THE ROLE OF THE GIANT CANADA GOOSE (*Branta Canadensis maxima*) CECUM IN NUTRITION

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled,

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(*Branta Canadensis maxima*) CECUM IN NUTRITION

Presented by Delia M. Garcia

A candidate for the degree of Doctor of Philosophy

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THE ROLE OF THE GIANT CANADA GOOSE
(*Branta Canadensis maxima*) CECUM IN NUTRITION

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ABSTRACT

Waterfowl are renowned for their ability to exploit a wide variety of food resources. They feed on fresh and marine water invertebrates, aquatic and terrestrial plants, and agricultural grains and crops. This nutritional flexibility has allowed them to exploit virtually every water environment and establish a world-wide distribution. Their nutritional flexibility can be attributed to the diversity of anatomical, behavioral, and physiological adaptations that exist within Anatidae. Of particular importance is the ability of intestinal organs to adjust to a changing diet. The intent of this research was to investigate the role of the Canada goose (*Branta candensis maxima*) cecum in facilitating nutritional flexibility. True metabolizable energy assays indicated that the cecum increases the ability of the digestive system to extract energy from nutritionally poor foods (i.e. high fiber foods). Carboxymethyl cellulose assays conducted on cecal contents, confirmed the presence of cellulose-splitting bacteria within the ceca. True amino acid digestibility assays failed to detect differences in amino acid digestibility between intact and cecectomized geese. The bioassay, however, might not have been the appropriate approach to use with Canada geese. With the exception of a decrease in pancreas weight, the removal of the cecum did not lead to compensatory growth in the remaining digestive organs. Post-mortem examination, however, led to the discovery that 8 out of the 9 cecectomized geese had one or both ceca in various stages of regeneration.

From an evolutionary perspective, our results indicate that the ability of the cecum to extract additional nutrients from low quality foods could make a difference in survival during periods of food shortages or food unavailability.

CHAPTER 1

GENERAL INTRODUCTION

Often described as the morphological equivalent of the human appendix, the ceca are more than just a vestigial organ in mammals and birds. The ceca are outpocketings of the alimentary canal that originate at the junction of the small and large intestine. Due to the presence of large populations of bacteria, they are usually thought of as fermentation chambers (Braun and Duke 1989). Initially the ceca were believed to have a single function, but it is now clear that the ceca has multiple functions (Clench and Mathias 1995). Depending on the species and ecological conditions the cecum may function in: (1) bacterial fermentation, (2) nitrogen recycling, (3) osmoregulation, (4) nutrient absorption, (5) vitamin synthesis, and (6) immunological response (DeGolier et al. 1999).

The function of the ceca in mammals is well defined; however, the role they play in avian metabolism is not well understood (Braun and Duke 1989). This is in part due to the study subjects on which cecal function studies have been conducted, and/or the methodology utilized. Most of the information on cecal function has been obtained from studies using domestic and semidomestic galliformes and waterfowl (Clench and Mathias 1995). The size, behavior, availability, and economic importance of domestic galliformes make them ideal subjects, but unfortunately, their domestication makes them poor models in cecal function studies. The domestication process has altered their genetic make-up to such a degree that their ceca have lost much of their natural microflora and -fauna and physiological capabilities (Clench and Mathias 1995).

Although several research studies used wild subjects, the older literature is filled with contradictory or erroneous reports arising from methodological problems. Unless

measures are taken to insure that birds are maintained on a natural diet, cecal integrity can be compromised. Moss (1972) showed that the lengths of ceca in captive red grouse (*Lagopus lagopus scoticus*) decreased in length the longer they were held in captivity. By the end of the 4th year, the ceca of captive birds were 52% shorter than the cecal lengths of birds in the wild. In a comparative study of willow grouse (*L. l. lagopus*), Hanssen (1979a,b) found that the cecal microanatomy and biology between captive and wild birds were different.

In addition, many studies failed to give birds the necessary time for gut adjustments to a new diet prior to assessment of function. Most studies only allowed for an adjustment period ranging from a few days to a week (e.g. Miller 1976). Several experiments demonstrated that the adjustment time necessary for microbiological and histological composition ranges from 8 to 12 weeks (Duke et al. 1984, Moss and Trenholm 1987, Redig 1989). Furthermore, Moss (1989) suggested that galliform digestion can take place in two modes: 1) a low-fiber mode, and 2) a high-fiber mode. In low-fiber mode, "bulk does not limit intake and fiber digestion is unimportant;" whereas in high-fiber mode, "bulk limits intake and fiber digestion may well be important." He noted that in digestion studies, captive galliforms usually function in low-fiber mode, while wild galliforms function in high-fiber mode most of the time, especially during winter.

A considerable amount of effort is being spent on increasing our knowledge of avian ceca and their function. In their concluding remarks after the First International Avian Cecal Symposium, Braun and Duke (1989) outlined several avian cecal function questions that need to be addressed by future research. Important cecal questions

included: 1) cecal motility, 2) volume and composition of cecal contents, 3) the relationship between cecal size and colonic motility and contents, 4) functional differences among species, 5) functional differences between adults, juveniles, males and females, and 5) lower gut adaptations after cecectomy. In their review of the avian cecum, Clench and Mathias (1995) identified additional questions that needed to be addressed. These include identifying and describing the ceca of various species, conducting histological studies to understand how intestinal ceca change their length and/or mass in response to dietary changes, and exploring the functions of nonintestinal types of ceca such as lymphatic ceca.

To date, research on cecal function has been confounded by inappropriate subjects and/or methodology. Most of our knowledge applies primarily to domesticated or semi-domesticated species, which leaves a considerable gap in our knowledge of cecal functions in wild birds. The information generated from domestic birds might not be applicable to birds in their natural habitat; however, we can build on the knowledge we have acquired from domestic birds. For example, cecectomy is a technique commonly used by the poultry industry as a means of minimizing error during feeding trials. A series of feeding trials utilizing cecectomized and unaltered wild-captured birds, maintained on relatively natural diets, would help further our understanding of the role of ceca in the ecology and distribution of birds (Sedinger 1997).

Waterfowl Cecal Function

Waterfowl are renowned for their ability to exploit a wide variety of food resources. They feed on fresh and marine water invertebrates, aquatic and terrestrial

plants, and agricultural grains and crops. This nutritional flexibility has allowed them to exploit virtually every water environment and establish a world-wide distribution.

Their nutritional flexibility can be attributed to the diversity of anatomical, behavioral, and physiological adaptations that exist within Anatidae. Of particular importance is the ability of intestinal organs to adjust to a changing diet. Miller (1975) demonstrated that the gizzards, ceca, and small and large intestines of captive mallards (*Anas platyrhynchos*) respond to increased fiber levels in the diet by increasing in size. These morphological changes were also noted in free living birds by Ankney (1977) and Paulus (1982) in breeding and wintering birds, respectively. In wood ducks (*Aix sponsa*) Drobney (1984) noted that changes in their digestive organs reflected adaptations to changes in diet quality, metabolism, and food intake.

The functional causes for physiological and anatomical responses of digestive organs to season and diet are only partially understood. Drobney (1984) and Miller (1975) demonstrated that changes in gizzard weight reflected dietary changes. In wood ducks, gizzard weights increased in the fall when high-fiber plant foods were the predominant foods in the diet, but decreased in the spring when the diet consisted primarily of invertebrates or soft, low-fiber seeds (Drobney 1984). Likewise, small and large intestine size is also directly linked to changes in the amount of food (Ankney 1977) and diet quality (Miller 1975, Drobney 1984). Ankney (1977) linked the decreased intestine length of incubating female lesser snow geese (*Chen Caerulescens caerulescens*) to reduced food consumption during incubation. In wood ducks (*Aix sponsa*), Drobney (1984) associated decreased intestine weights to changes in food intake and diet quality. Decreased intestine weights between fall and spring corresponded to

changes from hard-seeded, high-fiber plant foods during the fall to soft, low-fiber plant foods in the spring.

The functional responses of the ceca to dietary and seasonal changes, however, are not as clear-cut. For example, despite food quality being highest during egg laying, the cecal lengths of wood ducks were at their maximum during that period (Drobney 1984). That pattern was also reported by Anderson (1972) for female pheasants.

While the significance of changes in intestinal morphology are easy to interpret, the significance of cecal changes are difficult to interpret because the function or functions of the ceca in waterfowl are poorly understood. This is primarily due to the reasons listed in the previous section (i.e. inadequate study subjects and/or methodology utilized). For example, Mattocks (1971) did not find cellulose-splitting bacteria in the cecum; as a result he concluded that geese were incapable of digesting cellulose and that crude fiber was unavailable to waterfowl. Unfortunately that study was conducted on domestic geese; recent studies have shown that herbivorous waterfowl can metabolize 25-74% of hemicellulose in plant foods (Krapu and Reinecke 1992).

To date there has been little or no recent progress in understanding the functional role of ceca in waterfowl. We know that ceca respond to seasonal and dietary changes by increasing or decreasing in size, but that is the extent of our knowledge. We do not understand how or why cecal changes occur. In particular, we do not know if the ceca is involved in nutrient absorption in waterfowl. If the ceca is important in nutrient absorption, we do not know if its importance varies depending on the diet (i.e. herbivores vs omnivores). It is also not known whether the ceca is important for growth, development, and survival in waterfowl.

This study investigated the cecal role in nutrient absorption in Canada geese (*Branta canadensis*) by comparing the digestive capability of cecectomized and intact birds. Chapter 2 addresses the importance of ceca in nutrient digestion. We determined the true metabolizable energy values (TME) of 6 foods fed to intact and cecectomized geese. Because gut microflora within the ceca are suspected of playing a role in amino acid digestibility, we also calculated the true amino acid digestibility (TAAD) for the 6 foods fed during the feeding trials in Chapter 3. In Chapter 4 we determined if digestive organs played a compensatory role after ceca removal. We conducted a series of measurements on the digestive organs of cecectomized and intact birds. In our last chapter we conducted an in vitro analysis utilizing the cecal contents of ceca removed during the cecectomies. Collection, housing, surgeries, and experimental procedures conducted during this research were in compliance with a University of Missouri certified Animal Care and Use Protocol (#3476).

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CHAPTER 2

DETERMINATION OF FOOD METABOLIZABILITY USING CECECTOMIZED AND INTACT CANADA GEESE

INTRODUCTION

The role of the cecum in nutrient digestion has been extensively studied in gallinaceous birds (Thornburn and Wilcox, 1965; Moss and Parkinson, 1972; Hanssen, 1979; Anderson and Braun, 1984). For many years bacterial degradation of dietary fiber was believed to be the cecum's primary function, but it is now known that the cecum might have various functions including bacterial fermentation, nitrogen recycling, osmoregulation, nutrient absorption, vitamin synthesis, and immunological response (DeGolier et al. 1999).

Due to economical and logistical reasons most cecal function studies have focused on domestic and semidomestic species (chickens, quail, pheasants, ducks, and geese) (Clench and Mathias 1995). Unfortunately, the information gathered from those studies cannot always be extrapolated to birds in the wild. As a result of genetic changes that occurred through domestication, and the use of nutritionally complete feeds, the cecum of these birds have lost most or all of their natural microflora and –fauna and potential physiological capabilities (Thomas 1987). This is also true among captive bred game species, or even those that have been captured from the wild. Mean cecal lengths and cecal microanatomy and biology are known to differ between wild and captive birds (Clench and Mathias 1995). Moss (1972) found that after 4 years of being raised in captivity, the cecal length of red grouse (*Lagopus lagopus scoticus*) was 54% shorter than

those of wild birds. Hanssen (1979) found the cecal microbiology of captive willow grouse (*L. l. lagopus*) to be different than that of wild birds.

We have found no published information of studies on cecal function within wild waterfowl. Information on waterfowl ceca is usually incidental to other studies. Nevertheless, many investigators have noted changes that occur in the ceca of waterfowl in response to dietary or seasonal changes (Miller 1975, Ankney 1977, Drobney 1984). Miller (1975) demonstrated that the gizzard, cecum, and small and large intestines of captive mallards (*Anas platyrhynchos*) respond to increased fiber levels in the diet by increasing in size. These morphological changes were also noted in free living birds during breeding (Ankney 1977) and wintering (Paulus 1982). In wood ducks (*Aix sponsa*) Drobney (1984) noted that changes in their digestive organs reflected adaptations to changes in diet quality, metabolism, and food intake.

The ceca of waterfowl have been classified as intestinal, being long (4-38 cm) and histologically similar to the small intestine (Clench and Mathias 1995). Barnes and Thomas (1987) found a correlation between cecal size and diet among several species within *Anatidae*. Herbivore species had larger ceca than carnivores, and omnivore species were intermediate between herbivores and carnivores. These observational studies lead one to assume that waterfowl ceca must play a role in nutrient metabolism. However, to our knowledge, no investigation has focused on the role of ceca in nutrient digestion in wild waterfowl.

In order to understand the function of the ceca in wild waterfowl, we first needed to determine the ceca's role in metabolizing foods of varying fiber content. Therefore, our first objective was to investigate the difference in food metabolizability in intact

(sham operated) and cecectomized (treatment) Canada geese. Based on the TME literature, we hypothesized that: 1) intact birds would be able to extract more energy from food, and thus we expected TME values to be higher among them. We also expected intact geese to have higher body weights than cecectomized geese, and that the effects of cecectomy would be greater among higher fiber foods which would be reflected in larger TME differences between cecectomized and intact geese.

