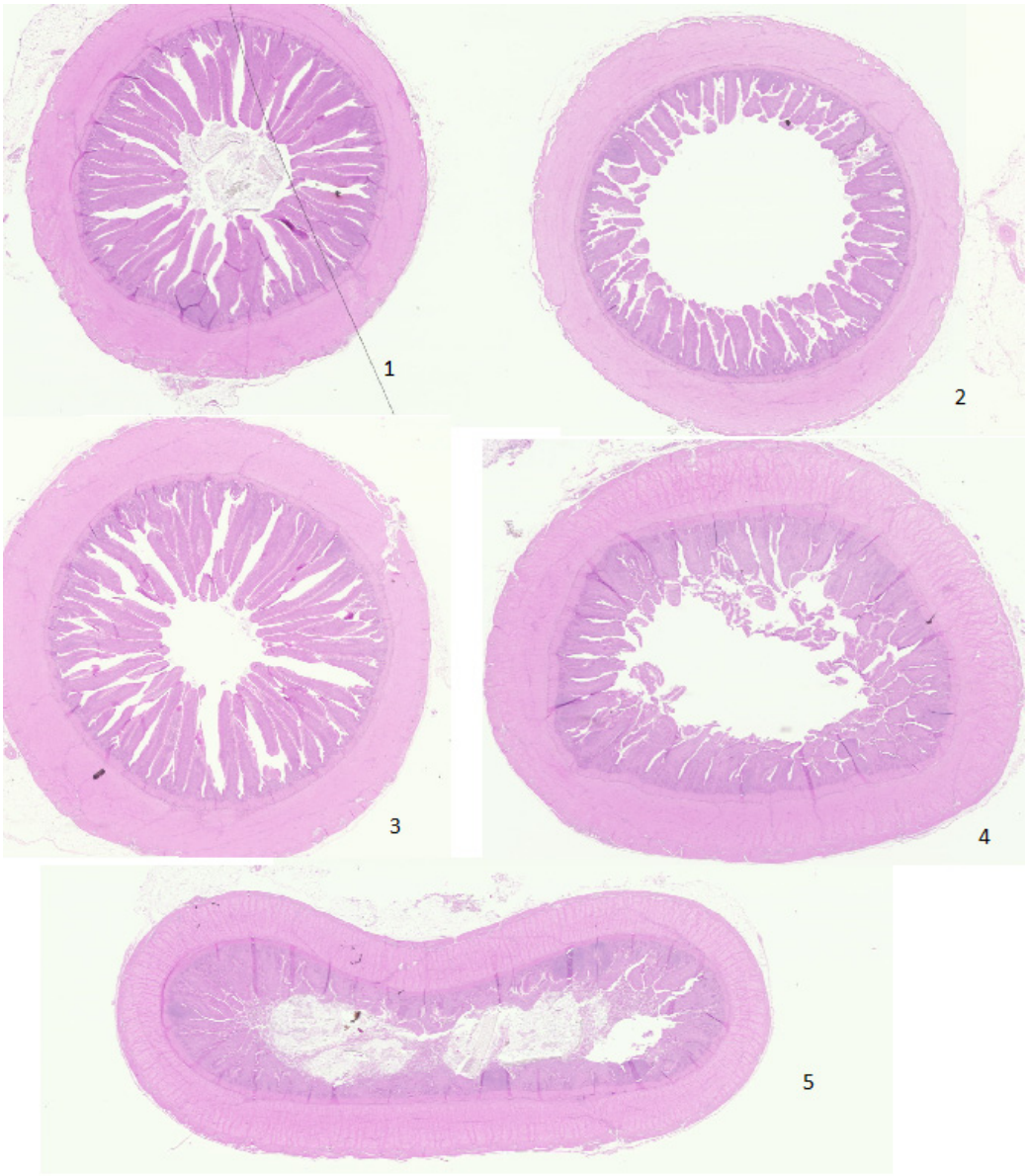

Figure 4.10: Intestinal Readability Scoring; Top Left: 0-25% Villi Unreadable in Cross Section (1), Top Right: 26-50% Villi Unreadable in Cross Section (2), Middle Left: 51-75% Villi Unreadable in Cross Section (3), Middle Right: Above 76% Unreadable Villi (4), Bottom: Cross Section Completely Unreadable (5)

Table 4.11: Effect of Transport and Dietary Supplement on the Duodenum Readability Score of Heavy Hen Turkeys

Duodenum Readability Score					
Trial 1					
	Age (days)				
Transport[z]	**14**		**28**	**56**	**70**
OAS	4.9 ±0.14[†]		4.9a ±0.10	4.8 ±0.12	4.9 ±0.04
OL	4.8 ±0.14		4.5b ±0.10	4.9 ±0.12	5.0 ±0.04
NO	4.8 ±0.14		5.0a ±0.10	5.0 ±0.12	5.0 ±0.04
P-value	*0.56*		*0.003*	*0.36*	*0.38*
Supplement[y]					
ANTI	4.9 ±0.16		4.8 ±0.12	5.0 ±0.14	5.0 ±0.04
AL	4.8 ±0.16		4.8 ±0.12	4.8 ±0.14	4.9 ±0.04
LYS	4.5 ±0.16		4.8 ±0.12	4.8 ±0.14	5.0 ±0.04
NS	5.0 ±0.16		4.8 ±0.12	5.0 ±0.14	5.0 ±0.04
P-value	*0.16*		*0.94*	*0.38*	*0.41*
Mean	***4.8***		***4.8***	***4.8***	***5.0***
Trial 2					
	Age (days)				
Transport[z]	**0**	**14**	**28**	**56**	**70**
OAS	4.9 ±0.10	5.0 ±0.05	4.8 ±0.09	5.0±0.00	4.9±0.04
OL	4.7 ±0.09	4.9 ±0.05	4.9 ±0.09	5.0±0.00	5.0±0.04
NO	5.0 ±0.10	5.0 ±0.05	4.9 ±0.09	5.0±0.00	5.0±0.04
P-value	*0.16*	*0.13*	*0.36*	.	*0.38*
Supplement[y]					
ANTI		4.9 ±0.06	4.9 ±0.11	5.0±0.00	5.0±0.04
AL		4.9 ±0.06	4.8 ±0.11	5.0±0.00	5.0±0.04
LYS		5.0 ±0.06	4.8 ±0.11	5.0±0.00	4.9±0.04
NS		5.0 ±0.06	4.9 ±0.11	5.0±0.00	5.0±0.04
P-value		*0.54*	*0.65*	.	*0.41*
Mean	***4.9***	***5.0***	***4.8***	***5.0***	***5.0***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

before measurement of weight and length. This removal of digesta may have included rough handling of the intestinal segment, which possibly caused breakage to the villi. Another possibility for the poor integrity seen in the cross sections is that the method of

digesta removal folded over the villi within the intestinal sections which made the villi appear broken in the cross sections. This breakage or folding has limited the histological analysis of the samples as there were not enough whole villi visible per sample to measure villi height, crypt depth, villi midwidth, or villi area. There is little information regarding the most appropriate method of intestinal sampling to maintain the villi integrity and decrease handling damage while collecting intestinal samples. Sims *et al.* (2004) found damage to duodenum histology sections. They noted sloughing of enterocytes or epithelial covering cells, and clumping or adherence to adjacent villi. They associated this damage across all treatments to possible gut inflammation (*Clostridia perfringens*), or post mortem changes. In broilers, Butcher *et al.* (2002) reported intestinal damage from heat stress, coccidiosis, parasitic infections, bacterial infections, mycotoxins, tannins, biogenic amines and consumption of contaminated litter. There was no evidence of a health related problem in the current study.

At day 28 during trial 1 birds fed OL had a lower duodenum readability score than birds that received OAS or NO during transport. This may indicate a resistance to intestinal breakage when birds are supplemented with OL during transport. This result was not observed during trial 2.

Jejunum mucosal width was similar ($P>0.05$) for birds regardless of transport or dietary supplements on days 14, 28 and 56 in trial 1 (Table 4.12). At day 70 there is an interaction between the transport and supplement groups. Birds fed NO/AL had a jejunum mucosal width of 598.8mm which is significantly higher than OAS/AL, OL/ANTI, OL/NS, and NO/LYS which ranged between 407.0-485.8μm (Table 4.12). During trial 2, the jejunum mucosal width was similar regardless of the transport or

Table 4.12: Interaction of Transport and Post-Placement Supplements on the Jejunum Mucosal Width of Heavy Hen Turkeys (Trial 1)

Jejunum Mucosal Width (µm)				
Transport Treatment				
Day 14				
Supplement	**OAS**	**OL**	**NO**	**Mean**
ANTI	254.1 ±30.38[†]	240.2 ±35.57	232.7 ±30.38	**242.3 ±18.59**
AL	212.2 ±30.38	267.2 ±30.38	281.2 ±30.38	**253.5 ±17.54**
LYS	296.3 ±30.38	246.5 ±30.38	239.1 ±35.57	**260.6 ±18.59**
NS	257.5 ±30.38	280.5 ±30.38	260.9 ±35.57	**266.3 ±18.59**
Transport Mean	*255.0* ±15.19	*258.6* ±15.88	*253.5* ±16.67	
Day 28				
ANTI	356.8 ±30.78	353.7 ±30.78	338.8 ±30.78	**349.8 ±17.77**
AL	360.0 ±30.78	298.6 ±30.78	345.9 ±30.78	**334.8 ±17.77**
LYS	301.2 ±30.78	317.4 ±30.78	332.3 ±35.94	**313.5 ±18.82**
NS	428.8 ±30.78	335.2 ±30.78	331.6 ±30.78	**365.2 ±17.77**
Transport Mean	*361.7* ±15.39	*326.2* ±15.39	*334.6* ±16.07	
Day 56				
ANTI	480.7 ±45.52	461.7 ±45.52	503.5 ±45.52	**482.0 ±26.28**
AL	448.2 ±45.52	403.2 ±45.52	495.1 ±45.52	**448.9 ±26.28**
LYS	441.8 ±45.52	412.3 ±45.52	415.9 ±45.52	**423.3 ±26.28**
NS	505.9 ±45.52	459.6 ±45.52	449.9 ±45.52	**471.8 ±26.28**
Transport Mean	*469.2* ±22.76	*434.2* ±22.76	*466.1* ±22.76	
Day 70				
ANTI	500.7abcd[*†]	474.4bcd	517.4abc	**497.5 ±21.88**
AL	485.8bcd	497.5abcd	598.8a	**527.4 ±21.88**
LYS	561.8ab	532.6abc	435.5cd	**509.9 ±21.88**
NS	520.5abc	407.0d	542.3abc	**490.0 ±21.88**
Transport Mean	*517.2* ±18.95	*477.9* ±18.95	*523.5* ±18.95	
ANOVA	**Day 14**	**Day 28**	**Day 56**	**Day 70**
Transport (T)	0.9736	0.2516	0.4923	0.1979
Supplement (S)	0.8156	0.2472	0.4117	0.6466
TxS	0.5120	0.4356	0.9018	0.0315

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[*] TxS interaction Standard error is 37.89 for all treatment combinations on day 70.

[†] Mean ± Standard Error

a-d Means within the same section with different letters differ significantly ($P \leq 0.05$).

dietary supplements (Table 4.13). The jejunum mucosal width initially was 132.8µm and increased to 553.4 µm by day 70.

Table 4.13: Effect of Transport and Dietary Supplement on the Jejunum Mucosal Width of Heavy Hen Turkeys (Trial 2)

Jejunum Mucosal Width (µm)					
	Age (days)				
Transport[z]	**0**	**14**	**28**	**56**	**70**
OAS	128.1b ±6.23[†]	236.4 ±13.33	293.9 ±12.15	471.7 ±17.42	565.1 ±29.60
OL	124.4b ±5.72	244.1 ±13.33	307.3 ±12.15	482.9 ±16.68	579.9 ±28.34
NO	146.0a ±6.44	221.8 ±13.33	295.1 ±12.15	479.2 ±16.68	512.7 ±28.34
P-value	*0.04*	*0.40*	*0.40*	*0.90*	*0.23*
Supplement[y]					
ANTI		252.0 ±15.61	307.3 ±14.03	463.7 ±20.39	555.4 ±34.65
AL		229.4 ±14.74	302.3 ±14.03	464.8 ±19.25	543.9 ±32.72
LYS		238.9 ±15.61	295.1 ±14.03	490.5 ±19.25	554.1 ±32.72
NS		216.3 ±15.61	287.5 ±14.03	492.8 ±19.25	557.0 ±32.72
P-value		*0.77*	*0.77*	*0.58*	*0.99*
Mean	***132.8***	***235.0***	***298.0***	***477.9***	***553.4***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

Similar to the duodenum mucosal width, there is little research on the effect of transport or dietary supplementation on the jejunum mucosal width. Jackson (2005) found that the jejunum mucosa width at 2 days was not different between birds fed Oasis® or subjected to a 24 hour fast. The difference in the jejunum mucosal width observed at day 70 followed the difference at day 70 for the duodenum mucosal width. Birds receiving NO/AL have a higher mucosal width. Although lysozyme has been removed from the diet, there was a residual effect in birds that remained un-supplemented during transport and then placed on AL.

The readability score for the jejunum was similar (P>0.05) for all birds on days 14 and 28 regardless of transport or dietary supplement provided during trial 1 (Table 4.14).

Table 4.14: Effect of Transport and Dietary Supplement on the Jejunum Readability Score of Heavy Hen Turkeys

Jejunum Readability Score					
Trial 1					
	Age (days)				
Transport[z]		**14**	**28**	**56**	**70**
OAS		4.4 ±0.13[†]	4.8±0.18	3.9 ±0.25	4.8 ±0.11
OL		4.6 ±0.14	4.4±0.18	3.7 ±0.25	4.6 ±0.11
NO		4.7 ±0.13	4.3±0.19	3.8 ±0.25	4.9 ±0.11
P-value		*0.41*	*0.14*	*0.86*	*0.07*
Supplement[y]					
ANTI		4.5 ±0.16	4.5 ±0.21	4.0a ±0.29	4.8 ±0.13
AL		4.7 ±0.15	4.7 ±0.21	3.7ab ±0.29	4.8 ±0.13
LYS		4.4 ±0.15	4.4 ±0.22	3.1b ±0.29	4.9 ±0.13
NS		4.8 ±0.15	4.5 ±0.21	4.3a ±0.29	4.7 ±0.13
P-value		*0.41*	*0.85*	*0.03*	*0.59*
Mean		***4.6***	***4.5***	***3.8***	***4.8***
Trial 2					
	Age (days)				
Transport	**0**	**14**	**28**	**56**	**70**
OAS	4.9 ±0.11	5.0 ±0.10	4.7±0.10	5.0±0.00	4.9±0.07
OL	4.6 ±0.11	4.8 ±0.10	4.6±0.10	5.0 ±0.00	4.9±0.07
NO	4.9 ±0.12	4.8 ±0.10	4.7±0.10	5.0 ±0.00	4.9±0.07
P-value	*0.09*	*0.32*	*0.68*	.	*0.79*
Supplement					
ANTI		4.8±0.11	4.6±0.10	5.0 ±0.00	5.0±0.08
AL		4.8±0.11	4.7±0.10	5.0 ±0.00	4.8±0.08
LYS		5.0±0.12	4.7±0.10	5.0 ±0.00	4.9±0.08
NS		4.9±0.11	4.7±0.10	5.0 ±0.00	4.9±0.08
P-value		*0.41*	*0.93*	.	*0.59*
Mean	***4.8***	***4.9***	***4.7***	***5.0***	***4.9***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

At day 56 the readability score was similar (P>0.05) for birds when provided the transport treatments, but the dietary supplements showed a significant (P≤0.05) difference (Table 4.14). Birds fed LYS had the best readability with a score of 3.1 which was significantly lower than those birds fed ANTI, or NS. Birds fed AL were similar to birds fed all other dietary supplements. On day 70 all readability scores were similar (P>0.05) regardless of transport or dietary supplement provided (Table 4.14). The jejunum readability score was not affected (P>0.05) by the transport or dietary supplements during trial 2 (Table 4.14). The score ranged from 4.7-5.0 throughout the trial.

Similar to the duodenum samples, the jejunum had many cross sections which were unreadable. This high level of unreadability again may be related to the sampling procedure used. The procedure was the same with the jejunum as the duodenum and resulted in similar levels of breakage or folding. The number of unreadable cross sections limited the histological analysis of the samples as the villi were not measurable for villi height, crypt depth, villi midwidth or villi area. During trial 1, day 56 there was a dietary supplement interaction where birds that received AL dietary supplement had the highest number of readable cross sections. Birds receiving this supplement had an average score of 3.1, which was significantly lower than birds provided with the NS or ANTI (4.3 and 4.0 respectively). Birds that received AL had similar readability scores than all other treatments with a score of 3.7. Perhaps birds receiving lysozyme in the diet had an increased number of readable cross sections at 56 days of age. In contrast to the low scores in trial 1, during trial 2 at day 56 many cross sections were unreadable for all transport or dietary supplements. This resulted in SAS (Littell *et al.*, 1996) not producing

an ANOVA results table as all treatments showed the same readability score. At day 70 the readability was similar between trial 1 and trial 2, indicating that any possible resistance found at day 56 from the addition of lysozyme did not continue to day 70.

During trial 1 on days 14 and 28 the ileum mucosal widths were similar regardless of the transport or dietary supplement provided (Trial 4.15). On day 56 there were no differences ($P>0.05$) among transport supplements but dietary supplements showed a difference ($P\leq0.05$) (Trial 4.15). Birds fed ANTI and LYS had a higher ileum mucosal width than the birds fed the AL supplement. Birds fed NS had similar mucosal widths to all other supplements fed. At day 70 there was an interaction ($P\leq0.05$) of both transport and dietary supplement (Table 4.15). Birds fed OAS/ANTI had an ileum mucosal width of 942.4μm, significantly higher than birds fed OAS/AL, OL/ANTI, OL/NS, NO/LYS and NO/NS which ranged from 680.1-800.5μm. During trial 2 the ileum mucosal width was similar ($P>0.05$) regardless of the transport or dietary supplements (Table 4.16). The ileum mucosal width initially was 144.1μm at day 0 and increased to 909.2μm by day 70.

Jackson (2005) found no differences at 2 days in ileal mucosa width when birds were supplemented with Oasis® or subjected to a 24 hour fast. The differences at day 56 in trial 1 corresponded to the increased mucosal width in the jejunum of birds consuming LYS after transport. There is an effect on multiple sections of the small intestine in regards to mucosal width when birds consume lysozyme as a dietary supplement. In the ileum during day 70 there was a similar affect when birds consumed the ANTI diet. These differences were not apparent in trial 2, where mucosal widths of all intestinal sections were similar regardless of transport or dietary supplement.

Table 4.15: Interaction of Transport and Post-Placement Supplements on the Ileum Mucosal Width of Heavy Hen Turkeys (Trial 1)

Ileum Mucosal Width (µm)				
	Transport Treatment[z]			
	Day 14			
Supplement[y]	**OAS**	**OL**	**NO**	**Mean**
ANTI	440.2 ±33.80[†]	395.3 ±33.80	399.6 ±33.80	**411.7 ±19.52**
AL	412.4 ±33.80	374.3 ±33.80	334.4 ±33.80	**373.7 ±19.52**
LYS	414.1 ±33.80	388.1 ±33.80	438.3 ±33.80	**413.5 ±19.52**
NS	432.7 ±33.80	449.7 ±33.80	322.8 ±33.80	**401.7 ±19.52**
Transport Mean	424.9 ±16.90	401.9 ±16.90	373.8 ±16.90	
	Day 28			
ANTI	566.1 ±40.87	611.2 ±40.87	538.5 ±40.87	**571.9 ±23.60**
AL	566.1 ±40.87	553.0 ±40.87	638.5 ±40.87	**585.8 ±23.60**
LYS	533.9 ±40.87	543.0 ±40.87	570.7 ±40.87	**549.2 ±23.60**
NS	577.3 ±40.87	514.5 ±40.87	541.0 ±40.87	**544.3 ±23.60**
Transport Mean	*560.8 ±20.44*	*555.4 ±20.44*	*572.1 ±20.44*	
	Day 56			
ANTI	789.2 ±52.18	835.3 ±52.18	791.7 ±52.18	**805.4 ±30.12**
AL	666.7 ±52.18	771.8 ±52.18	695.5 ±52.18	**711.3 ±30.12**
LYS	788.6 ±52.18	841.6 ±52.18	826.2 ±52.18	**818.8 ±30.12**
NS	702.1 ±52.18	739.3 ±52.18	808.8 ±52.18	**750.1 ±30.12**
Transport Mean	*736.7 ±26.09*	*797.0 ±26.09*	*780.5 ±26.09*	
	Day 70			
ANTI	942.4a[*†]	774.9cde	823.0abcd	**846.8 ±23.70**
AL	800.5bcde	839.6abcd	912.1ab	**850.8 ±23.70**
LYS	874.3abcd	890.4abc	760.1de	**841.6 ±23.70**
NS	886.6abc	680.1e	757.7de	**774.8 ±23.70**
Transport Mean	*876.0 ±20.52*	*796.3 ±20.52*	*813.2 ±20.52*	
ANOVA	**Day 14**	**Day 28**	**Day 56**	**Day 70**
T	0.12	0.84	0.25	0.02
S	0.46	0.57	0.06	0.10
TxS	0.23	0.50	0.86	0.005

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[*] TxS interaction Standard error is 41.04 for all treatment combinations on day 70.

[†] Mean ± Standard Error

a-e Means within the same section with different letters differ significantly ($P \leq 0.05$).

Table 4.16: Effect of Transport and Dietary Supplement on the Ileum Mucosal Width of Heavy Hen Turkeys (Trial 2)

Ileum Mucosal Width (µm)					
	Age (days)				
Transport[z]	**0**	**14**	**28**	**56**	**70**
OAS	137.2 ±9.16[†]	423.5 ±26.11	446.1 ±24.81	793.2 ±56.48	925.6 ±36.3
OL	148.4 ±8.41	377.6 ±24.99	480.2 ±23.75	896.3 ±54.07	959.2 ±36.3
NO	146.7 ±9.46	398.7 ±24.99	505.2 ±23.75	753.9 ±54.07	842.9 ±36.3
P-value	*0.6378*	*0.4557*	*0.2418*	*0.1748*	*0.0804*
Supplement[y]					
ANTI		429.4 ±30.56	522.0 ±27.42	775.9 ±66.12	935.7 ±41.87
AL		411.5 ±28.86	472.8 ±29.04	811.1 ±62.44	871.2 ±41.87
LYS		368.9 ±28.86	453.5 ±27.42	811.5 ±62.44	928.0 ±41.87
NS		390.0 ±28.86	460.4 ±27.42	859.4 ±62.44	901.9 ±41.87
P-value		*0.5096*	*0.3006*	*0.8853*	*0.6948*
Mean	***144.1***	***400.4***	***478.3***	***816.9***	***909.2***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

The ileum readability scores were not affected (P>0.05) by either transport or dietary supplement during trial 1 (Table 4.17). During trial 2, the jejunum score was affected (P≤0.05) by the transport treatments at day 0 (Table 4.17). Birds fed the OL treatment had the highest readability with a value of 4.0 which was more readable than birds fed OAS which had a score of 4.6. Birds that received NO during transport displayed similar results to both other transport treatments. On days 14, 28 and 56 birds there were no significant differences (P>0.05) among transport or dietary treatments (Table 4.17). On day 70 the transport supplements had similar (P>0.05) ileum readability scores, but there was a difference (P≤0.05) in the dietary supplements (Table 4.17). Birds fed AL had cross sections that were more readable than birds fed LYS or NS. Birds fed the ANTI showed similar scores to all other treatments.

Table 4.17: Effect of Transport and Dietary Supplement on the Ileal Readability Score of Heavy Hen Turkeys

Ileum Readability Score					
Trial 1					
	Age (days)				
Transport[z]		**14**	**28**	**56**	**70**
OAS		3.9 ±0.21[†]	4.0 ±0.19	4.5 ±0.15	3.7 ±0.24
OL		3.8 ±0.21	3.8 ±0.19	4.4 ±0.15	3.8 ±0.24
NO		3.6 ±0.21	3.9 ±0.19	4.7 ±0.15	3.7 ±0.24
P-value		*0.71*	*0.62*	*0.34*	*0.98*
Supplement[y]					
ANTI		3.5 ±0.25	3.8 ±0.21	4.6 ±0.17	3.8 ±0.28
AL		3.5 ±0.25	4.0 ±0.21	4.7 ±0.17	3.8 ±0.28
LYS		3.8 ±0.25	3.9 ±0.21	4.4 ±0.17	3.3 ±0.28
NS		4.2 ±0.25	3.9 ±0.21	4.4 ±0.17	3.9 ±0.28
P-value		*0.19*	*0.87*	*0.67*	*0.46*
Mean		***3.7***	***3.9***	***4.5***	***3.7***
Trial 2					
	Age (days)				
Transport	**0**	**14**	**28**	**56**	**70**
OAS	4.6a ±0.12	4.2±0.13	4.1 ±0.15	4.1a ±0.18	4.3±0.11
OL	4.1b ±0.12	4.2±0.13	3.9 ±0.14	4.3a ±0.17	4.1±0.11
NO	4.4ab ±0.13	4.2±0.13	3.9 ±0.14	3.6b ±0.17	4.1±0.11
P-value	*0.01*	*0.99*	*0.53*	*0.04*	*0.18*
Supplement					
ANTI		3.8±0.16	3.8 ±0.16	4.0 ±0.21	4.2ab±0.12
AL		4.3±0.15	3.9 ±0.17	4.0 ±0.20	3.8b±0.12
LYS		4.3±0.15	4.2 ±0.16	3.8 ±0.20	4.3a±0.12
NS		4.3±0.15	4.1 ±0.16	4.2 ±0.20	4.3a±0.12
P-value		*0.07*	*0.49*	*0.71*	*0.04*
Mean	***4.3***	***4.2***	***4.0***	***4.0***	***4.2***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

Overall the villi readability in the ileum was higher than the duodenum and jejunum. This increased readability allowed for analysis of villi height, crypt depth, villi midwidth and villi area. It is unknown why the villi of the ileum remained more readable

while there was such severe damage or folding occurring in the duodenum and jejunum. During trial 1 day 56 shows the lowest readability, which is in contrast to the jejunum during the same sampling time. During trial 2 there were differences at day 0 which were not reported in the duodenum or jejunum of the birds. The differences indicate that birds provided with OL during transport have a higher resistance to ileum breakage or folding than birds receiving OAS. Birds being transported with NO had similar ileum scores to both other treatments. Day 70 had an interaction in trial 2, which was not present in the duodenum or jejunum of the birds. Birds receiving AL had higher readability than birds receiving LYS or NS, while birds receiving ANTI were intermediate and similar to all others. Although there is no research on villi breakage to compare to, there may be a synergistic response with AL that improved the villi resistance to breakage.

During trial 1 the villi height of the ileum was affected ($P \leq 0.05$) by the transport treatments on day 14 (Table 4.18). Birds fed OAS showed the highest villi height with a value of 629.2 μm, which was significantly higher than OL and NO treatments with villi height of 540.1and 561.3 μm respectively. The dietary supplements at day 14 had no effect ($P > 0.05$) on the villi height. The average height was 576.9 μm. On days 28, 56 and 70 there was no effect ($P > 0.05$) of either transport or dietary supplement on the villi height of the ileum, with heights of 739.1, 861.7 and 885.5 μm respectively. During trial 2, the ileum villi heights were similar ($P > 0.05$) regardless of the transport or dietary supplements provided (Table 4.18). Initially at day 0 the villi height was 310.6μm increasing to 736.7 μm at day 70.

Table 4.18: Effect of Transport and Dietary Supplement on the Ileal Villi Height of Heavy Hen Turkeys

Ileum Villi Height (µm)					
		Age (days)			
		Trial 1			
Transport[z]		**14**	**28**	**56**	**70**
OAS		630.1a ±21.21[†]	723.6 ±34.83	792.5 ±56.43	887.5 ±56.43
OL		539.3b ±21.01	759.3 ±34.86	908.1 ±53.58	894.9 ±56.43
NO		560.5b ±21.01	748.5 ±36.75	848.5 ±51.62	830.8 ±56.43
P-value		*0.02*	*0.77*	*0.34*	*0.74*
Supplement[y]					
ANTI		612.3 ±24.07	745.0 ±39.43	886.8 ±60.47	946.6 ±56.43
AL		573.4 ±22.92	761.5 ±40.25	814.3 ±65.10	831.5 ±56.43
LYS		590.0 ±25.39	725.7 ±41.58	963.4 ±60.23	928.7 ±56.43
NS		531.0 ±25.41	743.0 ±41.74	734.4 ±63.63	777.4 ±56.43
P-value		*0.15*	*0.94*	*0.10*	*0.34*
Mean		***576.9***	***739.1***	***861.7***	***885.5***
		Trial 2			
		Age (days)			
Transport	**0**	**14**	**28**	**56**	**70**
OAS	290.9 ±26.05	388.6 ±28.00	618.7 ±86.02	626.8 ±65.47	720.9±57.85
OL	316.0 ±17.23	387.1 ±31.48	794.0 ±82.63	560.6 ±69.71	762.2±48.11
NO	324.8 ±24.37	358.7 ±27.74	582.9 ±80.00	668.9 ±62.65	725.3±47.46
P-value	*0.6166*	*0.6671*	*0.1712*	*0.5227*	*0.8157*
Supplement					
ANTI		394.5 ±30.95	596.4 ±90.36	627.2 ±85.90	770.5±56.15
AL		355.4 ±33.27	743.3 ±94.71	640.1 ±74.17	719.0±53.50
LYS		385.1 ±33.77	702.3 ±100.21	573.9 ±70.69	746.8±63.93
NS		377.5 ±33.58	618.8 ±94.69	633.8 ±74.17	708.2±62.4
P-value		*0.84*	*0.65*	*0.91*	*0.87*
Mean	***310.6***	***393.5***	***651.4***	***614.7***	***736.1***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

Villi height varied greatly between trial 1 and trial 2. In trial 1, the maximum villi height was 885.5µm at day 70, while during trial 2 the maximum villi height at day 70 was much less at 736.1µm. Applegate *et al.* (2005) found that the jejunum villi height of

turkey poults at day 0 to be 138.1µm, which was lower than the ileum villi height found in trial 2. Similarly, Uni *et al*. (1999) found an initial villi height of ~50µm at day 0 increasing to ~150µm by day 12; their heights were much lower than those found in the current trials. Humphrey *et al.* (2002) found that adding up to 10% modified rice (expressing lysozyme at 176mg/kg) and also up to 5% modified rice (expressing lactoferrin) to the diet had no effect on the duodenal, jejunal or ileal villi height of chicks at 17 or 19 days of age in one trial. In the second trial, Humphrey *et al.* (2002) found that birds fed 10% rice containing lysozyme, or a combination of 5% rice containing lactoferrin + 10% rice containing lysozyme or an antibiotic (bacitracin + roxarsone) had a greater villi height in the duodenum than chicks fed a control diet, this difference was not observed in the ileum of the birds. Similar to the result found in trial 1 at day 14, Potturi *et al.* (2005) found that birds denied access to feed for 48 hours had lower villus heights than birds that were supplemented immediately post hatch. Jackson (2005) found that ileum villi height was increased at 14 days when birds were provided with Oasis® post hatch, even when birds were subjected to a PEMS infection. This difference was not observed in the ileum villi at 21 days even though increased villi height was reported in both the duodenum and jejunum with Oasis® supplementation (Jackson, 2005). In contrast, and similar to the results of trial 2 day 0, Tabedian *et al.* (2010) saw no differences in villi height at 24 hours, in broiler chicks when there was a fast of 24 hours. But, increasing the fast to 48 hours resulted in decreased villi height.