METHODS

Experiment Birds

Geese were captured on 22 June 2000 during the flightless period at Stephens Lake and the Missouri Country Club in Boone County, Missouri. Upon capture, geese were sexed by cloacal examination. Seventeen and 13 female geese were retained from the Missouri Country Club and the Stephens College Lake, respectively. Two additional captures were conducted on September 03, 2000 at the City of Columbia Wetlands (4 geese) in Boone County, Missouri, and on December 08, 2000 at the University of Missouri South Farms (17 geese) in Boone County. Rocket nets aimed over a baited cracked corn site were used to capture geese on the latter two occasions. After capture, the geese were transported to the Charles Green Wildlife Area in Boone County, Missouri. They were weighed to the nearest gram using a spring scale and a cotton pillow case, then marked with numbered metal leg bands, and randomly assigned to one of the two following groups: 1) cecectomized (treatment), 2) intact (sham operated).

Husbandry

The geese were housed in 8' x 8' x 8' cages in an indoor facility equipped with windows to provide a natural photoperiod at the Charles Green Wildlife Area. Fresh water and grit (granite) were available to the birds at all times. Geese were initially maintained on a mixed diet consisting of 25% Purina Meat Builder (crude protein not less than 20.0%, crude fat not less than 3.0%, crude fiber not more than 5.0%) and 75% ground rabbit pellets (PMI Feeds, crude protein not less than 14.0%, crude fat not less than 1.5%, crude fiber not more than 25%) to ensure that their digestive system was operating in high fiber mode. After a month, we switched the geese to a commercial game-bird flight conditioner (crude protein <19.0%, crude fat no <2.0%, crude fiber not >12.0%; Purina Mills, St. Louis, Missouri, USA).

Surgical Procedure

Birds were fasted for 24 hours before surgery. Standard surgical procedures were followed during the cecectomies. Inhaled isoflourine (4-5% flow rate) was used to maintain a surgical plane of anesthesia. Birds were placed in a position of dorsal recumbency. Feathers were removed from the abdomen and the area was disinfected with iodine solution. A laparotomy was performed through an incision in the body wall. Each ceca was localized and the two distal ends were carefully detached by hand from the mesentery which joins them to the antimesenteric wall of the ileum. When both ceca were completely excised, each was transected and both cut surfaces sutured, as near as possible to the junctions of the ceca and the intestine. After the ceca were removed, the exposed intestine was returned to the peritoneal cavity, and the incision in the peritoneum and muscular layers closed by sutures. After the operation, solid food was withheld for

24 hours, but water was supplied *ad libitum*. During the sham procedure a laparotomy was performed and the viscera were manipulated. The ceca were taken out and the two distal ends were carefully detached by hand from the mesentery which join them to the antimesenteric wall of the ileum, the ceca were reinserted into the peritoneal cavity, and the incision in the peritoneum and muscular layers were closed by sutures.

The surgeries were conducted over four time periods. The first surgeries were conducted between August 14-20, 2000 by staff from the Department of Laboratory Animal Medicine at the University of Missouri, Columbia. With the exception of one bird, all cecectomized birds died as a result of complications from the surgery. All sham operated birds survived the surgery. Two additional birds were cecectomized on October 04, 2000, and both geese died from surgical complications. The remaining cecectomies were performed on November 14-16, 2000 and on March 22, 2001 by staff from the Department of Animal Sciences at the University of Missouri, Columbia. One of the geese died after surgery, but all others survived without complications.

Feeding Trials

Feeding trials were conducted between 02 June and 22 August 2001 in an unheated laboratory with a natural photoperiod. Six foods were tested: 1) corn (*Zea mays*), 2) milo (*Sorghum vulgare*), 3) game-bird flight conditioner, 4) curly dock (*Rumex crispus*), 5) tall fescue seed (*Festuca arundinacea*), and 6) buttonbush (*Cephalanthus occidentalis*). Mature curly dock seeds were harvested by hand from standing plants at the Thomas S. Baskett Wildlife Area in Boone County, Missouri. Buttonbush seeds were harvested by hand from standing plants at the Duck Creek Wildlife Area in Stoddard and Bollinger counties, Missouri. All other seeds were obtained from local vendors. Seeds

were allowed to air dry for >48 hours, and then stored at 5°C to minimize respiration prior to feeding trials.

Six intact and six cecectomized geese were precision-fed using the procedure described by Sibbald (1976). Feeding treatments were arranged in the form of two 6 X 6 Latin square designs; one for each bird group (intact or cecectomized). We conducted seven feeding trials (the last row of the 6th trial was repeated during the 7th trial to test for residual effects) and for each bioassay six experimental birds were precision-fed test foods. This experimental design resulted in each bird being fed each test food once. An additional three birds (for each group) were selected to serve as controls during each bioassay. Those birds served as controls in all 7 feeding trials.

Procedures for TME bioassays followed Sibbald (1986). All geese were weighed to the nearest 0.01 kg and fasted for 48 hr in individual metabolism cages (61 x 46 x 61 cm) with access to fresh water at all times. Experimental birds were then precision-fed a known amount of test food through a copper tube (1.2 x 40 cm) inserted down the esophagus. On average (± SE), birds were fed the following dry mass of each food: corn (20.83 ± 0.83 g), milo (21.54 ± 1.01 g), game-bird flight conditioner (13.50 ± 1.50 g), rumex (21.54 ± 1.01 g), fescue (12.3 ± 0.30 g), buttonbush (12.21 ± 0.29 g). Food was slowly poured into the funnel attached to the upper end of the tube and pushed down the tube with a wooden rod. The rod was of a diameter that allowed no foods to adhere to the tube or funnel. After the withdrawal of the tube from the esophagus, a stream of distilled water was forced into the bird's mouth to encourage swallowing. Cecectomized and intact birds were fed only once during the feeding trial, and precision feeding took 5-7 min/goose. For control birds, the feeding tube was inserted into their esophagus, but they

were not fed. Immediately after feeding the geese were returned to their individual metabolism cages. A 60 x 90 cm piece of plastic sheeting was attached beneath all cages to collect all fecal and urinary matter. Birds that regurgitated any portion of the test food were eliminated from the bioassay.

Excreta samples were collected for 48 hr and frozen for subsequent analysis. Feathers were removed from the excreta with forceps, and the excreta were sieved to remove any grit that had passed through the digestive tract (Biligi et al. 1982). If the samples contained intact seeds and could not be sieved, grit was removed by hand using forceps. Excreta was oven-dried at 60°C, weighed to the nearest 0.01 g, and ground in a blender for 3 minutes. Sub-samples of 1 g were oven-dried to a constant mass at 80°C to determine percent moisture. Gross energy of test foods and excreta from fed and fasted birds was determined on duplicate subsamples via a Parr adiabatic oxygen bomb calorimeter (30 atm O_2).

True metabolizable energy (kcal/g) was calculated after Sibbald (1976):

$$TME = [(GEF)\ (X) - (EEF - EEC)]/X$$

where GEF is the gross energy of the food (kcal/g), X is the dry weight fed (g), EEF is the energy voided as excreta by the experimental bird, and EEC is the energy voided as excreta by the control bird. The average energy excreted by control birds was used to estimate EEC because no correlation between body weight and excreta energy is apparent for Canada geese (Buckley 1989). Therefore, the values for energy and dry matter excreted by control birds in a feeding trial were pooled to calculate TME and TME_N. Use of pooled values reduces standard errors associated with TME, TME_N, and MEF values (Buckley 1989, Petrie 1994). True metabolizable energy values corrected to zero

nitrogen balance (TME_N) were calculated following procedures of Sibbald and Morse (1983). All TME_N values were converted to a measure of metabolic efficiency (MEF) using the equation (Hoffman and Bookhout 1985):

$$MEF = (TME_N / GEF)*100\%$$

MEF was determined for each food to facilitate comparisons with previous studies.

Nutrient content of each food was determined using proximate analysis. Crude protein was determined using a LECO FP-228 Nitrogen Determinator (Sweeney and Rexroad 1987) with 10 percent reruns by manual Kjeldahl with copper catalyst (AOAC 1984). Percent moisture was determined by drying in a vacuum oven at 95-100°C, crude fat was calculated using the indirect method, and crude fiber was estimated using the asbestos-free method (AOAC 1984). Ash content was determined by heating samples in a furnace at 625°C for 15 hours (AOAC 1984). Amino acid composition was determined with post-column derivitization on a Beckman 6300 amino acid analyzer with full computer integration following AOAC official method 982.30E (A,B,C) CHP 45.3052000.

Statistical analysis

We conducted a priori sample size determination by utilized theTME and TME_N values determined by Petrie (1994) for corn and milo. A sample size of 3.42 geese would be needed to estimate the TME of corn with a 95% level of precision. Whereas 4.10 geese would estimate TME of milo to a 90% level of precision. To estimate TME_N of corn at a 95% level of precision a sample size of 4.97 geese or more was needed. TME_N for milo could be estimated to a 90% level of precision with 2.25 geese or more.

At the initiation of the feeding trials, body mass of geese from each group was compared using a t-test. Changes in mass for both groups over the course of the study were evaluated using analysis of variance (ANOVA). Body masses used in the analyses were obtained from geese at the beginning of the food deprivation period during each trial. The experiment was designed in the form of two 6 x 6 Latin squares; one square for each bird group (intact and cecectomized). For each Latin square, an ANOVA was calculated using GLM (PROC GLM; SAS Institute 1989), and an F-test was conducted to determine if the data could be pooled. TME_N and weight data were analyzed using a mixed model analysis of variance (PROC MIXED; SAS Institute 1989) employing $\alpha = 0.05$. Treatment, period, food, and treatment-food interactions were treated as fixed effects. Significant differences among or between least square means were separated by repeated *t*-tests using the LSMEANS option of SAS. Statements of probability were based on $P \leq 0.05$. Confidence intervals (95%) were also estimated to provide an estimate of the effect size.

RESULTS

All birds remained healthy throughout the trials. Despite decreasing the amount of food fed after the first feeding trial, regurgitation was a problem throughout our feeding trials. Birds from a trial that regurgitated any portion of the test food were eliminated. Extra trials were not conducted to fill in missing values for birds that regurgitated a food type.

Body Mass

Body mass of Giant Canada geese used in this study ranged from 3.40 to 4.40 kg at the onset of feeding trials in June 2001 (mean = 3.87 ± .06 kg). Differences in body mass between treatments were significant (Table 1). Weight was not affected by trial, food fed, or treatment*food interaction. Cecectomized geese were generally heavier than intact geese (Table 2). Intact geese weighed 3.85 ± 0.10 kg (SE) at the beginning of feeding trials in June, and weighed 3.67 ± 0.14 kg at the termination of the trials in August. Cecectomized birds weighed 3.89 ± 0.08 kg and 3.98 ± 0.12 kg in June and August respectively. There were no significant differences in body weight between intact and cecectomized geese during the 1st, 2nd, 3rd, 6th and 7th feeding trials; however, cecectomized geese were significantly heavier than intact geese during the 4th and 5th feeding trials (Table 2).

Nutrient and Energy Content of Foods

On average (± SE), birds were fed the following dry mass of each food: corn (20.83 ± 0.83 g), milo (21.54 ± 1.01 g), game-bird flight conditioner (13.50 ± 1.50 g), rumex (21.54 ± 1.01 g), fescue (12.3 ± 0.30 g), and buttonbush (12.21 ± 0.29 g). The average gross energy of foods utilized in this study was 3.91 ± 0.06 kcal/g and ranged from 3.68 kcal/g to 4.05 kcal/g. Average gross energy of agricultural grains (3.90 ± 0.11 kJ/g) was similar to the gross energy of natural foods (3.90 ± 0.08 kcal/g). Game-bird flight conditioner contained less energy (3.68 kcal/g) than any other food item in either the agricultural or natural food groups (Table 3). Crude protein levels of game-bird flight conditioner (20.74%) and rumex (16.37%) exceeded that of all other foods which ranged from 5.78% (buttonbush) to 14.77% (fescue) (mean = 12.57 ± 2.30%). Crude fat varied

from 2.25% (fescue) to 4.89% (corn) with a mean of 3.59 ± 0.43%. Crude fiber levels were higher for natural foods and game-bird flight conditioner (relative to agricultural grains 1.58-1.62%) and ranged from 1.58 (corn) to 49.26% (buttonbush) with a mean of 12.94 ± 7.40%. Percent ash in game-bird flight conditioner (8.70%) exceeded all other food types which ranged from 1.61% (corn) to 5.66% (fescue) with a mean of 3.74 ± 1.17%.

TME, TME_N, Digestibility

Mean TME values for each food (buttonbush, corn, game-bird flight conditioner, fescue, milo, rumex) and bird group (intact, cecectomized) is summarized in Table 4. Buttonbush had the lowest TME for both intact (1.29 kcal/g) and cecectomized (0.66 kcal/g) groups. The highest food TME value varied between the intact and cecectomized groups. Corn (4.09 kcal/g) had the highest TME value for the intact birds, while milo (4.18 kcal/g) had the highest TME value for the cecectomized group. Mean TME_N values for each food and group is summarized in Table 4. Correcting to zero nitrogen balance (TME_N) decreased TME values of all foods for the intact group by 3.56% (range 0.51 – 7.75%; Table 4). For the cecectomized group, TME was reduced by 4.14% (range 0 – 15.15%) for buttonbush, game-bird flight conditioner, fescue, milo, and rumex (Table 4). In addition, standard error estimates generally decreased between 8.33% and 30.77% (Table 4). The standard error estimates decreased between 8.33% and 16.67% for intact birds fed corn, fescue, milo, and rumex. For cecectomized birds fed corn, fescue, and milo, standard error estimates decreased between 15% and 30.77 (Table 4).