Hoerr (1998) reported that the growth of the small intestine during the first 2 weeks is significant and villi height has been reported to double in the broiler chicken.

This was not found in trial 2, where villi height between day 0 and day 14 was not doubled, this could be due to a slower growth rate in turkeys compared to broiler chicks.

In trial 1 the ileum crypt depth of the birds was similar (P>0.05) regardless of the transport or dietary supplements (Table 4.19). During trial 2, at day 0, birds fed the OL or NO had a deeper crypt depth than those fed OAS (P≤0.05) (Table 4.20). At days 14 and 28 the birds (P>0.05) crypt depths were similar regardless of transport or dietary supplement (Table 4.20). On day 56 there is an interaction where birds receiving OAS/NS after transport show the deepest crypts at 156.3 µm (Table 4.20). This treatment combination was significantly higher than all other treatments except OL/ANTI, OL/LYS and NS/AL. At day 70, crypt depths were similar (P>0.05) regardless of transport or dietary supplement (Table 4.20).

Table 4.19: Effect of Transport and Dietary Supplement on the Ileum Crypt Depth of Heavy Hen Turkeys (Trial 1)

Ileum Crypt depth (µm)				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	102.5 ±5.50[†]	106.3±5.29	117.0 ±5.78	135.5 ±8.04
OL	100.3 ±5.50	100.9 ±5.29	118.4 ±5.49	123.5±8.12
NO	103.8 ±5.50	105.7 ±5.58	114.5±5.29	145.5 ±8.12
P-value	*0.90*	*0.73*	*0.87*	*0.18*
Supplement[y]				
ANTI	106.6 ±6.25	105.2 ±5.99	110.4±6.20	140.4 ±9.33
AL	99.7 ±5.95	103.0 ±6.11	122.8±6.67	138.9 ±9.48
LYS	97.1 ±6.59	108.9 ±6.31	125.6±6.17	138.0 ±9.34
NS	105.6 ±6.59	100.3 ±6.34	107.7±6.52	122.1 ±9.76
P-value	*0.68*	*0.80*	*0.16*	*0.52*
Mean	***102.5***	***104.5***	***115.7***	***134.9***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

Table 4.20: Effect of Transport and Dietary Supplement on the Ileum Crypt Depth of Heavy Hen Turkeys (Trial 2)

Ileum Crypt Depth (μm)				
	Transport Treatment[z]			
	OAS	**OL**	**NO**	**Mean**
		Day 0		
Day 0	66.6b ±3.07[†]	75.1a ±2.03	77.4a ±2.87	**73.0**
		Day 14		
Supplement[y]				
ANTI	74.6 ±10.07	107.4 ±10.32	86.2 ±10.32	**89.4 ±5.95**
AL	90.6 ±10.02	96.8 ±12.64	85.4 ±10.32	**90.9 ±6.39**
LYS	77.4 ±12.64	110.1 ±10.32	80.0 ±10.07	**89.2 ±6.49**
NS	107.9 ±10.32	75.9 ±10.32	99.1 ±10.32	**94.3 ±6.46**
Transport Mean	87.6 ±5.38	97.5 ±6.05	87.7 ±5.33	
		Day 28		
ANTI	91.3 ±7.87	91.5 ±7.87	94.7 ±9.26	**92.5 ±4.83**
AL	103.8 ±9.26	115.2 ±9.20	101.6 ±7.87	**106.9 ±5.06**
LYS	104.5 ±9.26	90.9 ±9.26	102.4 ±9.21	**99.2 ±5.35**
NS	95.1 ±9.26	99.8 ±9.21	98.0 ±7.87	**97.6 ±5.06**
Transport Mean	98.7 ±4.59	99.3 ±4.41	99.2 ±4.27	
		Day 56		
ANTI	106.4bc ±14.35	136.2abc ±17.79	115.9bc ±14.33	**119.5 ±9.10**
AL	105.4bc ±12.23	107.8bc ±14.30	130.5ab ±14.30	**114.6 ±7.86**
LYS	91.9c ±14.35	126.0abc ±12.23	116.1bc ±12.23	**111.3 ±7.49**
NS	158.8a ±14.33	111.5bc ±14.35	100.0bc ±12.23	**123.5 ±7.86**
Transport Mean	115.6 ±6.94	120.4 ±7.39	115.6 ±6.64	
		Day 70		
ANTI	131.1 ±13.31	123.0 ±13.33	127.0 ±11.35	**127.0 ±7.29**
AL	134.0 ±13.31	115.6 ±11.35	139.6 ±11.35	**129.7 ±6.95**
LYS	123.5 ±16.54	133.1 ±13.33	137.4 ±13.30	**131.3 ±8.30**
NS	138.5 ±16.53	124.3 ±11.35	105.5 ±13.33	**122.8 ±8.11**
Transport Mean	131.8 ±7.52	124.0 ±6.25	127.4 ±6.16	

ANOVA	**Day 0**	**Day 14**	**Day 28**	**Day 56**	**Day 70**
T	0.04	0.34	0.99	0.86	0.74
S		0.92	0.25	0.71	0.88
TxS		0.06	0.82	0.03	0.57

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-c Means within the same section different letters differ significantly ($P \leq 0.05$).

At day 0 during trial 2 birds supplemented with OL and NO had deeper crypts than birds supplemented with OAS. This increase in crypt depth did not impact the villi height of the birds, where all transport supplements were similar. In contrast to what was found in this study, Potturi *et al.* (2005) found birds denied access to feed for 48 hours had shallower crypts than birds that were supplemented immediately post hatch. Similarly Tabedian *et al.* (2010) saw no differences in crypt depth at 24 hours, in broiler chicks when fasted for up to 24 hours. When the fast was extended to 48 hours there was a significant decrease in the crypt depth. Humphrey *et al.* (2002) found that the addition of lysozyme (up to 10%) and lactoferrin (up to 5%) had no effect on the duodenal, jejunal or ileal crypt depth of chicks at 17 or 19 days of age. This is similar to the results found in all of trial 1 and in trial 2 after day 0, where lysozyme supplementation during transport or post transport did not impact the crypt depth of the villi in the ileum. Both Applegate *et al.* (2005) and Uni *et al.* (1999) found shallower crypts with an initial reading of 26.5µm and ~20µm, respectively whereas in trial 2 of this study initial mean was 73.0µmat day 0. Uni *et al.* (1999) found that the crypt depth of turkey poults changed little after day 6 of their 12 day trial. This was not observed in these trials where the crypt depth continued to increase over the course of both trials.

There was no effect ($P>0.05$) of transport or dietary treatment on the villi height/crypt depth ratio of the birds in either trial (Table 4.21).

Table 4.21: Effect of Transport and Dietary Supplement on the Ileum Villi Height Crypt Depth Ratio of Heavy Hen Turkeys

Villi height crypt depth ratio					
Trial 1					
	Age (days)				
Transport[z]		**14**	**28**	**56**	**70**
OAS		6.3 ±0.31[†]	6.9 ±0.46	6.9 ±0.64	6.9 ±0.68
OL		5.5 ±0.31	7.6 ±0.46	7.9 ±0.61	7.4 ±0.68
NO		5.7 ±0.31	7.2 ±0.48	7.6 ±0.59	5.8 ±0.68
P-value		*0.17*	*0.59*	*0.51*	*0.25*
Supplement[y]					
ANTI		6.0 ±0.36	7.1 ±0.52	8.0 ±0.69	6.9 ±0.79
AL		5.9 ±0.34	7.7 ±0.53	7.0 ±0.74	6.2 ±0.80
LYS		6.2 ±0.38	6.8 ±0.55	7.8 ±0.68	7.2 ±0.79
NS		5.1 ±0.38	7.5 ±0.55	7.0 ±0.72	6.6 ±0.64
P-value		*0.20*	*0.66*	*0.63*	*0.82*
Mean		***5.8***	***7.2***	***7.6***	***6.8***
Trial 2					
	Age (days)				
Transport	**0**	**14**	**28**	**56**	**70**
OAS	4.4 ±0.33	4.8 ±0.41	6.2 ±0.68	5.8 ±0.76	5.5±0.60
OL	4.2 ±0.22	4.1 ±0.46	7.6 ±0.65	4.8 ±0.81	6.2±0.50
NO	4.2 ±0.31	4.1 ±0.40	5.9 ±0.63	6.0 ±0.73	5.9±0.49
P-value	*0.89*	*0.41*	*0.15*	*0.47*	*0.66*
Supplement					
ANTI		4.7 ±0.45	6.4 ±0.71	5.3 ±1.00	6.2±0.58
AL		4.0 ±0.48	6.9 ±0.74	5.9 ±0.86	5.6±0.55
LYS		4.5 ±0.49	7.1 ±0.79	5.3 ±0.82	5.7±0.66
NS		4.2 ±0.49	5.9 ±0.74	5.7 ±0.86	6.0±0.64
P-value		*0.71*	*0.69*	*0.96*	*0.88*
Mean	***4.3***	***4.7***	***6.6***	***5.5***	***5.9***

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

There is little data reported on the villi height crypt depth ratio after supplementation during transport or post transport supplementation with lysozyme. Villi height crypt depth ratios were similar (P>0.05) regardless of transport or dietary supplement provided in either trial. It is noted that there is variation between the trials,

where trial 1 shows higher overall ratios than found in trial 2. The reason for this variation is unknown but could be related to the breeder flock of the poults or time of year that the trials took place. Similar to these trials, Tabedian *et al.* (2010) observed no differences in villi height crypt depth ratio with a 24 hour fast.

Ileum villi midwidth was not effected (P>0.05) by transport or dietary supplementation in either trial (Appendix Q). Humphrey *et al.* (2002) found that the addition of lysozyme (up to 10%) and lactoferrin (up to 5%) had no effect on the duodenal, jejunal or ileal villi midwidth of chicks at 17 or 19 days of age in trial 1. Potturi *et al.* (2005) reported that birds denied access to feed for 48 hours had a thinner villi midwidth than birds that were supplemented immediately post hatch. This effect was not found in either trial of this study, the longer hold time used by Potturi *et al.* (2005) may have a significant effect on the villi midwidth differences observed.

During trial 1, the ileum area of the birds was affected (P≤0.05) on day 14 by the transport supplements provided (Table 4.22). Birds fed OAS showed a significantly larger ileum area than birds fed OL. Birds fed NO were similar to birds fed both of the other transport supplements. The dietary supplements provided at day 14 resulted in a significant difference (P≤0.05) among treatments. Birds fed ANTI had a significantly higher ileal area than birds receiving NS. Birds fed the AL and LYS treatments were similar to both of the other treatments. On days 28, 56 and 70 birds ileum areas were similar (P>0.05) regardless of the transport or dietary supplements (Table 4.22). During trial 2, the ileum area was similar (P>0.05) among the transport and dietary supplements (Table 4.22). Initially at day 0, the average ileum area was 0.03, which increased to 0.17 by day 70 in the birds.

Table 4.22: Effect of Transport and Dietary Supplement on the Ileum Area of Heavy Hen Turkeys

Ileum area (mm^2)					
Trial 1					
	Age (days)				
Transport[z]	**14**		**28**	**56**	**70**
OAS	0.076a ±0.004[†]		0.100 ±0.006	0.161 ±0.014	0.209 ±0.015
OL	0.063b ±0.003		0.107 ±0.006	0.191 ±0.013	0.196 ±0.015
NO	0.066ab ±0.003		0.109 ±0.006	0.167 ±0.012	0.191 ±0.015
P-value	*0.03*		*0.58*	*0.24*	*0.67*
Supplement[y]					
ANTI	0.077a ±0.004		0.112 ±0.007	0.178 ±0.015	0.206 ±0.017
AL	0.070ab ±0.004		0.105 ±0.007	0.172 ±0.016	0.198 ±0.017
LYS	0.067ab ±0.004		0.101 ±0.007	0.187 ±0.014	0.225 ±0.017
NS	0.060b ±0.004		0.103 ±0.007	0.155 ±0.015	0.167 ±0.018
P-value	*0.05*		*0.69*	*0.50*	*0.16*
Mean	**0.068**		**0.103**	**0.173**	**0.202**
Trial 2					
	Age (days)				
Transport[z]	**0**	**14**	**28**	**56**	**70**
OAS	0.02 ±0.002	0.06 ±0.005	0.11 ±0.017	0.13±0.013	0.16±0.016
OL	0.03 ±0.002	0.06 ±0.006	0.13±0.017	0.12±0.014	0.17±0.013
NO	0.03 ±0.002	0.05 ±0.005	0.09±0.017	0.14±0.013	0.17±0.013
P-value	*0.69*	*0.22*	*0.16*	*0.58*	*0.96*
Supplement[y]					
ANTI		0.06 ±0.006	0.09±0.018	0.12±0.018	0.18±0.015
AL		0.05 ±0.006	0.13±0.019	0.13±0.015	0.18±0.015
LYS		0.06 ±0.006	0.11±0.020	0.13±0.014	0.15±0.017
NS		0.05 ±0.006	0.11±0.019	0.13±0.015	0.17±0.017
P-value		*0.90*	*0.42*	*1.00*	*0.66*
Mean	**0.03**	**0.05**	**0.11**	**0.13**	**0.17**

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same section and column with different letters differ significantly ($P \leq 0.05$).

Little research has shown the impact of Oasis® and lysozyme on the ileum villi area in poults. Jackson (2005) found no difference in morphological appearance or size of the villi at 2 days in the jejunum or ileum regions when Oasis® was fed. In contrast, in

trial 1 of the current study there was an effect of OAS supplementation increasing the villi area compared to birds supplemented with OL at day 14. At the same time, birds supplemented with ANTI had higher villi area than birds provided NS. Although there was no difference in the crypt depth or villi height crypt depth ratio, birds supplemented with OAS had a higher villi height at day 14 than birds remaining un-supplemented. There was improvement in villi height and area at day 14 when birds are supplemented with OAS. The post transport supplementation effect on villi area is unclear as there was no effect of ANTI on the villi height of the birds. Any differences reported at day 14 were not present at day 28 or beyond. The differences reported in trial 1 are not replicated in trial 2.

4.5 Intestinal Sampling Conclusions

Overall the effect of supplementing turkey poults with Oasis® and lysozyme during transport and lysozyme post transport on the intestinal characteristics produced varied results.

Intestinal weights and lengths are not affected by the transport supplements provided. There was no change in weight or length of intestinal parameters due to transport supplementations. There appears to be a significant effect of post transport supplementation. More specifically birds supplemented with ANTI post transport resulted in increased weights of some intestinal parameters, although this was not consistent across trials and was observed mainly during days 56 and 70 of sampling. Lysozyme increased the strength of the jejunum during early growth, but differences were not apparent at 70 days of age.

Intestinal histology was different between the two trials. Differences did not occur in trial 2 as often as they did in trial 1. In trial 1, the mucosal widths of the duodenum, jejunum and ileum resulted in an effect of transport and dietary supplementation measured at days 56 and 70. This was not found in trial 2. This effect on the mucosal width did not result in differences in the villi measurements. There appears to be some effect of transport supplementation on the early development of the villi, but results are not consistent for OAS or OL and did not show continued improvement through to 70 days.

Chapter 5: The Effect of Oasis® and Lysozyme Supplementation during Transport and Lysozyme after Transport on Behaviour of Turkey Poults

5.1 Objectives

To determine the effect of supplementing Oasis® and lysozyme during long transport and lysozyme after transport on the behavior and mortality of newly hatched turkey poults immediately post placement and up to 7 days after arrival.

To determine the effect of supplementing Oasis® and lysozyme during long transport and lysozyme after transport on the feeding preferences of newly hatched turkey poults.

5.2 Hypotheses

It is hypothesized that birds that received supplementation during transport would have higher energy levels and would be more hydrated than birds that remained un-supplemented. This would result in the birds being more active immediately post placement and potentially have lower mortality rates.