Overall, food metabolizability (TME_N) did not appear to be affected by treatment, period, or treatment*food interactions (Table 6). TestS foods differed in TME_N ($F_{5,48}$ =

124.39, $P < 0.0001$; Table 6). Pairwise comparisons of TME_N between foods indicated that geese metabolized significantly more energy from corn (3.97 kcal/g) and milo (4.03 kcal/g) ($P < 0.05$) than from game-bird flight conditioner, buttonbush , fescue, and rumex (Table 7). Game-bird flight conditioner (3.22 kcal/g) and fescue (3.18 kcal/g)) were intermediate in TME_N and provided significantly more metabolizable energy than buttonbush (0.90 kcal/g) and rumex (2.30 kcal/g). Buttonbush provided the least TME_N (0.90 kcal/g) (Table 7). Buttonbush was the only food to result in a significant treatment*food interaction (Table 8). The buttonbush TME_N of intact geese was more than twice that of cecectomized geese. No interactions occurred between treatment and corn, game-bird flight conditioner, fescue, milo, or rumex (Table 8).

Test foods also differed in digestibility (MEF) ($F_{11,63} = 65.45$. $P < 0.0001$). Both groups of geese metabolized a significantly higher percentage of the available energy from corn and milo than from game-bird flight conditioner, buttonbush, fescue, and rumex ($P < 0.05$; Table 9). With the exception of buttonbush, MEF was comparable between the two groups for all foods tested. The metabolic efficiency of buttonbush was twice as high among intact geese (Table 9). Cecectomized geese had slightly higher MEF values for game-bird flight conditioner and milo. MEF did not differ between corn and milo or between game-bird flight conditioner and fescue (Table 10). Buttonbush had the lowest metabolic efficiency of all foods tested (Table 10).

Regression analysis of each proximate measure on TME_N revealed that only crude fiber contributed significantly to variation in TME_N among foods (Fig. 1). None of the correlation coefficients of the remaining independent measures equaled or exceeded 0.20 (Fig. 2; Table 11). The coefficient of determination (r^2) for regression of TME_N on

percent of crude fiber was 0.82 (Table 11). Examination of error terms associated with the model indicated that transformation of crude fiber and TME_N was unwarranted.

DISCUSSION

As with previous studies (Buckley 1989, Petrie 1994, Checkett 2001) regurgitation of foods during the trial periods was a problem. As a result, we decreased the amount of food fed between the first and second trial, but some birds still regurgitated during the 4th, 5th, and 7th feeding trials. Milo was the only seed not regurgitated during any of the trials. All remaining foods were regurgitated at least once, despite following the recommendation of spraying a mist of water down the throat of each bird after feeding was complete to induce peristalsis (Checkett 2001). After the birds were placed in the cages, several geese regurgitated contaminating nearby cages. As a result, some samples were lost because of cross contamination.

Changes in Body Mass

Changes in goose body mass between June and August were similar to those reported for wild birds of the same age and class (Hanson 1962). Intact geese weighed less at the end of the study, but cecectomized geese had actually increased in weight. The Latin square design of this study in which all foods are fed during each feeding trial was conducted in order to reduce any variation caused by temporal changes in bird physiology and influence of climate. Regardless, the average body mass of geese used in this study varied by only 10% between June and August, and we detected no significant difference between the weights of geese at the beginning and end of the feeding trials.

We concluded that the nutritional status of experimental birds did not bias our estimates of digestibility for any of the 6 test foods.

There was a discrepancy in weight between cecectomized and intact birds, with cecectomized birds being generally heavier. Weight differences between treatments were only statistically significant during the 4th and 5th feeding trials. The correlation coefficient between TME_N and goose mass was -0.16 (P = .1557; n = 76), suggesting that TME_N was not a function of body mass in our experiment. Kaminski et al. (2003) found no relationship between acorn TME's and mean mass of wood ducks.

TME, TME_N and MEF

Available information on metabolizable energy and estimates of digestibility for foods consumed by waterfowl is limited. To date, there is no information on metabolizable energy and estimates of digestibility for foods consumed by cecectomized waterfowl. Previous studies that determined the TME of foods similar to those included in this study generally compared well with the results of our feeding trials. Our TME_N value for corn of 4.02 kcal/g (intact) was slightly higher than the 3.90 kcal/g and 3.86 kcal/g determined by Petrie (1994) and Buckley (1989), respectively for Canada geese. The TME_N value of 3.88 kcal/g for corn obtained from cecectomized geese was similar to the values obtained by Petrie (1994) and Buckley (1989). The TME_N value for milo of 3.93 kcal/g was higher than the 3.78 kcal/g determined by Petrie (1994), but lower than the 4.02 kcal/g value obtained by Buckely (1989). The cecectomized value of milo (4.13 kcal/g) was higher than both previous estimates. Our TME_N values for rumex of 2.37 (intact) and 2.23 kcal/g (cecectomized) were lower than the 2.68 kcal/g obtained by Checkett (2001) for mallard ducks. Our MEF values for corn and milo in intact birds

ranging from 88.35% to 88.91% were similar to those found by Buckely (1989) and Petrie (1994) (84.58% to 89.29%). The difference in MEF ranges suggest that intact Canada geese in this study were more efficient at metabolizing the energy in these food types.

Our higher TME_N and MEF values might be attributed to the pre-trial maintenance diet. During this study, we provided a pre-trial maintenance diet that contained high fiber (<12%) levels. Whereas the pre-trial maintenance diets provided by Petrie (1994) and Buckley (1989) contained very low fiber (<5%) levels. Maintaining birds on a pretrial maintenance diet with a high fiber level may have initiated changes in gut morphology and increased the TME_N and digestibility estimates of fibrous foods (Miller 1975). It has been suggested that TME bioassays of high fiber foods should be preceded by an acclimation period to the test foods (Buckley 1989). Petrie (1994) found no evidence that Canada geese maintained on a diet of alfalfa pellets (29% fiber) metabolized more energy from high fiber foods than geese maintained on a low fiber poultry ration (<5% fiber). However, the amount of time Petrie (1994) maintained the geese on the alfalfa pellets prior to the start of the feeding trials was not stated. Mallard ducks maintained on a high fiber diet did not metabolize more energy from high fiber foods (Checkett 2001); however, the mallards were maintained on the high fiber diet for 21 days prior to the initiation of feeding trials. In this study Canada geese were fed the maintenance diet for a minimum of 6 months prior to the start of feeding trials. The longer acclimation period to the high fiber diet may have contributed to the differences in TME.

In this study, we selected foods with a wide range of fiber content in order to determine differences in fiber processing between treatment groups. Crude fiber accounted for 82% of the variation in TME_N among foods included in this study. Overall, food metabolizability (TME_N) was not affected by treatment, but buttonbush had a significant treatment*food interaction. On average, buttonbush TME_N estimates for intact birds were more than twice that of cecectomized birds. Buttonbush had a much higher fiber (49%) content than any of the other test foods (1.58-9.42%). This is perhaps the reason buttonbush exhibited significant differences in TME_N between treatments. Geese were maintained on a relatively high fiber pre-trial maintenance diet, but relative to the higher fiber content of buttonbush it might not have been high enough. The fiber level of the maintenance diet was comparable to the fiber levels of fescue and the subsample of the maintenance diet (game-bird flight conditioner), which was set aside for use during the feeding trials. Waterfowl are known to undergo changes in gut morphology (increase or decrease in size of the upper and lower digestive tract organs) in response to changes in the fiber content of their diet (Moss 1972, Miller 1975, Ankey 1977). During the 6 month acclimation period, the gut morphology in geese might have acclimated to the fiber levels provided in their maintenance diet. This might have masked any treatment differences for all other foods (see Chapter 4).

Although a significant decrease in food metabolizability, after ceca removal, was not detected for the majority of the foods, the decreased TME_N values for buttonbush among cecectomized geese is of interest. During the fall, geese rely heavily on low fiber agricultural waste grain for the majority of their daily energy. Thus, having the ability to extract as much energy as possible from food is not as important. In times of food

shortages, however, the ability of the cecum to digest high fiber foods might make nutritionally poor foods available to geese. In a study of water absorption, Duke et al. (1981) found that cecectomized great horned owls (*Bubo virginianus*) drank more water during days 8-15 after surgery. Eventually, the owls' water intake levels equaled that of the sham-operated birds. This suggests a compensatory effect occurring as a result of ceca removal. Under temperature stress, however, Chaplin (1989) found that cecectomized great horned owls had a higher water turnover, which was offset by an increase in food intake for temperatures below 15ºC, and offset by an increase in food and water intake for temperatures below 27ºC. Without additional food and water, the cecectomized owls could not maintain their body mass and became dehydrated at 27ºC. Thus, the ceca's role in nutrient digestion might be of greater importance when food is either inaccessible or limited such as immediately following a heavy snow storm.

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Table 1. Results of mixed model ANOVA investigating effects of treatment, period, food, and treatment-food interactions on weight.

Effect	NDF	DDF	F Value	Pr > F
Treatment	1	10	4.76	0.0541
Period	6	56	1.35	0.2497
Food	5	56	0.50	0.7777
Treat*Food	5	56	1.38	0.2448

Table 2. Average body mass of Giant Canada geese during feeding trials.

Trial	Intact Group mean ± SE (kg)	95% CI	Cecectomized Group mean ± SE (kg)	95% CI	P-value
1	3.85 ± 0.10 (n[a] = 9)	(3.63, 4.07)	3.89 ± 0.08 (n = 9)	(3.72, 4.07)	0.7468
2	3.86 ± 0.10 (n = 9)	(3.62, 4.10)	4.08 ± 0.09 (n = 9)	(3.88, 4.29)	0.1225
3	3.71 ± 0.11 (n = 9)	(3.45, 3.97)	3.96 ± 0.10 (n = 9)	(3.74, 4.19)	0.1027
4	3.68 ± 0.11 (n = 9)	(3.41, 3.94)	4.11 ± 0.12 (n = 9)	(3.82, 4.40)	0.0208
5	3.67 ± 0.11 (n = 9)	(3.43, 3.91)	4.05 ± 0.14 (n = 9)	(3.72, 4.38)	0.0494
6	3.71 ± 0.10 (n = 9)	(3.49, 3.94)	3.98 ± 0.13 (n = 9)	(3.69, 4.26)	0.1194
7	3.67 ± 0.14 (n = 9)	(3.35, 4.00)	3.98 ± 0.12 (n = 9)	(3.70, 4.26)	0.1159

a = samples

Table 3. Proximate composition of foods fed to Canada geese on a dry mass basis.

Food	%N[1]	% Crude Protein	% Crude Fat	% Crude Fiber	%Ash	GE[2]
Buttonbush	0.93	5.78	4.80	49.26	2.34	4.03
Corn	1.45	9.09	4.89	1.58	1.67	4.05
Fescue	2.36	14.77	2.25	9.42	5.66	3.76
Game-Bird Flight Conditioner	3.32	20.74	3.25	8.47	8.70	3.68
Milo	1.39	8.69	3.48	1.62	1.61	3.97
Rumex	2.62	16.37	2.85	7.27	2.44	3.91
Average	2.01 ± 0.37	12.58 ± 2.30	3.59 ± 0.43	12.94 ± 7.40	3.74 ± 1.66	3.91 ± 0.06

[1]% Nitrogen = Protein/ 6.25
[2]GE = Gross Energy (kcal/g)

Table 4. True metabolizable energy (TME) and nitrogen corrected TME (TME_N)[1] of 6 foods fed to intact and cecectomized giant Canada geese. N is sample size, SE is standard error, and CI are the 95% confidence interval limits.

Food	TME										TME_N									
	Intact					Cecectomized					Intact					Cecectomized				
	N	Mean	SE	CI		N	Mean	SE	CI		N	Mean	SE	CI		N	Mean	SE	CI	
Buttonbush	5	1.29[a]	0.45	0.04	2.54	7	0.66[a]	0.14	0.32	1.00	5	1.19[a]	0.46	-0.09	2.48	7	0.56[a]	0.14	0.23	0.89
Corn	6	4.09[b]	0.07	3.92	4.25	6	3.88[b]	0.13	3.55	4.21	6	4.02[b]	0.06	3.87	4.17	6	3.88[b]	0.09	3.63	4.13
Game-bird	5	3.34[c]	0.17	2.87	3.81	7	3.40[c]	0.17	2.99	3.81	5	3.19[c]	0.17	2.71	3.66	7	3.25[c]	0.17	2.88	3.62
Fescue	6	3.39[c]	0.12	3.09	3.69	6	3.19[c]	0.20	2.69	3.69	6	3.24[c]	0.11	2.96	3.52	6	3.13[c]	0.17	2.70	3.55
Milo	7	3.95[b]	0.06	3.80	4.10	7	4.18[b]	0.04	4.09	4.26	5	3.93[b]	0.05	3.80	4.06	7	4.13[b]	0.03	4.05	4.22
Rumex	6	2.43[d]	0.12	2.13	2.72	7	2.28[d]	0.10	2.03	2.53	6	2.37[d]	0.11	2.08	2.76	7	2.23[d]	0.11	1.97	2.49

[1]TME_N : True Metabolizable Energy corrected to zero nitrogen balance.
[a-d]Means within a column with a common superscript do not differ significantly ($P < 0.05$).
balance.

Table 5. Results of mixed model ANOVA investigating effects of treatment, period, food, and treatment-food interactions on TME.

Effect	NDF	DDF	F Value	Pr > F
Treatment	1	10	3.41	0.0946
Period	6	48	2.12	0.0683
Food	5	48	110.63	<.0001
Treat*Food	5	48	1.97	0.1008

Table 6. Results of mixed model ANOVA investigating effects of treatment, period, food, and treatment-food interactions on TME_N.

Effect	NDF	DDF	F Value	Pr > F
Treatment	1	10	2.82	0.1240
Period	6	48	2.22	0.0572
Food	5	48	124.39	<.0001
Treat*Food	5	48	2.03	0.0915

Table 7. Least square means for true metabolizable energy (TME_N) of foods fed Canada geese and corrected to zero nitrogen balance.

Food	TME_N (Mean ± SE)
Corn	3.97 ± 0.11^{a}
Game-bird flight conditioner	3.22 ± 0.11^{b}
Buttonbush	0.90 ± 0.11^{c}
Fescue	3.18 ± 0.10^{b}
Milo	4.03 ± 0.10^{a}
Rumex	2.30 ± 0.10^{d}

*SE: Standard Error
**Different superscripts indicate significant differences between foods ($P < 0.05$)

Table 8. Least square means of true metabolizable energy (TME_N) of foods corrected to zero nitrogen balance for treatment*food interactions.

Food	Intact (Mean ± SE[1])	Cecectomized (Mean ± SE)	DF	t-value	Pr > t
Corn	4.05 ± 0.15[a]	3.87 ± 0.15[a]	48	0.86	0.3953
Game-bird flight conditioner	3.19 ± 0.17[a]	3.25 ± 0.14[a]	48	-0.30	0.7687
Buttonbush	1.24 ± 0.17[a]	0.56 ± 0.14[b]	48	3.12	0.0031
Fescue	3.24 ± 0.15[a]	3.13 ± 0.14[a]	48	13.04	0.5905
Milo	3.93 ± 0.14[a]	4.13 ± 0.14[a]	48	-1.04	0.3038
Rumex	2.39 ± 0.15[a]	2.23 ± 0.14[a]	48	0.76	0.4498

[1]SE: Standard Error
[a-b]Means with different subscripts across rows are significantly different ($P < 0.05$)

Table 9. Metabolic efficiencies (MEF) of foods fed to Canada geese in trials by treatment.

Food	Intact Group MEF (%) ± SE[1]	Cecectomized Group MEF (%) ± SE
Buttonbush	23.84 ± 9.26 (n = 5)[a]	11.23 ± 2.71 (n = 7)[a]
Corn	88.35 ± 1.27 (n = 6)[b]	85.13 ± 2.12 (n = 6)[b]
Game-bird flight conditioner	71.10 ± 3.81 (n = 5)[c]	72.54 ± 3.38 (n = 7)[c]
Fescue	73.2 ± 2.44 (n = 6)[c]	69.91 ± 4.52 (n = 6)[c]
Milo	88.91 ± 1.23 (n = 7)[b]	93.53 ± 0.78 (n = 7)[b]
Rumex	52.25 ± 2.50 (n = 6)[d]	49.19 ± 2.35 (n = 7)[d]

[1]Standard Error
[2]Means within a column with a common superscript do not differ significantly ($P < 0.05$)

Table 10. Least square means for metabolic efficiencies (MEF) of foods fed to Canada geese in trials.

Food	MEF (%) ± SE[1]
Buttonbush	16.82 ± 2.37 (n = 12)[a2]
Corn	87.07 ± 2.37 (n = 12)[b]
Game-bird flight conditioner	71.97 ± 2.37 (n = 12)[c]
Fescue	71.76 ± 2.37 (n = 12)[c]
Milo	91.22 ± 2.18 (n = 14)[b]
Rumex	50.78 ± 2.27 (n = 13)[d]

[1]Standard Error
[2]Different superscripts indicate significance between food metabolic efficiencies ($P < 0.05$)

Figure 1. Scatter plot of % crude fiber and TME_N.

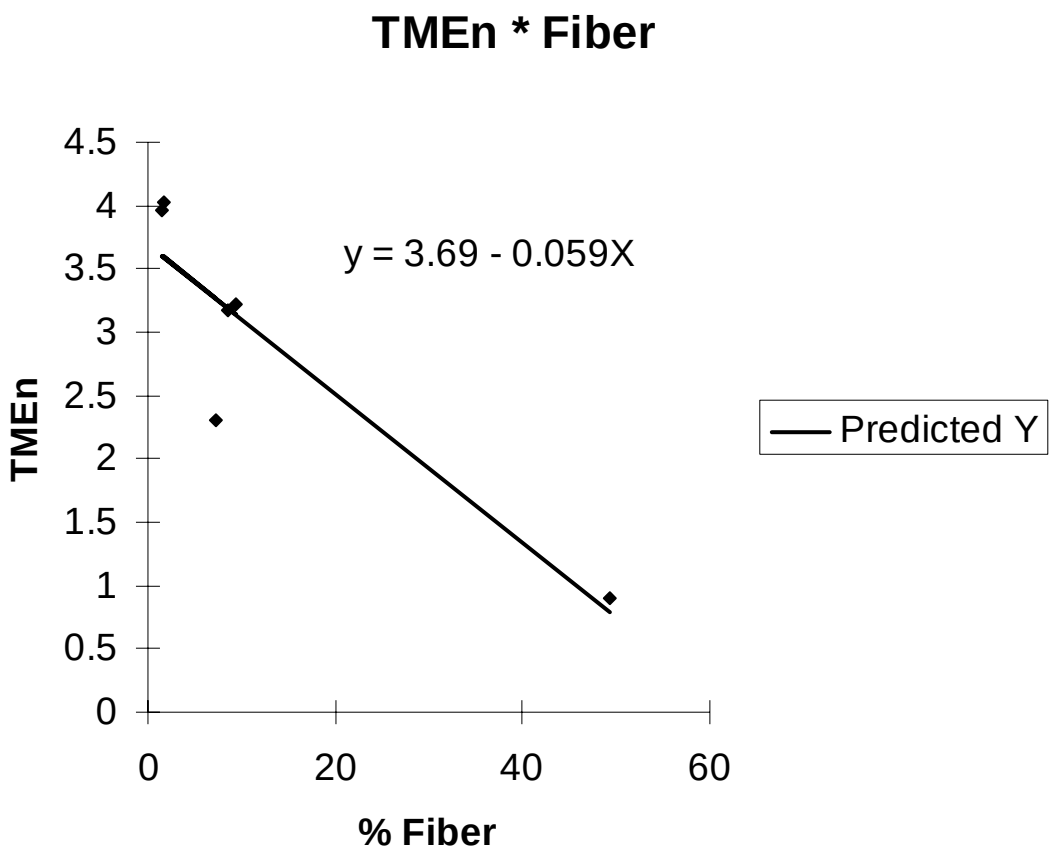
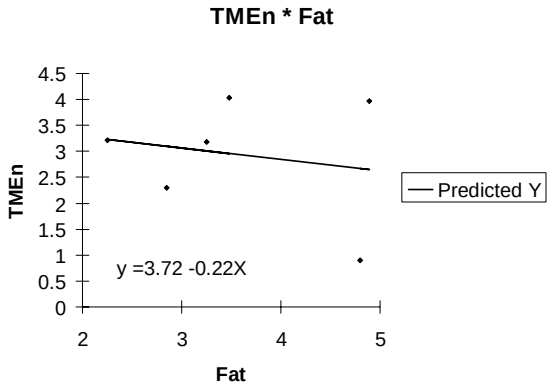
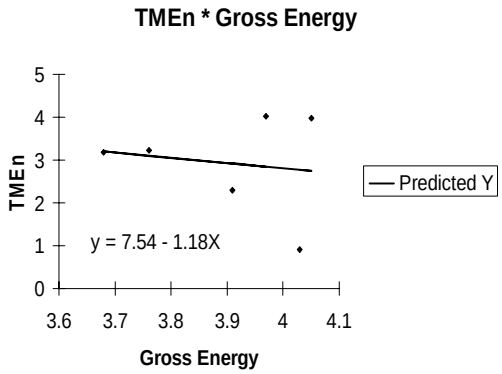
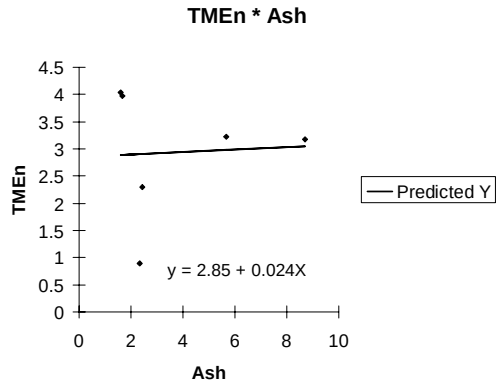

TMEn * Fiber
y = 3.69 - 0.059X
TMEn
% Fiber
Predicted Y
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60

Figure 2. Scatter plots of proximate measures and True Metabolizable Energy (TME_N) not included in the regression model.


TMEn * Fat
TMEn
Fat
y =3.72 -0.22X
Predicted Y


TMEn * Gross Energy
TMEn
Gross Energy
y = 7.54 - 1.18X
Predicted Y


TMEn * Ash
TMEn
Ash
y = 2.85 + 0.024X
Predicted Y

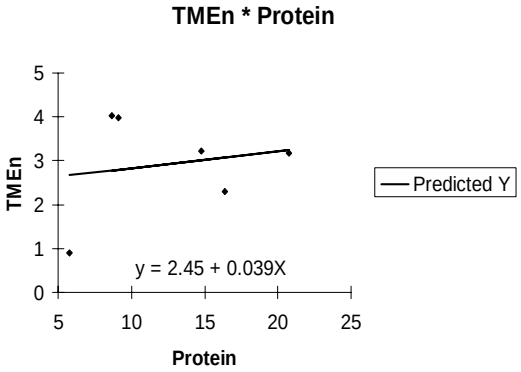

TMEn * Protein
TMEn
Protein
y = 2.45 + 0.039X
Predicted Y

Table 11. Correlation coefficients and P-values of TME_N proximate measures.

Proximate Measure	Correlation coefficient (r^2)	F	P-Value
% Crude Fat	0.0388	0.1616	0.7082
% Crude Protein	0.0345	0.1429	0.7245
% Ash	0.0032	0.0131	0.9143
% Fiber	0.8169	17.8503	0.0134
% Gross Energy	0.0225	0.0923	0.7764

CHAPTER 3. THE EFFECTS OF CECECTOMY ON AMINO ACID DIGESTIBILITY IN GIANT CANADA GEESE

INTRODUCTION

Waterfowl are renowned for their ability to exploit a wide variety of food resources. They feed on fresh and marine water invertebrates, aquatic and terrestrial plants, and agricultural grains and crops. Their nutritional flexibility has allowed them to exploit virtually every water environment, and with the exception of Antarctica, enjoy a world-wide distribution as a result of their behavioral, anatomical, and physiological adaptability.

Various studies have noted that digestive organs undergo physiological and/or anatomical changes in response to diet, season, or life stage. The waterfowl cecum is one of the digestive organs that respond to dietary and/or seasonal changes (Miller 1975, Ankney 1977, Drobney 1984). In Chapter 2, we evaluated the role of the cecum in the metabolization of food by comparing true metabolizable energy between foods varying in fiber content using precision-fed Canada geese (*Branta canadensis*). In five of the six foods fed, we found no differences between cecectomized and intact geese in their ability to extract nutrients from food. Intact geese, however, were able to obtain twice as much energy from buttonbush which had the highest fiber content. Cellulose splitting bacteria found within the ceca might be responsible for differences in fiber digestion (See Chapter 5).

It has been suggested that bacteria within the avian cecum might also synthesize amino acids, or consume undigested amino acids without benefit to the bird (Green et al. 1987). Nutritional studies focusing on amino acid digestibility use cecectomized

chickens (Payne et al. 1971, Sibbald 1979, Austic 1983, Parsons 1984, Raharjo and Farrell 1984, Johns et al. 1986, Green et al. 1987) to eliminate the possibility of bacterial action within the cecum which may affect results. Several studies have examined amino acid digestibility differences between intact and cecectomized chickens, but the results were inconclusive. After determining true amino acid and apparent amino acid digestibilities, Raharjo and Farrell (1984) concluded that there were only minor differences in amino acid digestibility between intact and cecectomized birds. However, Johns et al. (1986) concluded that the digestibility coefficients from cecectomized birds were lower than the digestibility coefficients from intact birds. Investigators have found that the availability coefficients of most amino acids in both meat-and-bone meal and distillers' dried grains are lower in cecectomized birds compared to intact birds (Green et al. 1987).

Bacteria capable of uric acid decomposition have also been found within the avian cecum (Barnes and Impey 1974). Anaerobic decomposition of uric acid by microorganisms within the ceca has been reported to yield ammonia, acetate, CO_2, glycine, formate, propionate, proteins, vitamins, and possibly some alcohols (Clench and Mathias 1995). Some of the ammonia released during the breakdown of uric acid, urea, and amino acids from dietary and urinary nitrogenous compounds is utilized in the synthesis of glutamine and other amino acids (Mortensen and Tinadall 1981, Karasawa 1989). Differences in amino acid digestibility between intact and cecectomized birds might be explained by the amino acid degradation that takes place within the cecum (Karasawa 1989).