5.3 Materials and Methods

The behavior of the birds was observed in association with the diets and housing conditions described in Chapter 3. Behaviour observations occurred during the first 7 days in trial 2.

5.3.1 Observation Guidelines and Schematics

To make observations a diagram was designed of each pen (Figure 5.1), showing location of the bell drinkers, feeders and temporary feed boxes. The diagrams can be defined into 3 sections to interpret movement within the pen. The circle in section 1 represents the bell drinker within the pen, while the square in section 2 is a representation

of the temporary feed box. This feed box only remained within the pen for the first 72 hours post placement. In section 3 the feeder is represented by the circle and remained in the same place throughout the trial.

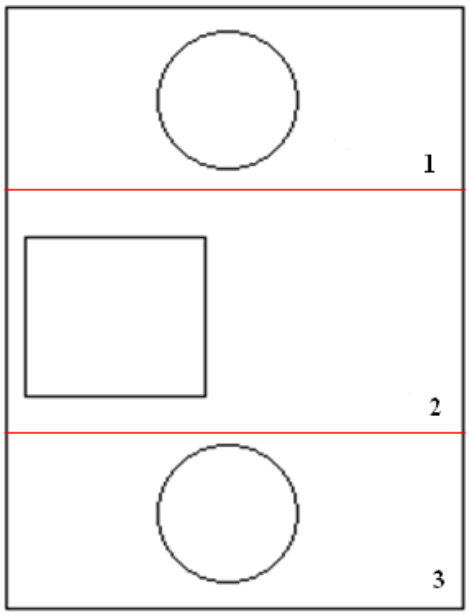

Figure 5.1: Schematic of the pen layout used in the behaviour analysis of turkey poults

Behaviour of poults was observed to track their actions immediately post-placement as well as throughout the first week. Prior to the arrival of the birds, the observed behaviours were clearly defined (Table 5.1). This ensured that all observers making the observations and recording data were using the same guidelines to minimize differences.

Temperature and lighting (lux) were recorded in each section of the pen at bird level the morning of placement so that any differences in these parameters that may affect behaviours were recognized. The three temperature and lux measurements from each pen were used to calculate the pen average.

Table 5.1: Definition of observed behaviours used during behavioural sampling of turkey poults

Behaviour	Abbreviation	Definition
Sitting Still	SS	birds that remained sitting down or sleeping while observations occurred
Standing	St	Birds that were standing up but not moving around during observation periods
Locomotion	L	Birds that were actively moving around the pen during observation periods
Drinking	D	Birds that were actively drinking from the bell drinker during observation periods
Feeding	F	Birds that were consuming feed from the feeders or pecking the litter of the pen while observations occurred

5.3.2 Observation Times and Frequency

The diagram created (Figure 5.1) was used for observing the behaviours of the birds immediately after placement. There was one observer per room observing the birds immediately after their placement. Upon arrival of the birds to the facility, the birds were weighed and then grouped for placement. When being placed all the birds were individually beak dipped and then placed by the bell drinker. The observer recorded the behaviours of the birds for a 2 minute period. The bird's behaviour was recorded at 30 second intervals, giving a total of 4 observations for each observation period. The 30 second observations were used to create an average of each behaviour for the 2 minute period. The behaviour sampling identified the number of birds active in behaviours as outlined in Table 5.1. Each bird's behaviour was recorded once for each of the 30 second intervals. Once the post placement observation was completed repeated observations were conducted again at 1 hour, 7 hours, 3 days, and 7 days post-placement. The post

placement observations began at approximately 1300h when the first pens were placed. The observations at 3 days and 7 days were performed by only 1 or 2 observers and were carried out in the early evening between 1800h and 2000h. This time was chosen to minimize disruptions during the observations.

After observations were completed an average was calculated for each behaviour over the 2 minute time frame. This average was expressed as the percentage of birds within the pen performing each behaviour. The average behaviours for each pen at each time point were used in the statistical analysis.

5.3.3 Statistical Analysis

All data was subjected to analysis of variance (ANOVA) using the Proc Mixed procedure of SAS (Littell *et al.*, 1996). Normality was checked and transformations were performed if necessary.

For behavior observations occurring on the first day of the trial (placement, 1-hour post placement, and 7-hours post placement) the statistical analysis was a completely randomized block design with the repeated measure of age:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\gamma_{ik} + \varepsilon_{ijkl}$$

Where Y= Response, μ= Population Mean, α= Factor 1 or transport supplement, i=Levels of factor 1 (O, OL, No), β= blocking factor or Room, j=Levels of blocking factor (151, 152, 153, 156), γ= Factor 2 or Age, k= levels of factor 3 (0, 0.1, 0.7), ε = 1, 2, 3…Error Effect, and l= number of replicates (16).

For behaviour observations occurring on day 3 and 7, the statistical analysis was a three factor completely randomized block design with the repeated measure of age:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\gamma_{ik} + \delta_l + \alpha\delta_{il} + \gamma\delta_{kl} + \alpha\gamma\delta_{ikl} + \varepsilon_{ijklm}$$

Where Y= Response, μ= Population Mean, α= Factor 1 or transport supplement, i=Levels of factor 1 (O, OL, No), β= blocking factor or Room, j=Levels of blocking factor (151, 152, 153, 156), γ= Factor 2 or dietary supplement, k=Levels of factor 2 (NS, Anti, Lys, AL), δ=Factor 3 or Age, l = levels of factor 3 (3, 7), ε = 1, 2, 3…Error Effect, and m= number of replicates (4).

The behaviour observation of feeding preference on day 3 was a completely randomized block design:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\gamma_{ik} + \varepsilon_{ijkl}$$

Where Y= Response, μ= Population Mean, α= Factor 1 or transport supplement, i=Levels of factor 1 (O, OL, No), β= blocking factor or Room, j=Levels of blocking factor (151, 152, 153, 156), γ= Factor 2 or dietary supplement, k=Levels of factor 2 (NS, Anti, Lys, AL), ε = 1, 2, 3…Error Effect, and l= number of replicates (4).

If significant main effects or interactions were found in the ANOVA in any of the models, the Tukey-Kramer option was used to compare differences among the least-square means ($P \leq 0.05$).

5.4 *Results and Discussion*

There is no published research on the overall behaviour patterns of turkey poults immediately post placement after a long transport. Research into the causes of early mortality and its reduction in turkey poults has been linked to behaviour observations (Bate, 1992, Aziz, 2001). Bate (1992) found that birds receiving sound stimuli of broody vocalizations had heavier body weights from day 3-21, but it is unclear whether the

sound stimuli decreased the presence of starve outs. Aziz (2001) found that the causes of high mortality in birds range from infectious disease, to the most common problem of starve outs. Changes in behaviour such as decreased movement, feeding and drinking may alert producers to problems within the flock. Knowing the behavioural changes resulting from long transport could benefit producers especially in Atlantic Canada due to routine long transport. A simulated long transport on 2 strains of laying hens found a decrease in competitiveness for food for one strain when birds were held for 14 hours, as well an increased perching response in birds that endured simulated long transport (Valros *et al.,* 2008).

The results of trial 2 placement behaviour observations are presented in table 5.2. Significant interactions ($P \leq 0.05$) of age and transport supplement occurred for feeding, drinking and locomotion behaviours, whereas only age showed significant ($P \leq 0.05$) effects for sitting or standing behaviours (Table 5.2). Co-variables of initial temperature, number of birds and lux were not found to significantly affect ($P > 0.050$) the behaviours observed throughout the trial (Appendix M). Blocking factor of room (where applicable) did not significantly ($P > 0.05$) affect the behaviours observed (Appendix M).

An interaction of age and transport supplement provided ($P \leq 0.05$) occurred in the feeding behaviour of the birds. At 1-hour post placement birds provided NO during transport had the highest feeding behaviours (36.3%) which was greater than birds fed any of the supplements at placement, 7-hours post placement, or birds supplemented with OL at 1-hour post placement, but was similar to birds fed O at 1-hour post placement. At 1-hour post placement birds that were un-supplemented (NO) during transport were hungrier than birds that received OL during transport and were hungrier than all birds at

placement or at 7-hours post placement. The lowest feeding behaviours were found at placement, birds supplemented with OL resulted in the lowest feeding behaviours (2.9%), which was lower than all birds at 1-hour post placement and birds supplemented with OL at 7-hours post placement. Lõhmus and Sundström (2004) found that in quail the decision

Table 5.2: Behavioural Observation of Turkey Poults Immediately After Placement and Throughout the First Day

Behavioural Sampling (% of birds performing each behaviour)					
Placement					
Transport Supplement[z]	**Feeding**	**Drinking**	**Sitting**	**Locomotion**	**Standing**
OAS	4.3cd ±3.50[†]	45.6a ±1.95	9.5 ±3.18	29.1abc ±3.01	11.9 ±3.54
OL	2.9d ±3.50	41.4a ±1.95	7.0 ±3.18	35.9a ±3.01	13.4 ±3.54
NO	8.4cd ±3.50	31.2b ±1.95	7.7 ±3.18	39.5a ±3.01	13.0 ±3.54
Age Mean	***5.2 ±2.02***	***39.1 ±1.13***	***8.0b ±1.84***	***34.8 ±1.74***	***12.8b ±2.04***
1 Hour Post-Placement					
OAS	28.6ab ±3.50	7.9c ±1.95	6.7 ±3.18	42.5a ±4.37	13.8 ±3.54
OL	15.4bcd ±3.50	11.4c ±1.95	10.2 ±3.18	44.7a ±4.37	17.3 ±3.54
NO	36.3a ±3.50	7.0c ±1.95	7.8 ±3.18	33.9ab ±4.37	14.7 ±3.54
Age Mean	***26.7 ±2.02***	***8.8 ±1.13***	***8.2b ±1.84***	***40.3 ±2.52***	***15.3b ±2.04***
7 Hours Post-Placement					
OAS	17.8bcd ±3.50	3.2c ±1.95	32.4 ±3.18	21.6bc ±3.13	24.8 ±3.54
OL	19.0bc ±3.50	3.6c ±1.95	32.2 ±3.18	19.1bc ±3.13	26.1 ±3.54
NO	17.4bcd ±3.50	2.4c ±1.95	38.8 ±3.18	15.1c ±3.13	25.9 ±3.54
Age Mean	***18.1 ±2.02***	***3.3 ±1.13***	***34.4a ±1.84***	***18.6 ±1.81***	***25.6a ±2.04***
ANOVA	**Feeding**	**Drinking**	**Sitting**	**Locomotion**	**Standing**
Transport (T)	0.03	0.01	0.66	0.44	0.70
Age (A)	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
T x A	0.03	0.002	0.60	0.04	1.00

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[†] Mean ± Standard Error
a-b Means within the same column with different letters differ significantly ($P \leq 0.05$).
Age Total: Placement=99.9%, 1 Hour Post placement = 99.3%, 7 hours post placement =100.0%

to leave cover and feed was more dependent on the behaviour of other members of the group, rather than individual hunger levels, but the duration of feeding was determined by the levels of individual hunger. Although there were differences seen, these

differences did not reflect improvements in growth performance such as body weight, weight gain or feed consumption. Differences in intestinal characteristics were also not reflective of these behaviour differences.

Drinking behaviour of the poults resulted in an age by transport supplement interaction ($P \leq 0.05$). Birds supplemented during transport with either O or OL had significantly higher drinking behaviour at placement than those birds that received NO during transport. All birds were placed at the bell drinker and beak dipped at placement to show them where the water was located. This significant interaction indicates that birds remaining un-supplemented during transport appeared more lethargic upon arrival and were not as quick to drink. This difference was no longer visible at 1h or 7h post placement, where all birds had similar drinking behaviour.

Sitting behaviour of the birds was not affected by transport supplement during the sampling periods of the first day, but resulted in a significant effect of age ($P \leq 0.05$). Birds had significantly more sitting behaviour at 7-hours post placement.

An interaction of age and transport supplement occurred in locomotion behaviour ($P \leq 0.05$). Birds provided with OL or NO at placement as well as birds provided with OAS or OL at 1-hour post placement were more active with higher locomotion behaviour than all birds at 7-hours post placement. Birds provided with NO at 1-hour post placement had higher locomotive behaviour ($P \leq 0.05$) than birds that remained un-supplemented at 7-hours post placement.

Standing behaviour of the birds was significantly higher ($P \leq 0.05$) at 7-hours post placement (25.6%) than at placement or 1-hour post placement (12.8, and 15.3%

respectively). There was no effect of transport supplement on the standing behaviour of the birds ($P>0.05$).

When behaviours were observed for days 3 and 7 there was no effect ($P>0.05$) of transport or dietary supplement on the behaviours of the birds, but age did have an effect ($P\leq0.05$) on the feeding and standing behaviours (Table 5.3).

Feeding behaviour of the birds was significantly ($P\leq0.05$) affected by the time of sampling. Higher feeding behaviour occurred in birds at day 3 than at day 7 with 9.5% of birds feeding during day 3 sampling and only 5.3% during day 7. Drinking, sitting and locomotion behaviour were not different ($P>0.05$) with birds performing behaviours similarly regardless of treatment or sampling day. There was an age difference ($P\leq0.05$) on the standing behaviour of the birds. Birds were standing more at day 7 than they were at day 3.

A transport supplement by dietary supplement and age interaction ($P\leq0.05$) occurred in both the feeding sitting behaviours. The Tukey-Kramer test was used to differentiate the means. It was found that no differences in means were observed using Tukey Kramer ($P>0.05$). Statistical consultation revealed that these interactions could be affected by the number of data pairs being used. In this analysis there are 12 dietary combinations plus 2 time periods, this creates 24 data point pairs. Tukey-Kramer controls the alpha value for the entire experiment. So as the number of pairs increases the probability of seeing differences due to treatment decreases. An ANOVA p-value for feeding and sitting behaviour was significant, but there was no ability of Tukey-Kramer

to differentiate the means. Slices of the interaction were performed using SAS, but the slices of the feeding or sitting interaction did not show any significant effects.

Table 5.3: Behaviour Observation of Turkey Poults during Day 3 and Day 7 after Placement

Behavioural Sampling (% of birds performing behaviour)					
Day 3					
Transport Supplement[z]	**Feeding**	**Drinking**	**Sitting**	**Locomotion**	**Standing**
OAS	9.9 ±2.12[†]	4.7 ±1.05	36.0 ±5.08	29.0 ±5.41	20.2 ±5.61
OL	8.1 ±2.12	3.1 ±1.05	30.6 ±5.08	32.6 ±5.41	25.1 ±5.61
NO	10.4 ±2.12	5.6 ±1.05	34.4 ±5.08	31.4 ±5.41	18.6 ±5.61
Dietary Supplement[y]					
ANTI	12.8 ±2.45	3.5 ±1.21	34.3 ±5.87	32.6 ±6.24	16.9 ±6.48
AL	5.5 ±2.45	4.6 ±1.21	37.5 ±5.87	31.1 ±6.24	21.2 ±6.48
LYS	8.3 ±2.45	4.1 ±1.21	31.9 ±5.87	27.4 ±6.24	27.6 ±6.48
NS	11.3 ±2.45	5.5 ±1.21	30.9 ±5.87	33.0 ±6.24	19.4 ±6.48
Age Mean	***9.5a ±1.23***	***4.5 ±0.60***	***33.7 ±2.93***	***31.0 ±3.12***	***21.3b ±3.24***
Day 7					
Transport Supplement					
OAS	6.8 ±2.12	4.0 ±1.03	28.8 ±5.08	24.3 ±5.41	36.3 ±5.61
OL	2.1 ±2.12	2.4 ±1.03	38.3 ±5.08	28.3 ±5.41	28.6 ±5.61
NO	7.0 ±2.12	3.5 ±1.03	24.2 ±5.08	37.6 ±5.41	28.3 ±5.61
Dietary Supplement					
ANTI	6.8 ±2.45	4.1 ±1.18	28.0 ±5.87	30.6 ±6.24	21.0 ±6.48
AL	6.7 ±2.45	2.3 ±1.18	32.1 ±5.87	29.3 ±6.24	29.4 ±6.48
LYS	3.1 ±2.45	2.4 ±1.18	27.3 ±5.87	26.6 ±6.24	40.4 ±6.48
NS	4.5 ±2.45	4.2 ±1.18	34.2 ±5.87	33.8 ±6.24	23.5 ±6.48
Age Mean	***5.3b ±1.23***	***3.3 ±0.59***	***30.4 ±2.93***	***30.0 ±3.12***	***31.1a ±3.24***
ANOVA	**Feeding**	**Drinking**	**Sitting**	**Locomotion**	**Standing**
Transport (T)	0.39	0.15	0.61	0.39	0.74
Supplement (S)	058	0.60	0.84	0.80	0.36
T x S	0.49	0.82	0.42	0.43	0.69
Age (A)	0.01	0.18	0.43	0.82	0.02
T x A	0.71	0.76	0.17	0.49	0.43
S x A	0.24	0.70	0.83	0.99	0.79
T x S x A	0.002	0.30	0.05	0.98	0.58

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

a-b Means within the same column with different letters differ significantly ($P \leq 0.05$).