Canada geese experience periods of food shortage or periods when nutritionally adequate food is unavailable to them; as a result, they must rely on nutritionally poor foods. During periods of stress when food is scarce or unavailable, the synthesis of amino acids or the release of precursors for amino acid synthesis within their ceca could be beneficial when they must rely on protein poor foods. Thus, our objective was to determine if differences in amino acid digestibility exist between cecectomized and intact Canada geese. Based on previous studies, we predicted that intact Canada geese would have higher digestibility values for some of the amino acids.

METHODS

Experiment Birds

Geese were captured on 22 June 2000 during the flightless period at Stephens Lake and the Missouri Country Club in Boone County, Missouri. Upon capture, geese were sexed by cloacal examination. We retained 17 and 13 female geese from the Missouri Country Club and the Stephens College Lake respectively. We conducted two additional captures on September 03, 2000 at the City of Columbia Wetlands (4 geese) in Boone County, Missouri, and on December 08, 2000 at the University of Missouri South Farms (17 geese) in Boone County. Rocket nets aimed over a baited cracked corn site were used to capture geese on the latter two occasions. After capture, we transported the geese to the Charles Green Wildlife Area in Boone County, Missouri. They were weighed to the nearest gram using a spring scale and a cotton pillow case, then marked with numbered metal leg bands, and randomly assigned to one of the two following groups: 1) cecectomized (treatment) and 2) intact (sham operated).

Husbandry

The geese were housed in 8' x 8' x 8' cages in an indoor facility equipped with windows to provide a natural photoperiod at the Charles Green Wildlife Area. Fresh water and grit (granite) were available to the birds at all times. Geese were initially maintained on a mixed diet consisting of 25% Purina Meat Builder (crude protein not less than 20.0%, crude fat not less than 3.0%, crude fiber not more than 5.0%) and 75% ground rabbit pellets (PMI Feeds, crude protein not less than 14.0%, crude fat not less than 1.5%, crude fiber not more than 25%) to ensure that their digestive system was operating in high fiber mode. After a month, we switched the geese to a commercial game-bird flight conditioner (crude protein <19.0%, crude fat no <2.0%, crude fiber not >12.0%; Purina Mills, St. Louis, Missouri, USA).

Surgical Procedure

Birds were fasted for 24 hours before surgery. Standard surgical procedures were followed during the cecectomies. Inhaled isoflourine (4-5% flow rate) was used to maintain a surgical plane of anesthesia. Birds were placed in a position of dorsal recumbency. Feathers were removed from the abdomen and the area was disinfected with iodine solution. A laparotomy was performed through an incision in the body wall. Each ceca was localized and the two distal ends were carefully detached by hand from the mesentery which joins them to the antimesenteric wall of the ileum. When both ceca were completely excised, each was transected and both cut surfaces sutured, as near as possible to the junctions of the ceca and the intestine. After the ceca were removed, the exposed intestine was returned to the peritoneal cavity, and the incision in the peritoneum and muscular layers closed by sutures. After the operation, solid food was withheld for

24 hours, but water was supplied *ad libitum*. During the sham procedure a laparotomy was performed and the viscera were manipulated. The ceca were taken out and the two distal ends were carefully detached by hand from the mesentery which joined them to the antimesenteric wall of the ileum, the ceca were reinserted into the peritoneal cavity, and the incision in the peritoneum and muscular layers were closed by sutures.

The surgeries were conducted over four time periods. The first surgeries were conducted between August 14-20, 2000 by staff from the Department of Laboratory Animal Medicine at the University of Missouri, Columbia. With the exception of one bird, all cecectomized birds died as a result of complications from the surgery. All sham operated birds survived the surgery. Two additional birds were cecectomized on October 04, 2000, and both geese died from surgical complications. The remaining cecectomies were performed on November 14-16, 2000 and on March 22, 2001 by staff from the Department of Animal Sciences at the University of Missouri, Columbia. One of the geese died after surgery, but all others survived without complications.

Feeding Trials

Feeding trials were conducted between 02 June and 22 August 2001 in an unheated laboratory with a natural photoperiod. Six foods were tested: 1) corn (*Zea mays*), 2) milo (*Sorghum vulgare*), 3) game-bird flight conditioner, 4) curly dock (*Rumex crispus*), 5) tall fescue seed (*Festuca arundinacea*), and 6) buttonbush (*Cephalanthus occidentalis*). Mature curly dock seeds were harvested by hand from standing plants at the Thomas S. Baskett Wildlife Area in Boone County, Missouri. Buttonbush seeds were harvested by hand from standing plants at the Duck Creek Wildlife Area in Stoddard and Bollinger counties, Missouri. All other seeds were obtained from local vendors. Seeds

were allowed to air dry for >48 hours, and then stored at 5°C to minimize respiration prior to feeding trials.

Six intact and six cecectomized geese were precision-fed using the procedure described by Sibbald (1976). Feeding treatments were arranged in the form of two 6 X 6 Latin square designs; one for each bird group (intact or cecectomized). We conducted seven feeding trials (the last row of the 6^{th} trial was repeated during the 7^{th} trial to test for residual effects) and for each bioassay six experimental birds were precision-fed test foods. This experimental design resulted in each bird being fed each test food once. An additional three birds (for each group) were selected to serve as controls during each bioassay. Those birds served as controls in all 7 feeding trials.

Procedures for TME bioassays followed Sibbald (1986). All geese were weighed to the nearest 0.01 kg and fasted for 48 hr in individual metabolism cages (61 x 46 x 61 cm) with access to fresh water at all times. Experimental birds were then precision-fed a known amount of test food through a copper tube (1.2 x 40 cm) inserted down the esophagus. On average (± SE), birds were fed the following dry mass of each food: corn (20.83 ± 0.83 g), milo (21.54 ± 1.01 g), game-bird flight conditioner (13.50 ± 1.50 g), rumex (21.54 ± 1.01 g), fescue (12.3 ± 0.30 g), buttonbush (12.21 ± 0.29 g). Food was slowly poured into the funnel attached to the upper end of the tube and pushed down the tube with a wooden rod. The rod was of a diameter that allowed no foods to adhere to the tube or funnel. After the withdrawal of the tube from the esophagus, a stream of distilled water was forced into the bird's mouth to encourage swallowing. Cecectomized and intact birds were fed only once during the feeding trial, and precision feeding took 5-7 min/goose. For control birds, the feeding tube was inserted into their esophagus, but they

were not fed. Immediately after feeding the geese were returned to their individual metabolism cages. A 60 x 90 cm piece of plastic sheeting was attached beneath all cages to collect all fecal and urinary matter. Birds that regurgitated any portion of the test food were eliminated from the bioassay.

Excreta samples were collected for 48 hr and frozen for subsequent analysis. Feathers were removed from the excreta with forceps, and the excreta were sieved to remove any grit that had passed through the digestive tract (Biligi et al. 1982). If the samples contained intact seeds and could not be sieved, grit was removed by hand using forceps. Excreta was oven-dried at 60°C, weighed to the nearest 0.01 g, and ground in a blender for 3 minutes. Sub-samples of 1 g were oven-dried to a constant mass at 80°C to determine percent moisture.

The excreta of 4 intact and 4 cecectomized birds/food were randomly selected for amino acid analysis. Apparent digestibility of a particular amino acid (AAAD) was calculated as the difference between amino acid intake and amino acid in excreta, expressed as a percentage of amino acid intake during the collection periods:

$$\%AAAD = [(AAF - AAV)/ AAF] * 100$$

where AAF is the total amount of amino acid consumed by the fed bird, AAV is the total amount of amino acid voided in the excreta by the fed bird in the 48 hour period after feeding. True amino acid digestibility (TAAD) was calculated using the following equation:

$$\%TAAD = [(AAF - (AAV - AAVF))/ AAF] * 100$$

AAVF is the amino acid voided in the excreta by the fasted control bird. The average amino acid value excreted by control birds was used to estimate AAVF because no

correlation between body weight and amino acids is apparent. Pooled values for an amino acid excreted by control birds in a feeding trial were therefore, used to calculate TAAD.

Nutrient content of each food was determined using proximate analysis. Crude protein of diets and excreta samples was determined using a LECO FP-228 Nitrogen Determinator (Sweeney and Rexroad 1987) with 10 percent reruns by manual Kjeldahl with copper catalyst (AOAC 1984). Percent moisture was determined by drying in a vacuum oven at 95-100°C, crude fat was calculated using the indirect method, and crude fiber was estimated using the asbestos-free method (AOAC 1984). Ash content was determined by heating samples in a furnace at 625°C for 15 hours (AOAC 1984). Amino acid composition was determined with post-column derivitization on a Beckman 6300 amino acid analyzer with full computer integration following AOAC official method 982.30E (A,B,C) CHP 45.3052000.

Statistical analysis

Statistical analysis of the data was accomplished using the GLM procedure of SAS (PROC GLM; SAS Institute 1989) based on a factorial arrangement of treatments, employing $\alpha = 0.05$. The least significant difference test was used to elucidate differences between treatment means. The excreta of 4 intact and 4 cecectomized birds/food were randomly selected for amino acid analysis.

RESULTS

All birds remained healthy throughout the trials. Despite decreasing the amount of food fed after the first feeding trial, regurgitation was a problem throughout our feeding trials. Birds from a trial that regurgitated any portion of the test food were

eliminated. Extra trials were not conducted to fill in missing values for birds that regurgitated a food type.

Body Mass

Body mass of Giant Canada geese used in this study ranged from 3.40 to 4.40 kg at the onset of feeding trials in June 2001 (mean = 3.87 ± .06 kg). Differences in body mass between treatments was significant (Table 1). Weight was not affected by trial, food fed, or treatment*food interaction. Cecectomized geese were generally heavier than intact geese (Table 2). Intact geese weighed 3.85 ± 0.10 kg (SE) at the beginning of feeding trials in June, and weighed 3.67 ± 0.14 kg at the termination of the trials in August. Cecectomized birds weighed 3.89 ± 0.08 kg and 3.98 ± 0.12 kg in June and August, respectively. There were no significant differences in body weight between intact and cecectomized geese during the 1st, 2nd, 3rd, 6th and 7th feeding trials; however, cecectomized geese were significantly heavier than intact geese during the 4th and 5th feeding trials (Table 2).

Nutrient and Energy Content of Foods

The proximate composition of foods fed during the feeding trials is shown in Table 3. On average (± SE), birds were fed the following dry mass of each food: corn (20.83 ± 0.83 g), milo (21.54 ± 1.01 g), game-bird flight conditioner (13.50 ± 1.50 g), rumex (21.54 ± 1.01 g), fescue (12.3 ± 0.30 g), buttonbush (12.21 ± 0.29 g). The average gross energy of foods utilized in this study was 3.91 ± 0.06 kcal/g and ranged from 3.68 kcal/g to 4.05 kcal/g. Average gross energy of agricultural grains (3.90 ± 0.11 kJ/g) was similar to the gross energy of natural foods (3.90 ± 0.08 kcal/g). Game-bird flight conditioner contained less energy (3.68 kcal/g) than any other food item in either the

agricultural or natural food groups. Crude protein levels of game-bird flight conditioner (20.74%) and rumex (16.37%) exceeded that of all other foods which ranged from 5.78% (buttonbush) to 14.77% (fescue) (mean = 12.57 ± 2.30%). Crude fat varied from 2.25% (fescue) to 4.89% (corn) with a mean of 3.59 ± 0.43%. Crude fiber levels were higher for natural foods and game-bird flight conditioner (relative to agricultural grains 1.58-1.62%) and ranged from 1.58 (corn) to 49.26% (buttonbush) with a mean of 12.94 ± 7.40%. Percent ash in game-bird flight conditioner (8.70%) exceeded all other food types which ranged from 1.61% (corn) to 5.66% (fescue) with a mean of 3.74 ± 1.17 %.

Amino Acid Composition of Foods

The amino acid composition of the foods is shown in Table 4. Of the essential amino acids, lysine content was high; and the tryptophan content was particularly low. Of the non-essential fraction, amounts of glutamate and proline were notably high. Of the foods fed, game-bird flight conditioner had the highest protein content; it was particularly high in glutamate, aspartic acid, and proline content. At only 0.27g/100g DM, tryptophan was the amino acid present in the lowest quantity in game-bird flight conditioner.

AAAD, *TAAD*, *Digestibility*

Mean AAAD values for each food (corn, game-bird flight conditioner, buttonbush, fescue, milo, rumex) and bird group (intact, cecectomized) are summarized in Tables 5 through 10. Differences between foods and groups were inconsistent. Higher coefficients were evident in corn, game-bird flight conditioner, and milo compared to buttonbush, fescue, and rumex. There were no significant differences in mean AAAD for either essential or nonessential amino acids between intact and

cecectomized geese for any of the foods fed. Many of the AAAD values were negative, especially for buttonbush and fescue (Tables 7 and 8).

Mean TAAD values for each food and group are summarized in Tables 11 through 16. When apparent digestibility was corrected for endogenous excretion, differences between groups were evident only for the true digestibility of methionine, histidine and tryptophan in buttonbush (Table 13). Intact geese had higher TAAD values for methionine, histidine, and tryptophan in buttonbush (Table 13). Cecectomy failed to influence true digestibility of indispensable and dispensable amino acids in corn, game-bird flight conditioner, fescue, milo, and rumex, as no differences were observed between cecectomized and intact geese. Correction of apparent to true digestibility influenced all amino acids in all foods, particularly among buttonbush and fescue.