Age totals across all behaviours: Day 3 = 100.0%, Day 7=100.1%

In relation to mortality of the birds, there were no significant effect of transport treatment affecting mortality in either trial (Tables 3.14 and 3.15). The behaviour differences observed during day of placement (Table 5.2) between transport treatments did not result in an effect on the mortality rates. Overall mortality was very low, with both trials highest mortality less than 2%. The mortality was only related to dietary treatment during trial 1 and did not have any relation to behaviour at days 3 or 7 as there is no effect of dietary treatment on behaviours of the poults at these time periods.

Figure 5.2 indicates the overall behaviours for each day of sampling. The differences in the charts show that the arrays of behaviours of the birds perform do not stay consistent from one sampling to the next.

Feeding behaviour is the lowest at placement (5.2%) and then at 1-hour post placement the feeding behaviour is at its highest (26.7%). At 7-hours post placement it had decreased to 18.1%, on day 3 and 7 it had further decreased to 9.5 and 5.3 % respectively. This observation was expected to be influenced by the time of sampling used within this trial. Savory (1980) found that in laying hens, food consumption is restricted to the light period, with peaks in consumption at either the beginning or end. Buyse *et al.* (1993) found that broiler chickens consumed the most feed in the beginning of a photoperiod. With a peak of consumption in late afternoon which was stored in the proventriculus and gizzard for utilization during the dark period fast (Buyse *et al*., 1993). Further studies aimed particularly at feeding behaviour should coordinate observations with the timing of the lights within the facility to ensure measurements are taken in relation to the lighting schedule. This was not done in the current study.

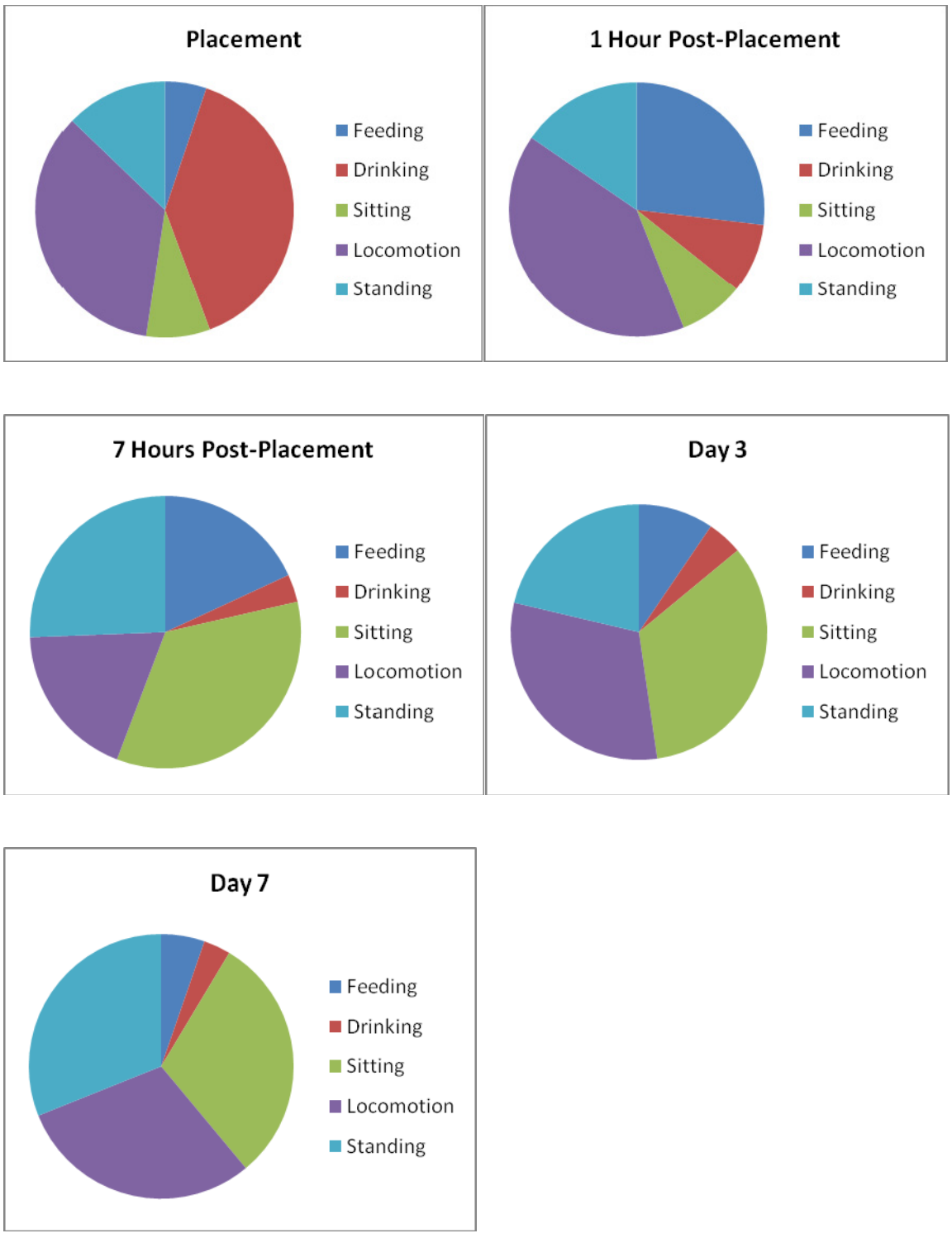

Figure 5.2: Mean Behaviours of Turkey Poults for Each Observation Period Regardless of Transport or Dietary Supplement Provided

Drinking behaviour of birds was at its highest immediately at placement with 39.1% of birds drinking. The birds were beak dipped and placed at the bell drinker, so it was expected that birds would spend the first few minutes drinking. By 1-hour post placement the total birds drinking was 8.8% which further dropped to 3.3% by 7-hours post placement. At day 3 and 7 drinking behaviour was similar with values of 4.5 and 3.3% respectively.

Sitting behaviour of the birds was initially low with 8.0 and 8.2% at placement and 1-hour post placement, but at 7-hours post placement birds were showing the highest sitting behaviour of 34.4%. At days 3 and 7, birds were similar in sitting behaviour with 33.7 and 30.4% respectively. Based on the observations in the current study sitting was equally occurring at 7h post placement, 3 and 7 days.

Locomotive behaviour is high immediately post placement, making up 34.8% of the behaviours observed. The locomotive behaviour increased to 40.3% at 1-hour post placement. By 7-hours post placement locomotive behaviour is at its lowest at 18.6%. On day 3 and 7 locomotive behaviour was similar at 31.0 and 30.0% representing almost 1/3 of the activity observed.

Standing behaviour was consistent between placement and 1-hour post placement with 12.8 and 15.3% respectively. At 7-hours post placement standing behaviour was 25.6%, while at 3 days 21.3% and at day 7 at 31.1%.

The trends in behaviour indicate that all birds at placement were hydrating themselves probably due to placement at the drinker and then moving around. A high percentage of birds were drinking after being beak dipped and placed in front of the

drinker. At 1-hour post placement birds changed behaviours to feeding and locomotion as the highest activities. This could be due to social facilitation in the birds. In group housing, Neilsen (2004) found that birds performed synchronized behaviours such as feeding and resting and that this synchrony is influenced by available feeding or resting space. Picard *et al.* (1992) reported that under group housing conditions, feeding sessions are reduced to approximately three or four per hour, but these sessions are longer in duration than when birds are individually housed and had increased intake that was associated with enough trough space for all birds at once. At 7-hours post placement birds were recorded to be sitting or standing the most. The high levels of sitting behaviour that begin at 7-hours post placement and continue through to 7 days are in agreement with a study by Bizeray *et al.* (2000) who performed scan sampling (days 1, 8, 15, 17) found that broilers spent 67% of their time lying down, with only 5% of their time devoted to locomotor behaviours and 28% of their time actively immobile (eating, drinking or standing). Febrer *et al.* (2006) found that 72% of broilers spent their observation time lying down, while 16% of time was spent feeding and drinking. In contrast to Bizeray *et al.* (2000), Febrer *et al.* (2006) found that birds spent up to 45% of time performing locomotor behaviours or comfort movements (stretching head or body, body shake). Buijs *et al.* (2010) found that stocking density of broilers did not affect the length of time spent standing, lying down, drinking or eating, but there was decreased time spent sitting, walking or preening when birds were at higher stocking densities. In contrast to our study, between days 1-8, Bizeray *et al.* (2000) found that locomotion behaviour increased slightly. In the current study the highest locomotion behaviour

occurred during day 1, and then decreased by day 7. Similarly to this trial, Bizeray *et al*. (2000) found that bird immobile behaviours decreased from day 1 to day 8.

Feeding of the birds was significantly different between the sampling times (P≤0.05) and feeding locations (Table 5.4) on day 1. Birds at both the feedbox and feeder showed the highest feeding behaviour at 1-hour post placement with 23.4 and 3.4% respectively. In feedbox feeding 7-hours post placement was greater than at placement (P≤0.05). This difference was not found in feeder feeding between 7-hours post

Table 5.4: Feeding Behaviour of Turkey Poults Given Different Feeding Locations on the Day of Placement

Feeding Preference		
Placement		
Transport Supplement[z]	**Feedbox**	**Feeder**
OAS	3.5 ±3.60[†]	0.7 ±0.83
OL	2.4 ±3.60	0.5 ±0.83
NO	7.2 ±3.60	1.0 ±0.83
Age Mean	***4.4c ±2.08***	***0.7b ±0.48***
1 Hour Post-placement		
OAS	25.3 ±3.60	3.3 ±0.83
OL	13.1 ±3.60	2.2 ±0.83
NO	31.8 ±3.60	4.5 ±0.83
Age Mean	***23.4a ±2.08***	***3.4a ±0.48***
7 Hours Post-placement		
OAS	16.9 ±3.60	0.9 ±0.83
OL	17.0 ±3.60	2.0 ±0.83
NO	15.8 ±3.60	1.6 ±0.83
Age mean	***16.6b ±2.08***	***1.5b ±0.48***
ANOVA	**Feedbox**	**Feeder**
Transport (T)	0.06	0.48
Age (A)	<0.0001	0.0004
TxA	0.07	0.49

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[†] Mean ± Standard Error
a-c Age means within the same column with different letters differ significantly (P ≤ 0.05).

placement and placement (P>0.05). Between the feedbox and feeder, during all sampling times on the day of placement, the birds favor the feedbox provided. Higher usage of the feedbox during the placement sampling time was expected as the birds were placed at the drinker which was in closer proximity to the feedbox than the feeder. This favorable feeding location carried through the entire placement day, with feeding at the feedbox higher by 6.8 fold at 1-hour post placement and 11 fold higher at 7-hours post placement.

On day 3 there was no significant effect of the transport or dietary supplement behaviours on the feeding behaviour of the birds in either the feedbox or feeder (Table 5.5). There was also no difference (P>0.05) between the feeding locations of the birds at day 3. The preference for using the feedbox did not exist when the birds were observed at 3 days. This was expected as no new feed was added to the feed box after placement.

There is no work readily available to confirm that turkey poults have a preference for feeding apparatus used. There has been work that shows that layer hens have a preference for feed color when it related to feed being palatable and hens can learn these preferences through social learning (Sherwin *et al.,* 2002). This may indicate that birds during the first day were feeding from the feed box, following cues set by the bolder poults within the pen. Allowing birds to display social learning of feeding location as well as synchrony of feeding behaviour was reported by Picard *et al*. (1992) in broiler chicks. In the feedbox, birds had more open space with feed, they could stand within the feed, and as well more birds could feed at one time. Although at up to 3 days birds could easily stand within the feeder, visual contact with other birds feeding was more limited due to the shape of the feeder itself. The accessibility to the feeder and feedbox were similar.

Table 5.5: Feeding Behaviour of Turkey Poults on Day 3 When Given Different Feeding Options within the Pen

Feeding Preferences		
Day 3		
Transport Supplement	**Feedbox**	**Feeder**
OAS	4.2 ±1.55[†]	6.0 ±1.57
OL	4.3 ±1.55	4.3 ±1.57
NO	5.7 ±1.61	4.9 ±1.63
Dietary Supplement		
ANTI	7.4 ±1.79	5.1 ±1.81
AL	2.1 ±1.79	3.5 ±1.81
LYS	5.0 ±1.89	4.8 ±1.91
NS	4.5 ±1.79	6.8 ±1.81
Age Mean	***4.8***	***5.1***
ANOVA	**Feedbox**	**Feeder**
Transport (T)	0.76	0.73
Supplement(S)	0.24	0.65
TxS	0.06	0.59

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL- Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.
[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.
[†] Mean ± Standard Error

5.5 Behaviour Conclusions

Overall birds showed significantly different behaviours when receiving transport supplements during the first day. Birds that had not received any supplementation during transport were feeding more at 1 hour post placement than birds that were supplemented with OAS or OL. Birds that received supplementation of OAS or OL were observed having higher drinking behaviour at placement than birds that remained un-supplemented. It was assumed that birds remaining un-supplemented were more lethargic upon arrival and did not have the energy to consume water, even when placed at the bell drinker. Activity of the birds was highest 1-hour post placement, whereas birds were sitting or standing most when observed at 7-hours post placement. After the initial day of

placement, there was no effect of the transport or post transport supplementation on the behaviours of the birds. In conclusion the transport supplements did have an effect on birds feeding and drinking behaviours on the day of placement, but these do not persist to days 3 or 7 of growth. The dietary supplements provided did not change the behaviours of turkey poults at days 3 or 7. Interestingly, feeding preferences were very clear on the day of placement, regardless of the time of sampling or transport supplements. Birds preferred to feed from the feedbox over the feeder. By day 3 this preference disappeared. Due to the very low mortality rates within the trial it was not possible to make any connections between mortality of the birds and their behaviour post placement.

Chapter 6: Overall Conclusions

Oasis® has been reported to improve production parameters as well as histological measurements, but has never been reported in association with an enzyme such as lysozyme. The combination of this supplement has provided varied results within this study. Growth production parameters (body weight, body weight gain, feed consumption and feed conversion) as well as mortality rates of poults were not overall affected by the supplementation of Oasis® alone or in combination with lysozyme. Oasis® alone increased resistance to breakage in the duodenum and ileum, increased the villi height and villi area. These improvements were not consistent between trials and did not have a significant effect on the growth performance of the birds. The drinking and locomotion behaviours of the birds was increased by Oasis® alone or in combination with lysozyme on the day of placement compared to the controls, but were not continued through to day 3 or 7.