DISCUSSION

As with previous studies (Buckley 1989, Petrie 1994, Checkett 2001) regurgitation of foods during the trial periods was a problem. As a result, we decreased the amount of food fed between the first and second trial; however, some birds still regurgitated during the 4th, 5th, and 7th feeding trials. Milo was the only seed not regurgitated during any of the trials. All remaining foods were regurgitated at least once, despite following the recommendation of spraying a mist of water down the throat of each bird after feeding to induce peristalsis (Checkett 2001). After the birds were placed in the cages, several geese regurgitated contaminating nearby cages. As a result some samples were lost due to cross contamination.

Changes in Body Mass

Changes in goose body mass between June and August were similar to those reported for wild birds of the same age and class (Hanson 1962). Intact geese weighed less at the end of the study, but cecectomized geese had actually increased in weight. The Latin square design of this study in which all foods are fed during each feeding trial was conducted in order to reduce any variation caused by temporal changes in bird physiology and influence of climate. Regardless, the average body mass of geese used in this study varied by only 10% between June and August, and we detected no significant difference between the weights of geese at the beginning and end of the feeding trials. We concluded that the nutritional status of experimental birds did not bias our estimates of digestibility for any of the 6 test foods.

There was a discrepancy in weight between cecectomized and intact birds, with cecectomized birds being generally heavier. Weight differences between treatments were only statistically significant during the 4^{th} and 5^{th} feeding trials. Mutzgar and Slinger (1981) concluded that endogenous amino acid excretion varied little with body weight. Green et al. (1987) noted that amino acids excreted by chicks (535g) are similar to those observed for adult (50 weeks of age) birds. Therefore, we believe that the weight differences between the two treatment groups did not have an effect on amino acid digestibility

AAAD, *TAAD*, *Digestibility*

Available information on estimates of digestibility for foods consumed by waterfowl is limited. To date, there is no information on estimates of amino acid digestibility for foods consumed by cecectomized wild waterfowl. Except for corn, we

were unable to find studies that determined the TAAD of foods fed in this study. The amino acid digestibility of corn in intact and cecectomized geese paralleled the results obtained by Ragland et al. (1999) for White Pekin ducks. Overall our TAAD coefficients for corn, however, were lower than those determined by Green *et al.* (1987) for chickens.

Overall, cecectomy failed to exert an effect on true amino acid digestibility of corn, game-bird flight conditioner, fescue, milo, or rumex. Intact geese, however, had higher digestibility coefficients for histidine, methionine, and tryptophan in buttonbush. The validity of TAAD values obtained for buttonbush, however, is questionable. Only seven of the buttonbush TAAD values were positive. Negative values indicate that endogenous losses were larger than amino acid intake. The amino acid content of buttonbush was lower than the content of the other foods tested, and it might have contributed to the negative values obtained. Buttonbush's high fiber content (49%), however, might have played a larger role in the low and/or negative TAAD values. Several studies have shown that excretion of N and amino acids is increased as dietary fiber is increased (Beames and Eggum, 1981; Parsons *et al*., 1983; Borges *et al*., 2003). In rats, true protein digestibility was significantly reduced when cellulose and barley hulls were included in the diet (Beames and Eggum 1981). Parsons *et al.* (1983) showed that roosters fed a high-fiber diet excreted substantially more amino acids than did fasted roosters or roosters fed a low-fiber diet. Borges *et al*. (2003) reported that high fiber foods had lower amino acid digestibility due to the interference of fiber with amino acid digestibility. Our results for amino acid digestibility in buttonbush might have been further compromised by our use of fasted birds to measure endogenous losses. Parsons *et*

al. (1983) reported that fasted birds may not provide an accurate estimate of endogenous amino acid excretion for birds fed high-fiber feedstuffs in amino acid digestibility trials.

Corrected amino acid availability values above 100%, and as high as127.51% were obtained for tryptophan. Likuski and Dorrell (1978) reported values above 100% for several amino acids including tryptophan, and attributed the values to errors that occur in analyzing foods and excreta for small concentrations of those amino acids. Tryptophan was the amino acid present in the lowest concentration amongst all the foods tested. Likuski and Dorrell (1978) suggested that the accuracy of TAAD values might improve if birds were fed a minimum of 40g of feedstuff. Sibbald (1977) reported that the optimum feed intake is 40g for determining TME values. Problems with regurgitation precluded us from being able to feed more than 20g of food. The TME estimates we obtained were comparable to those obtained by other researchers (Chapter 2), but the limited amount of food fed might have affected our TAAD results.

Although we were unable to detect differences in amino acid digestibility between intact and cecectomized geese, it does not rule out the potential role of the cecum in amino acid digestibility in Canada geese. The TAAD bioassay utilized in this study might not have been the appropriate approach for determining amino acid digestibility of foods fed to Canada geese. Problems with regurgitation precluded us from being able to feed the minimum amount (40 g) recommended for accurate results. Additionally, the removal of the ceca might have influenced degradation of dietary amino acids by microbes in the gastrointestinal tract anterior to the ceca (Sakata 1987). An in vitro study of bacteria within the Canada goose cecum, could elucidate whether or not bacteria capable of amino acid breakdown are present in the cecum.

We conducted a sample size determination based on our results to estimate the number of geese required to give a reasonable estimate of amino acid digestibility. The estimated number of geese that would be needed to achieve a certain level of accuracy varied considerably depending on food, amino acid, and bird treatment (intact, cecectomized). For example, when fed corn, the estimated number of cecectomized geese needed in order to achieve 10% accuracy at a 0.05 confidence level ranged from 15.64 to 19,828.38. For intact geese, the estimated number required for 10% accuracy at a 0.05 confidence level ranged from 0.05 to 5,401.70. The extreme range demonstrated by the sample size determination further supports our conclusion that the TAAD bioassay was an inappropriate test given the amount of food we fed.

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Table 1. Results of mixed model ANOVA investigating effects of treatment, period, food, and treatment-food interactions on weight.

Effect	NDF	DDF	F Value	Pr > F
Treatment	1	10	4.76	0.0541
Period	6	56	1.35	0.2497
Food	5	56	0.50	0.7777
Treat*Food	5	56	1.38	0.2448

Table 2. Average body mass of Giant Canada geese during feeding trials.

Trial	Intact Group mean ± SE (kg)	95% CI	Cecectomized Group mean ± SE (kg)	95% CI	P-value
1	3.85 ± 0.10 (n[a] = 9)	(3.63, 4.07)	3.89 ± 0.08 (n = 9)	(3.72, 4.07)	0.7468
2	3.86 ± 0.10 (n = 9)	(3.62, 4.10)	4.08 ± 0.09 (n = 9)	(3.88, 4.29)	0.1225
3	3.71 ± 0.11 (n = 9)	(3.45, 3.97)	3.96 ± 0.10 (n = 9)	(3.74, 4.19)	0.1027
4	3.68 ± 0.11 (n = 9)	(3.41, 3.94)	4.11 ± 0.12 (n = 9)	(3.82, 4.40)	0.0208
5	3.67 ± 0.11 (n = 9)	(3.43, 3.91)	4.05 ± 0.14 (n = 9)	(3.72, 4.38)	0.0494
6	3.71 ± 0.10 (n = 9)	(3.49, 3.94)	3.98 ± 0.13 (n = 9)	(3.69, 4.26)	0.1194
7	3.67 ± 0.14 (n = 9)	(3.35, 4.00)	3.98 ± 0.12 (n = 9)	(3.70, 4.26)	0.1159

a = samples

Table 3. Proximate composition of foods fed to Canada geese on a dry mass basis.

Food	%N[1]	%Protein	%Fat	%Fiber	%Ash	GE[2]
Buttonbush	0.93	5.78	4.80	49.26	2.34	4.03
Corn	1.45	9.09	4.89	1.58	1.67	4.05
Fescue	2.36	14.77	2.25	9.42	5.66	3.76
Game-Bird Flight Conditioner	3.32	20.74	3.25	8.47	8.70	3.68
Milo	1.39	8.69	3.48	1.62	1.61	3.97
Rumex	2.62	16.37	2.85	7.27	2.44	3.91
Average	2.01 ± 0.37	12.58 ± 2.30	3.59 ± 0.43	12.94 ± 7.40	3.74 ± 1.66	3.91 ± 0.06

[1]% N = Protein/ 6.25
[2]GE = Gross Energy (kcal/g)

Table 4. Amino acid analysis of foods fed to Canada geese on a dry mass basis.

Amino acids (g/ 100 g DM)	Buttonbush	Corn	Fescue	Game-bird Flight Conditioner	Milo	Rumex
Asparticacid	0.53	0.61	0.89	1.51	0.60	1.32
Threonine	0.23	0.32	0.45	0.67	0.29	0.58
Serine	0.24	0.37	0.48	0.67	0.36	0.71
Glutamate	0.96	1.70	3.48	3.49	1.83	2.60
Proline	0.27	0.77	1.22	1.24	0.71	0.58
Alanine	0.30	0.68	0.63	1.07	0.81	0.77
Cysteine	0.12	0.23	0.36	0.39	0.19	0.26
Valine	0.31	0.46	0.65	0.95	0.47	0.89
Methionine	0.11	0.21	0.23	0.36	0.18	0.36
Isoleucine	0.24	0.31	0.52	0.67	0.34	0.68
Leucine	0.42	1.07	0.98	1.37	1.15	1.14
Tyrosine	0.13	0.31	0.40	0.52	0.30	0.41
Phenelalanine	0.26	0.45	0.98	0.81	0.46	0.79
Histidine	0.12	0.27	0.34	0.50	0.21	0.44
Lysine	0.21	0.31	0.59	1.02	0.22	0.73
Arginine	0.38	0.49	0.77	1.32	0.37	1.87
Tryptophan	0.04	0.13	0.17	0.27	0.14	0.04

Table 5. Apparent digestibility of amino acids (AAAD) in corn fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	55.83	0.20	55.20	56.46	4	53.74	6.01	34.60	72.87	0.9156
Histidine	4	67.74	3.83	55.54	79.94	4	59.73	7.13	37.04	82.42	0.5353
Isoleucine	4	32.53	2.23	25.43	39.64	4	14.17	15.86	-36.32	64.66	0.4734
Leucine	4	68.03	1.72	62.56	73.49	4	60.79	8.38	34.12	87.46	0.7899
Lysine	4	5.23	6.04	-14.00	24.46	4	-5.69	12.59	-45.74	34.36	0.7211
Methionine	4	64.05	1.62	58.90	69.20	4	60.82	3.78	48.79	72.84	0.8007
Phenylalanine	4	52.38	3.33	41.79	62.97	4	43.48	11.66	6.37	80.59	0.7433
Threonine	4	23.51	3.33	12.90	34.12	4	-5.25	9.07	-34.10	23.60	0.3432
Tryptophan	4	58.52	8.17	32.52	84.51	4	68.45	6.30	48.42	88.49	0.6260
Valine	4	40.49	2.94	31.12	49.86	4	21.38	19.88	-41.89	84.66	0.4802
Nonessential AA, %											
Alanine	4	58.98	2.15	52.13	65.82	4	53.39	6.44	32.90	73.87	0.8326
Aspartate	4	25.91	2.55	17.78	34.04	4	16.37	8.74	-11.43	44.17	0.6543
Cysteine	4	38.31	5.92	19.48	57.13	4	32.44	9.84	1.12	63.75	0.9184
Glutamate	4	67.77	1.31	63.62	71.93	4	61.73	4.37	47.83	75.63	0.7332
Proline	4	69.04	3.31	58.50	79.79	4	62.58	4.43	48.49	76.68	0.8893
Serine	4	38.21	5.32	21.28	55.13	4	25.27	9.29	-4.30	54.83	0.7148
Tyrosine	4	44.23	3.98	31.56	56.90	4	34.24	9.30	4.66	63.83	0.8702

[1]SE: Standard Error

Table 6. Apparent digestibility of amino acids (AAAD) in game-bird flight conditioner fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	69.23	1.32	65.04	73.42	4	76.88	4.20	63.52	90.23	0.6994
Histidine	4	71.83	1.55	66.90	76.76	4	79.30	4.15	72.70	85.90	0.5633
Isoleucine	4	38.17	2.08	31.56	44.78	4	54.62	6.71	33.25	75.98	0.5206
Leucine	4	50.69	2.04	44.21	57.17	4	63.73	5.04	47.68	79.77	0.6314
Lysine	4	52.64	3.14	42.65	62.62	4	66.37	4.40	52.37	80.38	0.6536
Methionine	4	60.67	2.44	52.92	68.42	4	70.69	2.26	63.51	77.87	0.4364
Phenylalanine	4	49.98	1.67	44.67	55.29	4	63.75	4.25	50.21	77.29	0.6127
Threonine	4	31.50	3.16	21.44	41.55	4	42.03	8.43	15.19	68.86	0.7270
Tryptophan	4	79.42	3.24	69.12	89.71	4	80.64	5.80	62.19	99.08	0.9522
Valine	4	42.51	2.30	35.20	49.81	4	56.64	5.82	38.10	75.17	0.6010
Nonessential AA, %											
Alanine	4	50.33	2.96	40.92	59.73	4	62.86	3.94	50.31	75.40	0.6362
Aspartate	4	43.27	2.47	35.41	51.12	4	56.37	4.92	40.71	72.03	0.5391
Cysteine	4	18.14	2.17	11.25	25.03	4	34.85	19.66	-27.71	97.41	0.7708
Glutamate	4	69.52	1.57	64.53	74.50	4	77.75	2.48	69.87	85.62	0.6298
Proline	4	56.23	1.03	52.97	59.50	4	17.13	8.57	34.23	88.75	0.9098
Serine	4	22.06	8.64	8.31	35.81	4	37.64	14.12	-7.30	82.58	0.6601
Tyrosine	4	38.68	3.38	27.94	49.42	4	54.47	7.00	32.20	76.74	0.3511