Post placement dietary supplementation of lysozyme to turkey poults produced more varied results. Increases in body weight and feed consumption of birds fed combination of lysozyme and BMD during trial 1 occurred. A possible synergistic response existed in birds even after the lysozyme had been removed at day 28. Birds displayed similar body weight gain, feed conversion, and mortality rates regardless of dietary supplementation. Birds supplemented with BMD showed the highest mortality rates during trial 2, but no differences were observed in trial 1. With intestinal measures there appeared to be a significant effect of post transport supplementation. More specifically birds supplemented with BMD post transport had increased weights of the ileum, gizzard and proventriculus, although this result was not consistent across trials or

day of sampling. Lysozyme appeared to have an effect on the strength of the jejunum during early growth, but differences are not apparent by 70 days of age. With intestinal histology some improvements were linked to dietary supplementation. In trial 1 the mucosal widths of the duodenum, jejunum and ileum were thicker during days 56 and 70 with transport and dietary supplementation. Other intestinal parameters did not show differences when birds were provided with lysozyme up to 28 days of age. Determining the mechanism of synergism between BMD and lysozyme as combined supplement needs further investigation since some effects on body weights and feed consumption occurred.

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Appendix A: Diet Formulations for Starter Period (Day 0-14, 29% CP and 2850 kcal ME/kg) Trial 1

Ingredient	No Supplement (%)	Antibiotic & Lysozyme (%)	Antibiotic (%)	Lysozyme (%)
Soybean Meal	42.2	42.2	42.2	42.3
Corn	33.8	33.8	33.8	33.8
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.6	1.6	1.6	1.6
Poultry Fat	1.1	1.2	1.1	1.1
Mineral and Vitamin Premix[z]	1.0	1.0	1.0	1.0
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.3	0.3	0.3	0.3
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	-----	0.01
Total	100	100	100	100

Calculated Values			
Metabolizable Energy (Kcal/Kg)	2850	*Manganese (mg/Kg)*	89.6
Crude Protein (%)	28.0	*Selenium(mg/Kg)*	0.4
Linoleic Acid (%)	1.4	*Thiamin (mg/Kg)*	5.6
Crude Fiber (%)	2.5	*Arginine (%)*	2.0
Calcium (%)	1.4	*Histidine (%)*	0.7
Total Phosphorus (%)	0.9	*Methionine (%)*	0.6
Potassium (%)	1.1	*Methionine + Cystine (%)*	1.1
Magnesium (%)	0.2	*Sodium (%)*	0.2
Lysine (%)	2.0	*Dry Matter (%)*	90

Analyzed Values

	No Supplement	Antibiotic & Lysozyme	Antibiotic	Lysozyme
Dry Matter (%)	88.64	88.59	88.47	88.31
Crude Protein (%)	26.78	26.54	26.32	26.66
Calcium (%)	1.72	1.68	1.58	1.66
Phosphorus (%)	0.93	0.91	0.85	0.90
Sodium (%)	0.20	0.27	0.21	0.20
Potassium (%)	1.11	1.05	1.06	1.08
Magnesium (%)	0.20	0.19	0.19	0.19
Manganese (ppm)	176.64	134.36	136.19	135.63
Copper (ppm)	37.24	33.14	33.78	28.62
Zinc (ppm)	113.39	115.01	112.47	115.50
Crude Fat (%)	5.16	4.96	5.09	5.03

[z]Supplied per kg starter diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 2871 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 3045 mg; ethoxyquin, 50 mg; wheat middlings, 1049 mg; ground limestone, 500 mg.

[y]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

[w]Lysozyme, Neova Technologies Inc. (Providing 10.0mg $tonne^{-1}$ mixed feed).

Appendix B: Diet Formulations for Grower 1 Period (Day 15-28, 26.5%CP and 3000 kcal ME/kg) Trial 1

Ingredient	No Supplement (%)	Antibiotic & Lysozyme (%)	Antibiotic (%)	Lysozyme (%)
Soybean Meal	38.3	38.3	38.3	38.3
Corn	37.1	37.0	37.0	37.0
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.1	1.1	1.1	1.1
Poultry Fat	2.9	2.9	2.9	2.9
Vitamin and Mineral Premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	------	0.01
Total	100	100	100	100

Calculated Values			
Metabolizable Energy (Kcal/Kg)	3000	*Manganese (mg/Kg)*	88.6
Crude Protein (%)	26.5	*Selenium(mg/Kg)*	0.4
Linoleic Acid (%)	1.8	*Thiamin (mg/Kg)*	5.5
Crude Fiber (%)	2.5	*Arginine (%)*	1.9
Calcium (%)	1.3	*Histidine (%)*	0.7
Total Phosphorus (%)	0.8	*Methionine (%)*	0.5
Potassium (%)	1.1	*Methionine + Cystine (%)*	1.0
Magnesium (%)	0.2	*Sodium (%)*	0.2
Lysine (%)	1.6	*Dry Matter (%)*	90

Analyzed Values

	No Supplement	Antibiotic & Lysozyme	Antibiotic	Lysozyme
Dry Matter (%)	88.79	88.72	88.67	88.90
Crude Protein (%)	26.17	26.65	25.47	26.32
Calcium (%)	1.42	1.46	1.82	1.62
Phosphorus (%)	0.84	0.82	0.76	0.83
Sodium (%)	0.17	0.23	0.15	0.20
Potassium (%)	1.04	1.01	1.01	1.04
Magnesium (%)	0.19	0.19	0.18	0.19
Manganese (ppm)	107.83	125.94	106.96	137.09
Copper (ppm)	30.90	33.44	27.33	39.80
Zinc (ppm)	98.55	122.31	104.82	119.56
Crude Fat (%)	6.49	6.73	6.29	6.64

[z]Supplied per kg grower diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 1079 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 29.7 mg; ethoxyquin, 50 mg; wheat middlings, 905 mg; ground limestone, 500 mg.

[y] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

[w]Lysozyme, Neova Technologies Inc. (Providing 10.0mg $tonne^{-1}$ mixed feed).

Appendix C:Diet Formulations for Grower 2 (23%CP and 3200 kcal ME/kg) and Finisher (19%CP and 3250 kcal ME/kg) Period Trial 1

	Grower 2 (day 29-56)		Finisher (day 57-70)	
Ingredient	**NS (%)**	**Anti (%)**	**NS (%)**	**Anti (%)**
Soybean Meal	29.5	29.5	22.5	22.5
Corn	43.8	43.8	53.1	53.1
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	5.6	5.6
Limestone (ground)	1.6	1.6	1.7	1.7
Mono-Dicalcium Phosphate	0.8	0.8	0.6	0.6
Poultry Fat	4.8	4.8	4.8	4.8
Vitamin and Mineral Premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
Ameri-bond 2x[x]	0.5	0.5	0.5	0.5
BMD[w]	---	0.004	---	0.004
Total	100.0	100.004	99.8	99.804

Calculated Values

	Grower 2	Finisher		Grower 2	Finisher
Metabolizable Energy (Kcal/Kg)	3200	3250	*Manganese (mg/Kg)*	86.3	84.3
Crude Protein (%)	23	19	*Selenium(mg/Kg)*	0.5	0.5
Linoleic Acid (%)	1.3	1.4	*Thiamin (mg/Kg)*	5.3	5.2
Crude Fiber (%)	2.4	2.4	*Arginine (%)*	1.6	1.3
Calcium (%)	1.2	1.1	*Histidine (%)*	0.6	0.5
Total Phosphorus (%)	0.7	0.6	*Methionine (%)*	0.5	0.5
Potassium (%)	0.9	0.8	*Methionine + Cystine (%)*	0.9	0.8
Magnesium (%)	0.2	0.2	*Sodium (%)*	0.2	0.2
Lysine (%)	1.3	1.2	*Dry Matter (%)*	90	90

Analyzed Values

	Grower 2		Finisher	
	No Supplement	**Antibiotic**	**No Supplement**	**Antibiotic**
Dry Matter (%)	88.90	88.87	88.40	88.20
Crude Protein (%)	22.63	22.66	18.89	19.48
Calcium (%)	1.26	1.16	1.02	1.09
Phosphorus (%)	0.70	0.67	0.56	0.58
Sodium (%)	0.20	0.19	0.17	0.19
Potassium (%)	0.92	0.90	0.74	0.76
Magnesium (%)	0.18	0.19	0.14	0.16
Manganese (ppm)	120.04	111.06	107.30	115.99
Copper (ppm)	35.18	39.13	34.20	29.13
Zinc (ppm)	111.11	108.14	100.11	111.23
Crude Fat (%)	8.38	8.80	8.74	8.70

[z]Supplied per kg diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.03 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.3 mg; pyridoxine, 4.9 mg; thiamine, 2.9 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1296 mg; ground limestone, 500 mg.

[y]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]Ameri-bond 2x, Ligno Tech, Rothschild, WS, USA (providing 6.25mg tonne^{-1} mixed feed).

[w]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne^{-1} mixed feed).

APPENDIX D: Diet Formulations for Starter Period (Day 0-14, 29% CP and 2850 kcal ME/kg) Trial 2

Ingredient	No Supplement (%)	Antibiotic & Lysozyme (%)	Antibiotic (%)	Lysozyme (%)
Soybean Meal	42.2	42.2	42.2	42.3
Corn	33.8	33.8	33.8	33.8
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.6	1.6	1.6	1.6
Poultry Fat	1.1	1.2	1.1	1.1
Mineral and Vitamin Premix[z]	1.0	1.0	1.0	1.0
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.3	0.3	0.3	0.3
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	-----	0.01
Total	100	100	100	100

Calculated Values			
Metabolizable Energy (Kcal/Kg)	2850	*Manganese (mg/Kg)*	89.6
Crude Protein (%)	28.0	*Selenium(mg/Kg)*	0.4
Linoleic Acid (%)	1.4	*Thiamin (mg/Kg)*	5.6
Crude Fiber (%)	2.5	*Arginine (%)*	2.0
Calcium (%)	1.4	*Histidine (%)*	0.7
Total Phosphorus (%)	0.9	*Methionine (%)*	0.6
Potassium (%)	1.1	*Methionine + Cystine (%)*	1.1
Magnesium (%)	0.2	*Sodium (%)*	0.2
Lysine (%)	2.0	*Dry Matter (%)*	90

Analyzed Values

	No Supplement	Antibiotic & Lysozyme	Antibiotic	Lysozyme
Dry Matter (%)	92.27	93.79	93.13	92.65
Crude Protein (%)	30.71	30.09	30.28	31.4
Calcium (%)	1.32	1.43	1.38	1.34
Phosphorus (%)	0.87	0.91	0.90	0.88
Sodium (%)	0.17	0.21	0.18	0.19
Potassium (%)	1.23	1.13	1.19	1.18
Magnesium (%)	0.20	0.21	0.20	0.21
Manganese (ppm)	116.69	132.92	115.07	128.14
Copper (ppm)	36.98	35.47	30.14	38.70
Zinc (ppm)	124.63	118.34	120.76	119.83
Crude Fat (%)	4.50	4.92	5.03	4.76

[z]Supplied per kg starter diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 2871 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 3045 mg; ethoxyquin, 50 mg; wheat middlings, 1049 mg; ground limestone, 500 mg.

[y]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

[w]Lysozyme, Neova Technologies Inc. (Providing 10.0mg $tonne^{-1}$ mixed feed).

Appendix E: Diet Formulations for Grower 1 Period (Day 15-28, 26.5%CP and 3000 kcal ME/kg) Trial 2

Ingredient	No Supplement (%)	Antibiotic & Lysozyme (%)	Antibiotic (%)	Lysozyme (%)
Soybean Meal	38.3	38.3	38.3	38.3
Corn	37.1	37.0	37.0	37.0
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	8.0	8.0
Limestone (ground)	1.6	1.6	1.6	1.6
Mono-Dicalcium Phosphate	1.1	1.1	1.1	1.1
Poultry Fat	2.9	2.9	2.9	2.9
Mineral and Vitamin Premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
BMD[x]	-----	0.004	0.004	-----
Lysozyme[w]	-----	0.01	------	0.01
Total	100	100	100	100

Calculated Values			
Metabolizable Energy (Kcal/Kg)	3000	*Manganese (mg/Kg)*	88.6
Crude Protein (%)	26.5	*Selenium(mg/Kg)*	0.4
Linoleic Acid (%)	1.8	*Thiamin (mg/Kg)*	5.5
Crude Fiber (%)	2.5	*Arginine (%)*	1.9
Calcium (%)	1.3	*Histidine (%)*	0.7
Total Phosphorus (%)	0.8	*Methionine (%)*	0.5
Potassium (%)	1.1	*Methionine + Cystine (%)*	1.0
Magnesium (%)	0.2	*Sodium (%)*	0.2
Lysine (%)	1.6	*Dry Matter (%)*	90

Analyzed Values

	No Supplement	Antibiotic & Lysozyme	Antibiotic	Lysozyme
Dry Matter (%)	87.88	88.29	88.19	88.88
Crude Protein (%)	26.61	26.61	25.83	26.43
Calcium (%)	1.22	1.28	1.54	1.37
Phosphorus (%)	0.76	0.75	0.79	0.77
Sodium (%)	0.15	0.17	0.16	0.17
Potassium (%)	1.04	1.07	0.96	0.99
Magnesium (%)	0.18	0.18	0.17	0.18
Manganese (ppm)	88.56	128.79	90.26	117.07
Copper (ppm)	28.32	40.46	24.72	30.68
Zinc (ppm)	95.85	123.88	91.93	105.74
Crude Fat (%)	5.98	6.59	6.49	6.53

[z]Supplied per kg grower diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.015 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.6 mg; pyridoxine, 5.9 mg; thiamine, 2.9 mg; methionine, 1079 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; lysine, 29.7 mg; ethoxyquin, 50 mg; wheat middlings, 905 mg; ground limestone, 500 mg.

[y] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg $tonne^{-1}$ mixed feed).

[w]Lysozyme, Neova Technologies Inc. (Providing 10.0mg $tonne^{-1}$ mixed feed).

Appendix F: Diet Formulations for Grower 2 (23%CP and 3200 kcal ME/kg) and Finisher (19%CP and 3250 kcal ME/kg) Period Trial 2

	Grower 2 (day 29-56)		Finisher (day 57-70)	
Ingredient	**NS (%)**	**Anti (%)**	**NS (%)**	**Anti (%)**
Soybean Meal	29.5	29.5	22.5	22.5
Corn	43.8	43.8	53.1	53.1
Wheat	10.0	10.0	10.0	10.0
Poultry By-Product	8.0	8.0	5.6	5.6
Limestone (ground)	1.6	1.6	1.7	1.7
Mono-Dicalcium Phosphate	0.8	0.8	0.6	0.6
Poultry Fat	4.8	4.8	4.8	4.8
Vitamin and Mineral Premix[z]	0.5	0.5	0.5	0.5
Iodized Salt	0.3	0.3	0.3	0.3
Methionine Premix[y]	0.2	0.2	0.2	0.2
Ameri-Bond 2x[x]	0.5	0.5	0.5	0.5
BMD[w]	---	0.004	---	0.004
Total	100.0	100.004	99.8	99.804

Calculated Values

	Grower 2	Finisher		Grower 2	Finisher
Metabolizable Energy (Kcal/Kg)	3200	3250	*Manganese (mg/Kg)*	86.3	84.3
Crude Protein (%)	23	19	*Selenium(mg/Kg)*	0.5	0.5
Linoleic Acid (%)	1.3	1.4	*Thiamin (mg/Kg)*	5.3	5.2
Crude Fiber (%)	2.4	2.4	*Arginine (%)*	1.6	1.3
Calcium (%)	1.2	1.1	*Histidine (%)*	0.6	0.5
Total Phosphorus (%)	0.7	0.6	*Methionine (%)*	0.5	0.5
Potassium (%)	0.9	0.8	*Methionine + Cystine (%)*	0.9	0.8
Magnesium (%)	0.2	0.2	*Sodium (%)*	0.2	0.2
Lysine (%)	1.3	1.2	*Dry Matter (%)*	90	90

Analyzed Values

	Grower 2		Finisher	
	No Supplement	**Antibiotic**	**No Supplement**	**Antibiotic**
Dry Matter (%)	86.74	86.95	91.60	91.65
Crude Protein (%)	23.21	23.6	20.41	19.55
Calcium (%)	1.13	1.22	1.14	1.25
Phosphorus (%)	0.66	0.67	0.59	0.60
Sodium (%)	0.16	0.17	0.17	0.18
Potassium (%)	0.87	0.87	0.79	0.76
Magnesium (%)	0.15	0.16	0.15	0.15
Manganese (ppm)	97.83	97.95	85.97	106.64
Copper (ppm)	27.63	29.10	30.23	29.73
Zinc (ppm)	113.35	104.45	112.92	110.49
Crude Fat (%)	8.49	8.45	8.64	8.72

[z]Supplied per kg diet; vitamin A, 10,000 IU; vitamin D_3, 2000 IU; vitamin E, 35 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 27 mg; vitamin B_{12}, 0.03 mg; niacin, 76.2; folic acid, 4.9 mg, choline chloride, 801 mg; biotin, 0.3 mg; pyridoxine, 4.9 mg; thiamine, 2.9 mg; manganous oxide, 70. 2 mg; zinc oxide, 80.0 mg; selenium, 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 1296 mg; ground limestone, 500 mg.

[y]Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[x]Ameri-bond 2x, Ligno Tech, Rothschild, WS, USA (providing 0.00625% tonne^{-1} mixed feed).

[w]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne^{-1} mixed feed).

APPENDIX G: Temperature data from data logger. Trial 1

		151			152			153			156		
Day	Temp Setting	Average Temp	Min Temp	Max Temp	Average Temp	Min Temp	Max Temp	Average Temp	Min Temp	Max Temp	Average Temp	Min Temp	Max Temp
0	35	34.73	34	35	34.75	34	35	34.37	34	35	32.63	31	34
3	34	33.43	32	34	32.89	32	34	32.97	32	34	32.32	30	34
5	33	31.45	31	32	31.26	30	32	31.20	30	32	31.48	30	33
10	31	31.41	30	32	31.47	30	33	31.38	30	32	30.68	27	34
12	30	29.90	29	31	29.72	29	30	29.65	29	30	29.56	28	31
15	29	28.65	27	30	29.72	29	30	28.72	28	30	28.46	25	30
17	28	28.16	27	33	28.76	28	30	28.00	28	28	28.02	27	30
19	27	27.18	26	31	28.04	27	30	27.00	27	27	27.01	26	28
21	26	26.14	26	32	25.96	25	27	26.00	26	26	26.02	25	27
24	25	25.02	25	26	25.00	25	25	25.00	25	25	25.00	25	25
26	24	24.39	24	27	24.21	24	26	24.16	24	26	24.27	24	27
29	23	25.55	23	30	25.15	23	29	24.90	23	29	25.34	23	30
33	22	23.00	23	23	24.80	24	26	21.90	21	22	22.30	22	23
35	21	23.88	21	27	23.06	21	27	22.97	21	26	23.80	21	27
57	21	22.32	21	26	22.13	21	25	22.02	21	25	22.36	21	25
70	21	21.18	21	22	21.00	21	21	21.01	21	22	21.12	21	22

n=98 for each day. Temperature measures were taken every 15 minutes

APPENDIX H: Temperature data from data logger. Trial 2

		Room										
		151			152			153			156		
Day	Temp Setting	Avg Temp (ºC)	Min Temp (ºC)	Max Temp (ºC)	Avg Temp (ºC)	Min Temp (ºC)	Max Temp(º C)	Avg Temp (ºC)	Min Temp (ºC)	Max Temp (ºC)	Avg Temp (ºC)	Min Temp (ºC)	Max Temp (ºC)
0	35	33.5	32	34	33.4	31	34	32.7	29	34	33.1	32	34
3	34	33.0	33	33	33.0	33	33	31.9	31	32	33.0	32	32
5	33	33.6	32	35	32.1	31	33	31.8	31	32	31.9	31	32
10	31	30.7	30	31	31.0	30	31	29.6	29	30	30.3	30	31
12	30	29.8	29	30	30.0	29	30	29.8	29	30	29.8	29	30
15	29	28.7	28	29	28.8	28	29	28.0	28	29	28.8	28	29
17	28	27.8	27	28	28.0	27	28	27.8	27	28	27.8	27	28
19	27	26.9	26	27	26.9	26	27	26.9	26	27	26.9	26	27
21	26	26.1	25	27	26.0	25	27	25.9	25	26	25.9	25	26
24	25	24.9	24	25	25.0	25	26	24.8	24	25	24.9	24	25
26	24	24.0	23	24	24.0	24	24	23.9	23	24	24.0	24	24
29*	23	23.0	22	24	22.6	22	23	22.6	23	23	22.7	23	23
33	22	23.2	21	22	21.6**			21.5	21	22	21.0	20	22
35	21	21.1	21	21	21.2	21	21	21.4	21	22	21.3	21	21
57	21	21.1	21	21	21.3	21	22	21.1	21	21	21.3	21	22
70	21	21.0	21	21	21.1	21	21	21.6	21	22	20.8	21	21

n=98 for each day. Temperature measures were taken every 15 minutes

*Values from day 29 on are represented by measures taken morning and afternoon by hand (n=2).

** On Day 33 in room 152 only one value was measured

APPENDIX I: Mortalities occurring in Trial 1 with pathological diagnosis

Mort #	Pen #	Transport Supplement[z]	Dietary Supplement[y]	Date	Age (days)	Weight (g)	Notes	Pathological Diagnosis
1	42	OL	Anti	July 1	1	53		No specific diagnosis
2	42	OL	Anti	July 1	2	59		Omphalitis, klebsiella pneumonia
3	44	NO	AntiL	July 2	2	71		No specific diagnosis, Entrapment?
4	9	OL	AntiL	July 4	4	51		Omphalitis, E. Coli
5	46	NO	Anti	July 5	5	80		Omphalitis, E. coli
6	4	OL	L	July 5	5	73		No specific diagnosis
7	45	NO	L	July 5	5	60		Advanced autolysis
8	43	O	L	July 5	5	47		"starve out"
9	5	NO	Anti	July 7	7	57		Omphalitis, klebsiella ozanae, E. Coli
10	21	OL	Anti	July 9	9	65		Intestinal torsion
11	42	OL	Anti	July 11	11	145		Possible acute E. Faecalis septicemia
12	40	O	NS	July 29	29	264	Culled	Emaciation/Dehydration
13	17	O	Anti	Aug 2	33	501	Culled	Tibial head Osteomyelitis- E.Coli
14	4	OL	L	Aug 9	41	1590	Culled	Severe Eye infection
15	27	OL	Anti	Aug 11	43	1366		Tibial head Osteomyelitis

[z] Transport supplements: NO = no supplement, O = Oasis®, OL = Oasis® + Lysozyme

[y]Dietary Supplements: Anti = Antibiotic, AntiL = Antibioitic + Lysozyme, NS = No Supplement, L= Lysozyme

APPENDIX J: Mortalities occurring in Trial 2 with pathological diagnosis

Mort #	Pen #	Transport Supplement[z]	Dietary Supplement[y]	Date	Age (days)	Weight (g)	Notes	Pathological Diagnosis
1	13	O	AntiL	Dec 17	2	57	Culled	Eye Infection, E. Coli isolated
2	47	NO	Anti	Dec 17	2	54	Culled	Dehydration
3	4	NO	NS	Dec 17	2	39		Dehydration
4	12	O	Anti	Dec 17	2	42		Omphalitis, E. Coli, Strep. Faecalis Isolated
5	24	NO	NS	Dec 17	2	45		Omphalitis, E. Coli, Strep. Faecalis Isolated
6	13	O	AntiL	Dec 17	2	46		Omphalitis, Enterobacter avium isolated
7	5	OL	L	Dec 18	3	41		Dehydration
8	37	NO	L	Dec 18	3	40		Dehydration
9	45	NO	NS	Dec 18	3	38		Dehydration
10	45	NO	NS	Dec 18	3	41		Dehydration
11	13	O	AntiL	Dec 18	3	49		Omphalitis, Enterobacter avium and Streptococcus sp. Isolated
12	29	OL	NS	Jan 9	25	346		Very autolyzed- prolapsed cloaca
13	23	NO	L	Jan 12	28	335	Culled	No visible lesions
14	43	OL	AntiL	Jan 12	28	293	Culled	Large impacted gizzard- litter eating
15	43	OL	AntiL	Jan 12	28	469	Culled	Large impacted gizzard- litter eating
16	46	OL	Anti	Jan 12	28	263	Culled	Very soft bones – Possible Rickets

[z] Transport supplements: NO = no supplement, O = Oasis®, OL = Oasis® + Lysozyme

[y]Dietary Supplements: Anti = Antibiotic, AntiL = Antibiotic + Lysozyme, NS = No Supplement, L= Lysozyme

APPENDIX K: Typical Nutrient Profile of Oasis® Hatchling Supplement as Provided by Novus International Inc.

Oasis® Typical Nutrient Profile[z]			
Basic components	**g/100g**	**Vitamins**	
Moisture	26.2	Folic Acid	0.12mg/100g
Crude Protein	23.7	Niacin	1.38mg/100g
Fiber	1.9	Biotin	0.02mg/100g
Ash	3.5	Vitamin A	<30 IU/g
Crude Fat	1.2	Vitamin B1	0.22mg/100g
Carbohydrates	44.8	Vitamin B2	0.21mg/100g
Total	*101.3*	Vitamin B6	0.79mg/100g
Minerals		Vitamin B12	<0.44mg/100g
Calcium	0.14%	Vitamin C	<0.44mg/100g
Copper[y]	7 ppm	Vitamin E	2.67 IU/100g
Iron[y]	117 ppm	Vitamin K2	0.01mg/kg
Potassium	1.08%	Vitamin D3	36.30 IU/g
Magnesium	0.17%	Vitamin K3	0.01mg/kg
Manganese[y]	19 ppm	Vitamin D2	36.30 IU/g
Selenium	0.33 ppm	Vitamin K	0.84mg/kg
Zinc[y]	25 ppm		
Phosphorous	0.35%		
Sodium	0.02%		
Animal Acid Profile (w/w%)			
Methionine	0.40	Proline	1.35
Cystine	0.37	Glycine	1.18
Methionine + Cystine	0.76	Alaline	0.27
Lysine	1.39	Valine	1.25
Arginine	1.85	Isoleucine	1.15
Tryptophan	0.33	Leucine	2.21
Tyrosine	0.86	Histidine	0.69
Threonine	1.10	Hydroxyproline	0.04
Serine	1.28	Hydroxylysine	0.03
Phenylalanine	1.41	Taurine	0.06
Aspartic Acid	3.05	Lanthionine	0.03
Glutamic Acid	4.98	Ornithine	0.03

[z] – All information as provided by Novus International Inc. 2011.
[y]- Measured on an as-is basis

APPENDIX L: P-Values for Blocking Factor of Room on Production Parameters, and Intestinal Sampling.

Chapter 3- Production Parameter	Trial 1 P-Value	Trial 2 P-Value
Body Weight	0.92	0.92
Body Weight Gain	0.61	0.43
Feed Consumption	0.19	0.44
Feed Conversion	0.15	0.06
Percent Mortality	0.10	0.32
Chapter 4- Intestinal Sampling		
Gizzard Weight -Day 14	0.23	0.05
Gizzard Weight -Day 28	0.87	0.28
Gizzard Weight -Day 56	0.36	0.93
Gizzard Weight -Day 70	0.20	0.62
Proventriculus Weight- Day 14	0.12	0.01
Proventriculus Weight- Day 28	0.82	0.27
Proventriculus Weight- Day 56	<0.0001	0.004
Proventriculus Weight- Day 70	0.01	0.05
Duodenum Weight-Day 14	0.18	0.17
Duodenum Weight-Day 28	0.94	0.08
Duodenum Weight-Day 56	0.01	0.02
Duodenum Weight-Day 70	0.001	0.18
Duodenum Length -Day 14	0.47	0.10
Duodenum Length -Day 28	0.50	0.004
Duodenum Length -Day 56	0.18	0.73
Duodenum Length -Day 70	0.11	0.23
Jejunum Weight-Day 14	<0.0001	0.10
Jejunum Weight-Day 28	0.01	0.01
Jejunum Weight-Day 56	0.28	0.30
Jejunum Weight-Day 70	0.001	0.25
Jejunum Length- Day 14	0.02	0.42
Jejunum Length- Day 28	0.87	0.04
Jejunum Length- Day 56	0.09	0.45
Jejunum Length- Day 70	0.57	0.21
Ileum Weight-Day 14	0.11	0.30
Ileum Weight-Day 28	0.02	0.13
Ileum Weight-Day 56	0.23	<0.0001
Ileum Weight-Day 70	0.17	0.03
Ileum Length-Day 14	0.05	0.16
Ileum Length-Day 28	0.31	0.03
Ileum Length-Day 56	0.22	0.14
Ileum Length-Day 70	0.22	0.81

Chapter 4- Intestinal Sampling	Trial 1 P-Value	Trial 2 P-Value
Intestinal Breaking Strength-Day 28	0.23	0.66
Intestinal Breaking Strength-Day 70	0.90	0.001
Chapter 4- Intestinal Histology		
Duodenum Mucosal Width-Day 14	0.05	0.04
Duodenum Mucosal Width-Day 28	0.88	0.55
Duodenum Mucosal Width-Day 56	0.14	0.02
Duodenum Mucosal Width-Day 70	0.11	0.02
Duodenum Breakage - Day 14	0.87	0.11
Duodenum Breakage - Day 28	0.25	0.65
Duodenum Breakage - Day 56	0.38	.
Duodenum Breakage - Day 70	0.41	0.41
Jejunum Mucosal Width- Day 14	0.05	0.06
Jejunum Mucosal Width- Day 28	0.04	0.28
Jejunum Mucosal Width- Day 56	0.40	0.46
Jejunum Mucosal Width- Day 70	0.45	0.51
Jejunum Breakage- Day 14	0.02	0.18
Jejunum Breakage- Day 28	0.18	0.39
Jejunum Breakage- Day 56	0.75	.
Jejunum Breakage- Day 70	0.07	0.59
Ileum Mucosal Width -Day 14	0.06	0.70
Ileum Mucosal Width -Day 28	0.43	0.30
Ileum Mucosal Width -Day 56	0.53	0.13
Ileum Mucosal Width -Day 70	0.08	0.73
Ileum Breakage- Day 14	0.48	0.001
Ileum Breakage- Day 28	0.43	0.25
Ileum Breakage- Day 56	0.03	0.49
Ileum Breakage- Day 70	0.46	0.10
Ileum Villi Height- Day 14	0.91	0.02
Ileum Villi Height- Day 28	0.49	0.43
Ileum Villi Height- Day 56	0.03	0.09
Ileum Villi Height- Day 70	0.02	0.62
Ileum Crypt Depth-Day 14	0.07	0.12
Ileum Crypt Depth-Day 28	0.84	0.05
Ileum Crypt Depth-Day 56	0.83	0.62
Ileum Crypt Depth-Day 70	0.61	0.52
Ileum Villi Height/Crypt Depth Ratio -Day 14	0.07	0.02
Ileum Villi Height/Crypt Depth Ratio -Day 28	0.47	0.86
Ileum Villi Height/Crypt Depth Ratio -Day 56	0.06	0.39
Ileum Villi Height/Crypt Depth Ratio -Day 70	0.08	0.34
Ileum Midwidth-Day 14	0.85	0.18
Ileum Midwidth-Day 28	0.75	0.38

Chapter 4- Intestinal Histology	Trial 1 P-Value	Trial 2 P-Value
Ileum Midwidth-Day 56	0.36	0.01
Ileum Midwidth-Day 70	0.07	0.62
Ileum Villi Area- Day 14	0.70	0.14
Ileum Villi Area- Day 28	0.48	0.33
Ileum Villi Area- Day 56	0.12	0.08
Ileum Villi Area- Day 70	0.01	0.82

APPENDIX M: P-Values for Blocking Factor of Room on Behaviour Parameters.