[1]SE: Standard Error

Table 7. Apparent digestibility of amino acids (AAAD) in buttonbush fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	-49.84	21.41	-117.97	18.28	4	-72.16	34.77	-182.82	38.49	0.2633
Histidine	4	-61.77	11.63	-98.78	-24.76	4	-86.63	25.51	-167.83	-5.23	0.0599
Isoleucine	4	-108.52	18.33	-166.84	-50.20	4	-140.77	50.74	-302.25	20.71	0.2114
Leucine	4	-108.94	31.12	-208.00	-9.89	4	-140.25	53.53	-310.60	30.09	0.2528
Lysine	4	-184.62	25.81	-266.75	-102.49	4	-224.36	58.71	-411.21	-37.50	0.1989
Methionine	4	-62.45	11.58	-99.30	-25.61	4	-86.54	25.12	-166.47	-6.60	0.0665
Phenylalanine	4	-102.12	27.36	-189.19	-15.06	4	-133.08	55.32	-309.14	42.97	0.2585
Threonine	4	-148.36	28.10	-237.78	-58.93	4	-201.02	59.05	-388.93	-13.11	0.0871
Tryptophan	4	-92.01	27.63	-179.96	-4.07	4	-115.57	33.77	-223.03	-8.10	0.2517
Valine	4	-120.64	25.47	-201.69	-39.59	4	-150.86	50.00	-309.99	8.27	0.2666
Nonessential AA, %											
Alanine	4	-125.75	29.97	-221.13	-30.36	4	-157.41	50.39	-317.78	2.96	0.2358
Aspartate	4	-100.23	15.63	-149.96	-50.49	4	-128.13	40.76	-257.83	1.57	0.1949
Cysteine	4	-299.13	67.38	-513.56	-84.70	4	-319.87	110.54	-671.65	31.91	0.7177
Glutamate	4	-53.18	18.29	-111.39	5.03	4	-73.57	34.71	-184.01	36.88	0.2363
Proline	4	-176.14	62.06	-373.64	21.36	4	-228.59	89.48	-513.35	56.18	0.2628
Serine	4	-161.71	42.29	-296.29	-27.14	4	-183.87	63.50	-385.96	18.23	0.5323
Tyrosine	4	-203.95	62.03	-401.37	-6.53	4	-251.15	91.92	-543.69	41.40	0.3438

[1]SE: Standard Error

Table 8. Apparent digestibility of amino acids (AAAD) in fescue fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	35.22	9.54	4.85	65.59	4	59.27	6.31	39.19	79.35	0.2287
Histidine	4	53.38	5.82	34.85	71.90	4	64.07	3.40	53.26	74.89	0.4087
Isoleucine	4	7.99	10.92	-26.76	42.75	4	44.49	10.10	12.34	76.65	0.1585
Leucine	4	11.69	11.77	-25.78	49.16	4	45.37	9.19	16.12	74.62	0.2193
Lysine	4	3.33	12.57	-36.67	43.32	4	30.15	8.51	3.08	57.23	0.3828
Methionine	4	29.09	7.48	5.30	52.89	4	50.14	5.18	33.64	66.63	0.1069
Phenylalanine	4	45.59	6.70	24.27	66.91	4	65.54	5.95	46.61	84.47	0.4641
Threonine	4	-25.73	17.10	-80.14	28.68	4	-5.03	9.80	-36.23	26.17	0.4938
Tryptophan	4	62.61	11.09	27.33	97.89	4	63.37	7.78	38.61	88.14	0.9700
Valine	4	2.63	12.96	-38.62	43.87	4	33.76	8.69	6.10	61.42	0.2527
Nonessential AA, %											
Alanine	4	-7.02	13.89	-51.22	37.18	4	29.76	9.82	-1.48	61.00	0.1700
Aspartate	4	-10.13	13.19	-52.09	31.83	4	21.56	10.18	-10.83	53.95	0.1424
Cysteine	4	-12.65	15.81	-62.95	37.66	4	29.77	11.80	-7.78	67.32	0.4610
Glutamate	4	63.88	4.53	49.45	78.31	4	75.89	3.26	65.51	86.27	0.4823
Proline	4	38.03	9.45	7.95	68.11	4	63.79	6.49	43.14	84.44	0.5798
Serine	4	-26.29	18.74	-85.92	33.34	4	17.64	13.16	-24.25	59.23	0.2192
Tyrosine	4	-14.70	17.72	-71.11	41.70	4	31.29	12.89	-9.74	72.32	0.3315

[1]SE: Standard Error

Table 9. Apparent digestibility of amino acids (AAAD) in milo fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	16.34	19.60	-46.04	78.72	4	51.81	7.67	27.41	76.21	0.0793
Histidine	4	55.59	7.08	33.05	78.13	4	67.58	3.57	56.21	78.95	0.3550
Isoleucine	4	20.48	18.28	-37.69	78.65	4	48.54	6.22	28.74	68.34	0.2756
Leucine	4	54.41	10.41	21.29	87.52	4	74.77	3.91	62.33	87.20	0.4546
Lysine	4	-50.80	28.29	-140.81	39.22	4	4.81	11.23	-30.91	40.54	0.0753
Methionine	4	54.29	8.51	27.21	81.36	4	68.13	3.53	56.88	79.38	0.2839
Phenylalanine	4	32.58	14.89	-14.81	79.97	4	59.51	6.16	39.91	79.10	0.3246
Threonine	4	-10.72	20.65	-76.44	55.00	4	22.21	8.19	-3.86	48.28	0.2787
Tryptophan	4	69.13	4.55	54.65	83.61	4	81.89	6.93	59.84	103.94	0.5319
Valine	4	17.18	18.09	-40.40	74.75	4	50.72	6.81	29.06	72.38	0.2184
Nonessential AA, %											
Alanine	4	51.80	10.13	19.56	84.03	4	71.99	4.15	58.77	85.20	0.4471
Aspartate	4	10.54	17.70	-45.80	66.87	4	37.72	6.28	17.72	57.72	0.2064
Cysteine	4	-52.94	35.61	-166.27	60.40	4	14.86	13.00	-26.52	56.23	0.2415
Glutamate	4	58.33	8.90	29.99	86.67	4	74.32	3.48	63.25	85.39	0.3512
Proline	4	25.89	19.02	-34.63	86.42	4	63.59	6.94	41.49	85.68	0.4190
Serine	4	-7.83	23.72	-83.30	67.65	4	35.51	9.28	5.98	65.03	0.2254
Tyrosine	4	0.19	23.88	-75.80	76.17	4	45.48	8.94	17.03	73.93	0.7438

[1]SE: Standard Error

Table 10. Apparent digestibility of amino acids (AAAD) in rumex fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	26.92	3.03	17.28	36.55	4	21.35	2.90	12.11	30.58	0.7782
Histidine	4	15.92	3.70	4.12	27.71	4	11.75	1.41	7.26	16.23	0.7464
Isoleucine	4	5.74	5.41	-11.49	22.96	4	-5.85	3.94	-18.39	6.70	0.6505
Leucine	4	0.54	5.43	-16.73	17.81	4	-8.27	6.41	-28.68	12.15	0.7456
Lysine	4	-3.98	4.11	-17.05	9.08	4	-9.60	4.47	-23.81	4.61	0.8542
Methionine	4	13.24	2.73	4.55	21.94	4	9.12	1.99	2.79	15.45	0.7479
Phenylalanine	4	7.76	4.89	-7.79	23.31	4	1.12	4.69	-13.81	16.05	0.8070
Threonine	4	-14.93	4.44	-29.05	-0.80	4	-29.49	5.12	-45.79	-13.19	0.6296
Tryptophan	4	27.58	11.16	-7.92	63.08	4	27.47	4.60	12.84	42.11	0.9958
Valine	4	3.47	5.31	-13.44	20.38	4	-11.57	8.15	-37.50	14.37	0.5781
Nonessential AA, %											
Alanine	4	-2.68	4.15	-15.88	10.52	4	-15.61	14.29	-61.08	29.86	0.6255
Aspartate	4	1.73	3.35	-8.94	12.40	4	-2.91	4.32	-16.66	10.85	0.8276
Cysteine	4	-41.26	9.04	-70.03	-12.48	4	-48.26	15.88	-98.79	2.28	0.9028
Glutamate	4	18.61	3.59	7.20	30.02	4	13.86	3.86	1.59	26.13	0.7806
Proline	4	-19.40	11.27	-55.27	16.47	4	-34.16	11.04	-69.28	0.96	0.7506
Serine	4	-11.18	7.13	-33.87	11.52	4	-11.39	5.36	-28.43	5.65	0.9952
Tyrosine	4	-25.70	10.56	-59.31	7.92	4	-33.58	9.33	-63.29	-3.88	0.8361

[1]SE: Standard Error

Table 11. True digestibility of amino acids (TAAD) in corn fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	86.13	2.55	78.00	94.26	4	83.44	5.85	64.84	102.05	0.8854
Histidine	4	86.05	3.74	74.14	97.97	4	77.95	7.02	55.59	100.30	0.4847
Isoleucine	4	76.07	2.60	67.81	84.35	4	59.22	15.66	9.38	109.06	0.4789
Leucine	4	90.06	1.84	84.19	95.92	4	84.32	8.28	57.97	110.68	0.8219
Lysine	4	63.45	5.08	47.30	79.61	4	52.05	12.22	13.16	90.94	0.6841
Methionine	4	86.69	1.64	81.48	91.89	4	82.36	3.66	70.70	94.02	0.7140
Phenylalanine	4	86.17	3.37	75.43	96.91	4	79.32	11.51	42.69	115.96	0.7879
Threonine	4	75.79	3.61	64.30	87.28	4	57.45	8.58	30.13	84.77	0.5012
Tryptophan	4	90.09	7.33	66.75	113.43	4	100.23	6.35	80.03	120.42	0.5901
Valine	4	90.08	3.23	79.81	100.36	4	72.70	19.67	10.09	135.30	0.4831
Nonessential AA, %											
Alanine	4	87.50	2.25	80.35	94.65	4	82.89	6.28	62.91	102.87	0.8518
Aspartate	4	72.35	2.62	64.00	80.70	4	64.42	8.47	37.47	91.37	0.6841
Cysteine	4	87.90	6.30	67.84	107.97	4	89.73	9.56	59.30	120.17	0.9728
Glutamate	4	89.69	1.39	85.26	94.12	4	84.29	4.24	70.81	97.78	0.7334
Proline	4	94.47	3.53	83.23	105.71	4	91.96	4.24	78.47	105.45	0.9545
Serine	4	85.99	5.75	67.68	104.30	4	80.55	8.93	52.15	108.96	0.8684
Tyrosine	4	88.39	4.26	74.84	101.93	4	81.20	9.04	52.41	109.98	0.8736

[1]SE: Standard Error

Table 12. True digestibility of amino acids (TAAD) in game-bird flight conditioner fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	86.65	1.37	82.28	91.02	4	92.43	2.91	83.17	101.18	0.7568
Histidine	4	88.44	1.62	83.27	93.61	4	93.17	1.31	88.99	97.35	0.6831
Isoleucine	4	72.02	2.14	65.20	78.84	4	84.02	5.16	67.59	100.45	0.6137
Leucine	4	79.60	2.09	72.96	86.24	4	89.65	3.26	79.27	100.03	0.6935
Lysine	4	83.32	3.21	73.10	93.55	4	91.13	4.60	76.49	105.77	0.7804
Methionine	4	82.86	2.52	74.84	90.87	4	88.42	2.52	80.39	96.44	0.6378
Phenylalanine	4	81.34	1.84	75.47	87.21	4	91.84	2.73	83.17	100.51	0.6801
Threonine	4	73.45	3.31	62.91	83.98	4	84.27	3.86	72.00	96.54	0.6909
Tryptophan	4	106.63	3.28	96.17	117.08	4	102.22	4.85	86.77	117.66	0.8145
Valine	4	82.86	2.38	75.28	90.43	4	91.69	3.08	81.89	101.48	0.7210
Nonessential AA, %											
Alanine	4	80.78	3.02	71.16	90.39	4	89.31	2.70	80.71	97.91	0.7297
Aspartate	4	74.79	2.58	66.57	83.00	4	83.75	3.36	73.04	94.46	0.6458
Cysteine	4	67.28	2.06	60.72	73.84	4	82.52	14.38	36.77	128.28	0.7769
Glutamate	4	87.46	1.62	82.32	92.60	4	93.25	1.20	89.42	97.09	0.7144
Proline	4	82.77	0.96	79.70	85.84	4	88.38	4.66	73.57	103.20	0.8985
Serine	4	66.57	4.55	52.09	81.04	4	80.71	9.30	51.12	110.30	0.6667
Tyrosine	4	82.91	3.49	71.82	94.00	4	93.96	3.00	84.41	103.50	0.8069