	Blocking Factor	**Co-Variables**		
	Room	*Average Temperature*	*Average Lux*	*Number of Birds*
Day 0 Feeding	.	0.88	0.16	0.60
Day 0 Drinking	.	0.81	0.58	0.15
Day 0 Sitting	.	0.10	0.56	0.69
Day 0 Locomotion	.	0.84	0.74	0.26
Day 0 Standing	.	0.14	0.36	0.97
Day 3-7 Feeding	0.38	0.56	0.72	0.28
Day 3-7 Drinking	0.84	0.57	0.82	0.92
Day 3-7 Sitting	0.71	0.11	0.80	0.95
Day 3-7 Locomotion	0.82	0.13	0.77	0.21
Day 3-7 Standing	0.92	0.94	0.93	0.09
Day 0 Feedbox Feeding	.	0.72	0.14	0.65
Day 0 Feeder Feeding	.	0.36	0.53	0.86
Day 3 Feedbox Feeding	0.11	0.62	0.07	0.25
Day 3 Feeder Feeding	0.97	0.70	0.68	0.14

APPENDIX N: Effect of Transport and Dietary Supplement on the Duodenum Length of Heavy Hen Turkeys

Duodenum Length (cm)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	18.0 ±0.25[†]	22.7 ±0.27	30.7 ±0.39	32.2 ±0.38
OL	18.1 ±0.25	22.5 ±0.27	30.0 ±0.40	32.4 ±0.38
NO	18.1 ±0.25	22.4 ±0.27	30.0 ±0.39	33.0 ±0.38
P-value	*0.9436*	*0.7083*	*0.3945*	*0.3909*
Supplement[y]				
ANTI	18.3 ±0.29	22.1 ±0.31	29.7 ±0.46	32.6 ±0.44
AL	18.0 ±0.28	23.2 ±0.30	30.5 ±0.44	32.6 ±0.42
LYS	18.1 ±0.31	22.6 ±0.33	30.1 ±0.48	32.7 ±0.46
NS	17.9 ±0.29	22.3 ±0.31	29.7 ±0.45	32.2 ±0.44
P-value	*0.7872*	*0.0827*	*0.0734*	*0.8610*
Mean	**18.1**	**22.5**	**30.0**	**32.5**
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	17.2 ±0.25	22.3 ±0.23	31.2 ±0.39	32.8 ±0.37
OL	16.6 ±0.25	22.9 ±0.23	30.8 ±0.39	33.1 ±0.37
NO	17.0 ±0.25	22.2 ±0.23	30.4 ±0.39	32.6 ±0.37
P-value	*0.1740*	*0.0857*	*0.3271*	*0.6792*
Supplement				
ANTI	17.1 ±0.29	22.5 ±0.26	30.8 ±0.46	32.0 ±0.42
AL	16.7 ±0.29	22.7 ±0.26	30.9 ±0.46	33.5 ±0.42
LYS	16.8 ±0.29	22.6 ±0.26	30.5 ±0.46	33.1 ±0.42
NS	17.2 ±0.29	22.1 ±0.26	31.0 ±0.46	32.8 ±0.42
P-value	*0.6131*	*0.4249*	*0.8726*	*0.1164*
Mean	**16.9**	**22.5**	**30.8**	**32.8**

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

APPENDIX O: Effect of Transport and Dietary Supplement on the Jejunum Length of Heavy Hen Turkeys

Jejunum Length (cm)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	68.9 ±0.98[†]	89.2 ±0.97	127.0 ±1.75	138.6±2.32
OL	70.1 ±1.00	89.4 ±0.99	125.5 ±1.78	142.2±2.36
NO	68.9 ±0.98	91.7 ±0.97	125.1 ±1.75	138.1±2.32
P-value	*0.6227*	*0.1541*	*0.7184*	*0.4118*
Supplement[y]				
ANTI	69.7 ±1.14	89.3 ±1.13	124.5 ±2.04	139.6±2.70
AL	71.5 ±1.10	91.4 ±1.08	127.1 ±1.96	142.4±2.60
LYS	67.6 ±1.20	89.7 ±1.18	126.5 ±2.14	140.0±2.84
NS	68.5 ±1.13	90.1 ±1.12	125.5 ±2.02	136.5±2.68
P-value	*0.0898*	*0.5364*	*0.8026*	*0.4868*
Mean	**69.3**	**90.1**	**125.9**	**139.6**
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	68.3 ±0.88	90.1 ±1.27	132.0 ±2.06	141.7 ±1.39
OL	66.2 ±0.88	92.7 ±1.27	134.4 ±2.06	144.6 ±1.39
NO	66.6 ±0.88	90.9 ±1.27	130.1 ±2.06	142.2 ±1.39
P-value	*0.2165*	*0.3195*	*0.2418*	*0.2907*
Supplement				
ANTI	66.4 ±1.01	90.2 ±1.46	130.1 ±2.38	142.5 ±1.61
AL	67.5 ±1.01	93.2 ±1.46	131.1 ±2.38	143.7 ±1.61
LYS	67.5 ±1.01	90.9 ±1.46	131.6 ±2.38	143.2 ±1.61
NS	66.7 ±1.01	90.6 ±1.46	135.9 ±2.38	142.5 ±1.61
P-value	*0.8302*	*0.4812*	*0.2201*	*0.9838*
Mean	**67.0**	**91.2**	**132.2**	**143.0**

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

APPENDIX P: Effect of Transport and Dietary Supplement on the Ileum Length of Heavy Hen Turkeys

Ileum Length (cm)				
Trial 1				
	Age (days)			
Transport[z]	**14**	**28**	**56**	**70**
OAS	14.3 ±0.19[†]	18.3 ±0.23	26.3 ±0.36	27.8 ±0.39
OL	14.6 ±0.19	17.9 ±0.24	25.8 ±0.36	27.6 ±0.39
NO	14.2 ±0.19	18.4 ±0.23	25.7 ±0.36	28.1 ±0.39
P-value	*0.4421*	*0.2962*	*0.4476*	*0.6035*
Supplement[y]				
ANTI	14.3 ±0.22	17.9 ±0.27	26.0 ±0.42	27.8 ±0.45
AL	14.7 ±0.21	18.3 ±0.26	25.8 ±0.40	28.5 ±0.43
LYS	14.3 ±0.23	17.9 ±0.28	26.3 ±0.44	27.6 ±0.47
NS	14.2 ±0.21	18.6 ±0.27	25.6 ±0.41	27.5 ±0.45
P-value	*0.3040*	*0.2882*	*0.5861*	*0.3788*
Mean	**14.4**	**18.2**	**25.9**	**27.8**
Trial 2				
	Age (days)			
Transport	**14**	**28**	**56**	**70**
OAS	13.3 ±0.22	16.8 ±0.23	25.4 ±0.46	27.0 ±0.39
OL	13.3 ±0.22	16.7 ±0.23	24.8 ±0.46	26.7 ±0.39
NO	13.1 ±0.22	16.4 ±0.23	25.5 ±0.46	27.2 ±0.39
P-value	*0.7779*	*0.6465*	*0.4608*	*0.7120*
Supplement				
ANTI	13.1 ±0.22	16.6 ±0.26	26.2 ±0.53	26.8 ±0.44
AL	13.2 ±0.22	16.3 ±0.26	24.8 ±0.53	27.5 ±0.44
LYS	13.4 ±0.22	16.6 ±0.26	25.3 ±0.53	26.5 ±0.44
NS	13.2 ±0.22	17.1 ±0.26	24.6 ±0.53	27.1 ±0.44
P-value	*0.8699*	*0.2283*	*0.1719*	*0.4326*
Mean	**13.2**	**16.7**	**25.2**	**27.0**

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

APPENDIX Q: Effect of Transport and Dietary Supplement on the Ileum Midwidth of Heavy Hen Turkeys

Ileum midwidth (μm)					
		Age (days)			
Transport[z]		**14**	**28**	**56**	**70**
OAS		127.0 ±4.46[†]	141.8 ±5.30	213.5 ±12.09	253.1 ±11.50
OL		118.4 ±4.42	138.1 ±5.30	229.3 ±11.48	229.6 ±11.62
NO		125.7 ±4.42	142.8 ±5.59	206.0 ±11.06	240.8 ±11.62
P-value		*0.3472*	*0.8045*	*0.3344*	*0.3746*
Supplement[y]					
ANTI		133.1 ±5.06	155.0 ±6.00	218.0 ±5.30	237.4 ±13.34
AL		125.5 ±4.82	133.5 ±6.13	226.6 ±5.30	248.1 ±13.56
LYS		118.0 ±5.34	133.3 ±6.33	201.6 ±5.30	246.4 ±13.36
NS		118.1 ±5.34	141.7 ±6.35	219.0 ±5.30	232.6 ±13.97
P-value		*0.1522*	*0.0656*	*0.6235*	*0.8430*
Mean		**123.6**	**141.3**	**212.1**	**242.7**
Trial 2					
		Age (days)			
Transport	**0**	**14**	**28**	**56**	**70**
OAS	81.8 ±3.23	143.6 ±10.26	170.8±7.52	201.0±10.04	234.5±16.65
OL	76.5 ±2.13	170.5 ±11.54	167.6±7.22	212.4±10.69	238.5±13.85
NO	75.6 ±3.02	137.6 ±10.17	154.8±6.99	226.8±9.61	240.1±13.66
P-value	*0.3115*	*0.0703*	*0.2635*	*0.1992*	*0.9668*
Supplement					
ANTI		150.7 ±11.34	154.7±7.90	227.4±13.17	241.5±16.16
AL		147.8 ±12.19	178.9±8.28	193.9±11.37	251.4±15.40
LYS		155.1 ±12.37	155.5±8.76	211.3±10.84	208.7±18.40
NS		148.9 ±12.31	168.4±8.28	221.1±11.37	249.4±17.96
P-value		*0.9732*	*0.1448*	*0.2522*	*0.3145*
Mean	**78.0**	**150.6**	**164.4**	**213.4**	**238.8**

[z] OAS - Oasis® (Hydrated Hatchling Supplement, Novus International, Inc., St. Louis, Mo, USA), OL-Oasis® + Lysozyme (Inovapure™ 213 (active ingredient lysozyme at 20%, 24,000 Shugar units mg^{-1}) Neova Technologies, Inc., Abbotsford, BC, Canada), NO – No supplement provided during transport.

[y] NS- No supplement, ANTI –Antibiotic (BMD® 110 G (active ingredient methylene disalicylate, 110 g kg^{-1}) Alpharma Canada Corporation, Mississauga, ON., Canada), LYS – Lysozyme, AL – Antibiotic + Lysozyme.

[†] Mean ± Standard Error

APPENDIX R: Visual observation of Birds Dissected Immediately Post Transport (Day 0) Receiving No Supplement (NS).

Bird	Weight(g)	Esophagus	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Large Intestine	Ceca	Notes
1	44.24	-*	-	-	-	-	-	-	-	-	Nothing found
2	58.01	-	-	-	-	-	-	-	-	-	Nothing found
3	50.26	-	-	-	-	-	-	-	-	-	Nothing found
4	47.68	-	-	-	-	-	-	-	-	-	Nothing found
5	48.71	-	-	-	-	-	-	-	-	-	
6	59.82	-	-	-	-	Light yellow	-	-	-	-	
7	53.76	-	-	-	-	-	-	-	-	-	Nothing found
8	56.58	-	-	-	-	-	-	-	-	Pale & foamy	
9	58.33	-	-	-	-	-	-	-	-	-	Nothing found
10	53.28	-	-	-	-	-	-	-	-	-	Nothing found
11	59.91	-	-	Yellow	-	--	-	-	-	Pale	
12	53.01	-	-	-	-	-	-	-	-	-	Nothing found
13	49.29	-	-	-	-	-	-	-	-	-	Nothing found
14	51.73	-	-	-	-	-	-	-	-	-	Nothing found
15	50.35	-	-	-	-	-	-	-	-	-	Nothing found

*Indicates that there was nothing found present in this section

APPENDIX S: Visual observation of Birds Dissected Immediately Post Transport (Day 0) Receiving Oasis (OAS).

Bird	Weight(g)	Esophagus	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Large Intestine	Ceca
1	58.72	-*	-	-	-	-	-	-	-	Particles present
2	57.19	-	Green	Full	Full	Empty	Food Present	Food Present	-	Dark Green
3	51.40	-	Full	Full	Full	Green	-	-	-	Green & Foamy
4	54.74	-	Empty[z]	Empty	Food Present	Empty	-	Green Color	-	Green & Foamy
5	53.42	-	Empty	Empty	Green Color	Empty	Empty	Empty	-	Small
6	54.47	-	Full	Full	-	Empty	Empty	Green Color	Green Color	Light Green
7	54.04	-	Full	Full	Full	Empty	Empty	Green Color	Full	Green & Foamy
8	46.21	-	Full	Food Present	-	-	Empty			Green & Foamy
9	55.89	-	Full	Food Present	Full	Empty	Green Color			Green & Foamy
10	43.78	-	Full	Full	Full	Full	Full	Full	Full	Green & Foamy
11	55.35	-	Empty	Empty	Empty	Full	Empty	Empty	Empty	Empty
12	52.96	-	Empty	Green color	Green color	Food Present	Empty	Empty	Green Material	Green & Foamy
13	54.14	-	-	-	-	-	-	-	-	-
14	57.11	-	-	-	-	-	-	-	-	Green & Foamy
15	53.70	-	Food Present	Full	Full	Light green	Green	Green	-	Green & Foamy
16	55.40	-	Empty	Light green, Little food	Green, little food	Empty	Empty	-	-	-
17	51.44	-	Full	Full	Full	Light Green	Green	Green	Full	-

*Indicates that there was nothing found present in this section

[z] All sections indicated as "Empty" were found to have no feed within the section.

APPENDIX T: Visual observation of Birds Dissected Immediately Post Transport (Day 0) Receiving Oasis and Lysozyme (OL).

Bird	Weight(g)	Esophagus	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Large Intestine	Ceca
1	49.49	One Pellet	Little food and liquid	One pellet	Green food	-	-	-	-	Dark Green Foam
2	54.08	-	Full	Light green	Green food	Green	Green	Green	Food	Dark Green
3	54.85	Empty	One pellet	-	Empty	Empty	Empty	Empty	Green liquid, white soft deposits	Dark Green
4	49.31	Empty	Full	Green	Green	Green liquid	Green	Green	Dark green	Dark Green Foam
5	51.66	-	Full	Green pellets	Full	Food	Food	Food	Full	Green & Foamy
6	54.22	-	-	Empty	Empty	Clear liquid	-	-	Little green	Green Liquid
7	58.10	-	-	Empty	Empty	Clear liquid	-	-	Uric Acid	Brownish Green
8	50.21	Empty	Full	Light green	Green	Food	Little food	Empty	Full	Green & Foamy
9	58.66	Empty	Full	Light green	Light green	Clear liquid	Green	Green	Full; Uric Acid	Dark Green & Foamy
10	51.97	Empty	Half full	Green food	Light green and food	-	Little food	Little food	-	Dark Green & Foamy
11	56.52	-	Full	Green food	Light green and food	Green liquid	Pale green	Green	Full and green	Green Liquid
12	54.92	-	White foam	Empty	Empty	Empty	Empty	Empty	Little Uric Acid	Green Liquid
13	56.06	-	Full	Green food	Full	Green liquid	Green food	Green food	Full	Dark Green & Foamy
14	51.32	-	Blood	-	Yellow-green Color	-	-	-	Full	Green & Foamy
15	59.41	-	Full	Green Food	Food	Yellow green liquid	Green	Little green	Empty	Green & Foamy
16	49.46	-	Full	Green Food	Food	Green liquid	Little green	Little green	-	Green
17	49.79	-	Yellow paste	Empty	Empty	Empty	Empty	Empty	Empty	Green Liquid
18	53.56	-	Full	-	Full	Clear liquid	Green food	Green food	Full	Green & Foamy
19	46.21	-	Full	-	-	Yellow liquid	-	Green	-	Green & Foamy

*Indicates that there was nothing found present in this section

[z] All sections indicated as "Empty" were found to have no feed within the section.*9*