[1]SE: Standard Error

Table 13. True digestibility of amino acids (TAAD) in buttonbush fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	10.77	20.60	-54.79	76.33	4	-9.75	32.27	-112.45	92.95	0.2747
Histidine*	4	7.54	10.41	-25.58	40.66	4	-19.86	22.63	-91.88	52.17	0.0224
Isoleucine	4	-13.88	16.89	-67.64	39.88	4	-45.95	46.90	-195.22	103.31	0.1819
Leucine	4	-14.49	29.95	-109.79	80.82	4	-42.57	49.77	-200.95	115.81	0.2742
Lysine	4	-35.34	23.56	-110.30	39.63	4	-85.46	53.46	-255.59	84.67	0.0797
Methionine*	4	10.25	10.36	-22.72	43.22	4	-19.52	22.91	-92.43	53.39	0.0155
Phenylalanine	4	-3.69	26.24	-87.21	79.83	4	-32.00	51.48	-195.83	131.84	0.2700
Threonine	4	-25.96	26.20	-109.41	57.35	4	-58.88	52.82	-226.98	109.22	0.2317
Tryptophan*	4	91.93	25.13	11.96	171.90	4	52.71	29.26	-40.42	145.83	0.0426
Valine	4	3.19	23.77	-72.45	78.83	4	-26.79	44.91	-169.72	116.13	.2297
Nonessential AA, %											
Alanine	4	-16.98	28.72	-108.38	74.42	4	-48.43	46.39	-196.08	99.22	02073
Aspartate	4	-10.29	14.11	-55.19	34.61	4	-38.00	37.11	-156.10	80.09	0.1605
Cysteine	4	-139.18	65.08	-346.31	67.94	4	-140.92	102.82	-468.12	186.29	0.9743
Glutamate	4	12.13	17.41	-43.29	67.55	4	-8.46	32.02	-110.36	93.44	0.1983
Proline	4	-54.10	60.68	-247.22	139.01	4	-92.06	83.93	-359.16	175.03	0.3909
Serine	4	-37.75	41.49	-168.54	95.54	4	-44.97	57.66	-228.48	138.53	0.7962
Tyrosine	4	-26.79	60.08	-218.00	164.43	4	-68.70	84.68	-338.18	200.78	0.3565

[1]SE: Standard Error
*Means within a row differ significantly ($P < 0.05$)

Table 14. True digestibility of amino acids (TAAD) in fescue fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	64.05	9.44	34.01	94.10	4	87.69	5.74	69.41	105.96	0.2096
Histidine	4	76.95	5.71	58.79	95.12	4	85.51	2.74	77.08	94.55	0.4454
Isoleucine	4	50.10	10.78	39.26	84.42	4	82.37	8.87	54.14	110.59	0.1793
Leucine	4	50.71	11.65	15.78	87.80	4	84.00	8.46	57.07	110.93	0.1963
Lysine	4	54.54	12.28	15.44	93.63	4	75.76	7.23	52.76	98.75	0.4501
Methionine	4	62.61	7.34	18.86	85.95	4	79.71	5.06	63.59	95.82	0.1531
Phenylalanine	4	70.76	6.62	49.71	91.81	4	90.28	5.29	73.44	107.13	0.4448
Threonine	4	34.57	16.86	-19.08	88.22	4	62.00	7.19	39.12	84.88	0.3165
Tryptophan	4	104.32	10.88	69.70	138.95	4	99.90	8.85	71.75	128.05	0.8138
Valine	4	59.55	12.79	-11.20	100.25	4	88.35	7.49	64.52	112.19	0.2483
Nonessential AA, %											
Alanine	4	42.90	13.68	-0.62	86.43	4	77.63	8.78	49.70	105.56	0.1649
Aspartate	4	41.49	12.94	0.33	82.66	4	71.06	8.85	42.90	99.22	0.1351
Cysteine	4	38.74	15.69	-11.20	88.68	4	84.80	9.78	53.67	115.94	0.3939
Glutamate	4	81.24	4.46	67.03	95.45	4	92.46	2.79	83.58	101.35	0.4797
Proline	4	64.06	9.40	34.15	93.98	4	91.66	5.66	73.65	109.68	0.5318
Serine	4	33.45	18.59	-25.71	92.62	4	81.71	11.20	46.07	117.34	0.7962
Tyrosine	4	41.55	17.34	-13.64	96.74	4	85.99	11.67	48.86	123.12	0.3286

[1]SE: Standard Error

Table 15. True digestibility of amino acids (TAAD) in milo fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	53.44	19.24	-7.81	114.69	4	88.00	5.16	71.58	104.42	0.0699
Histidine	4	79.19	6.85	57.39	101.00	4	89.12	1.65	83.86	94.38	0.3929
Isoleucine	4	60.29	17.91	3.30	117.28	4	88.82	3.71	77.02	100.63	0.2338
Leucine	4	74.96	10.21	42.46	107.47	4	94.90	2.69	86.34	103.46	0.4356
Lysine	4	34.13	27.44	-53.20	121.46	4	79.66	5.94	60.75	98.57	0.1101
Methionine	4	80.77	8.24	54.54	107.00	4	91.25	2.62	82.89	99.60	0.3772
Phenylalanine	4	65.74	14.57	19.35	112.12	4	91.76	3.84	79.55	103.97	0.3099
Threonine	4	47.13	20.16	-17.03	111.30	4	88.85	3.73	73.99	97.72	0.1602
Tryptophan	4	100.45	4.33	86.67	114.23	4	109.03	8.48	82.04	136.03	0.6482
Valine	4	65.85	17.65	9.68	122.02	4	96.92	3.71	85.11	108.72	0.2137
Nonessential AA, %											
Alanine	4	75.81	9.89	44.32	107.29	4	94.77	2.80	85.85	103.70	0.4438
Aspartate	4	57.88	17.27	2.92	112.85	4	82.66	2.78	73.80	91.51	0.2084
Cysteine	4	7.27	35.18	-104.69	119.23	4	81.16	9.41	51.19	111.13	0.1748
Glutamate	4	78.75	8.72	51.01	106.49	4	93.60	2.04	87.11	100.09	0.3509
Proline	4	53.55	18.79	-6.25	113.35	4	92.89	4.80	77.62	107.17	0.3741
Serine	4	41.43	23.33	-32.83	115.68	4	87.78	4.99	71.91	103.65	0.1471
Tyrosine	4	45.94	23.45	-28.67	120.56	4	90.12	5.98	71.08	109.16	0.3314

[1]SE: Standard Error

Table 16. True digestibility of amino acids (TAAD) in rumex fed to cecectomized and intact Canada geese.

	Intact					Cecectomized					
	N	Mean	SE[1]	95% CI		N	Mean	SE	95% CI		P-value
Essential AA, %											
Arginine	4	33.61	2.76	24.82	42.40	4	28.40	3.13	18.45	38.35	0.7799
Histidine	4	26.18	3.26	15.81	36.56	4	21.88	1.45	17.27	26.49	0.7099
Isoleucine	4	23.88	4.87	8.40	39.36	4	12.78	3.07	3.00	22.55	0.6403
Leucine	4	19.45	4.88	3.91	34.98	4	11.76	5.84	-6.84	30.36	0.7629
Lysine	4	19.35	3.43	8.44	30.24	4	12.63	3.45	1.64	23.62	0.8105
Methionine	4	25.31	2.42	17.62	33.00	4	20.51	1.65	15.27	25.75	0.6846
Phenylalanine	4	25.35	4.31	11.62	39.09	4	19.63	4.34	5.81	33.45	0.8220
Threonine	4	11.44	3.85	-0.82	23.70	4	1.87	3.65	-9.74	13.49	0.7251
Tryptophan	4	127.51	12.23	88.58	166.43	4	121.10	5.91	102.28	139.92	07332
Valine	4	26.90	4.62	12.18	41.61	4	12.48	7.30	-10.74	35.71	0.5607
Nonessential AA, %											
Alanine	4	20.34	3.68	8.62	32.06	4	8.01	13.35	-34.47	50.49	0.6178
Aspartate	4	21.35	3.10	11.50	31.20	4	17.22	3.25	6.89	27.56	0.8323
Cysteine	4	-0.40	8.20	-26.48	25.68	4	-2.30	14.51	-48.46	43.87	0.9719
Glutamate	4	31.71	3.27	21.30	42.12	4	27.33	3.18	17.10	37.37	0.7773
Proline	4	11.46	10.15	-20.85	43.78	4	1.20	9.33	-28.51	30.90	0.8155
Serine	4	11.58	6.36	-8.65	31.82	4	14.73	4.01	1.97	27.49	0.1632
Tyrosine	4	4.83	9.09	-24.11	33.75	4	-1.39	8.49	-28.40	25.61	0.8906

[1]SE: Standard Error

CHAPTER 4. COMPARATIVE GUT MORPHOLOGY OF GIANT CANADA GEESE AFTER CECA REMOVAL

INTRODUCTION

Diet quality and quantity, reproductive state, and food habits are factors known to influence the size of digestive organs in birds. Changes in one or more of those factors can lead to changes in the morphology of the digestive tract (Kehoe et al. 1988). Gut morphology changes in response to diet quality and quantity have been well documented in gallinaceous birds (Anderson 1972, Moss 1972, Pendergast and Boag 1973). They have also been studied extensively in waterfowl (Miller 1975, Korschgen 1976, Ankney 1977, Paulus 1982, Drobney 1984). Miller (1975) demonstrated that the gizzards, ceca, and intestines of captive mallards (*Anas platyrhynchos*) respond to increased dietary fiber levels by increasing in length and weight. These morphological changes allow birds to increase their digestive efficiency. These morphological changes were also noted in free living birds by Ankney (1977) and Paulus (1982), in breeding and wintering birds respectively. In wood ducks (*Aix sponsa*) Drobney (1984) noted that changes in digestive organs reflected adaptations to changes in diet quality, metabolism and food intake. Drobney (1984) found that the size of the intestine, ceca, and liver of males decreased between fall and spring, which correlated with a reduction in the fiber content of the diet. The mean size of the intestine, liver, and ceca of female wood ducks increased in response to higher dietary fiber in the fall, and hyperphagia during laying (Drobney 1984).

The response of the cecum to dietary changes is difficult to interpret because its function in waterfowl is poorly understood. Most researchers have primarily focused on

the effects of dietary fiber on cecal length. Drobney (1984), however, found that the quantity of food ingested influences cecal length as much or more than food quality. He found that cecal lengths of female wood ducks increased between prebreeding and laying, a period when diets were high in fiber content. He also suggested that increased cecal length of females was caused by increased food intake due to hyperphagia.

One approach to studying the function of the ceca in nutrition is to examine the effects of their removal. Cecectomized birds are regularly used in domestic nutritional and digestive studies (Thornburn and Willcox 1965; Sibbald 1979; Johns et al. 1986; Green et al. 1986). Despite the regular use of cecectomized birds, little information is available about the responses of the digestive tract to cecectomy in general. We are unaware of any waterfowl research that has experimentally removed a digestive organ and examined the response of other digestive organs. Physical and physiological adaptations may occur among the remaining organs in response to the surgical removal of an organ.

We had the opportunity of studying the effects of ceca removal on other digestive organs by comparing a group of cecectomized and intact Giant Canada geese (*Branta canadensis*) previously utilized in our study of nutrient metabolizability. Given that the digestive organs have the capacity to adapt in response to changes in diet quality and quantity, reproductive state, and food habits, we would expect them to have the flexibility to make compensatory adjustments after loss or injury to a digestive organ. Our objective was to compare sizes of digestive organs between cecectomized and intact birds, and assess the remaining digestive organs' capacity to make compensatory adjustments.

METHODS

Gut morphology weights and measurements were obtained from Giant Canada geese captured on several locations around Boone County, Missouri for a feeding ecology study (Chapter 2). At the end of the feeding trials, we euthanized 18 females to conduct our gut morphology study. The ceca of nine birds were surgically removed prior to the feeding trials, and the ceca of the remaining nine birds underwent a sham operation. The sham operation consisted of manual manipulation of the ceca, which were reinserted into the peritoneal cavity (see *Surgical Procedure*).

Experiment Birds

Geese were captured on 22 June 2000 during the flightless period at Stephens Lake and the Missouri Country Club in Boone County, Missouri. Upon capture, geese were sexed by cloacal examination. We retained 17 and 13 female geese from the Missouri Country Club and the Stephens College Lake respectively. We conducted two additional captures on September 03, 2000 at the City of Columbia Wetlands (4 geese) in Boone County, Missouri, and on December 08, 2000 at the University of Missouri South Farms (17 geese) in Boone County. Rocket nets aimed over a baited cracked corn site were used to capture geese on the latter two occasions. After capture, we transported the geese to the Charles Green Wildlife Area in Boone County, Missouri. They were weighed to the nearest gram using a spring scale and a cotton pillow case, then marked with numbered metal leg bands, and randomly assigned to one of the two following groups: 1) cecectomized (treatment), 2) intact (sham operated).

Husbandry

The geese were housed in 8' x 8' x 8' cages in an indoor facility equipped with windows to provide a natural photoperiod at the Charles Green Wildlife Area. Fresh water and grit (granite) were available to the birds at all times. Geese were initially maintained on a mixed diet consisting of 25% Purina Meat Builder (crude protein not less than 20.0%, crude fat not less than 3.0%, crude fiber not more than 5.0%) and 75% ground rabbit pellets (PMI Feeds, crude protein not less than 14.0%, crude fat not less than 1.5%, crude fiber not more than 25%) to ensure that their digestive system was operating in a high fiber mode. After a month, we switched the geese to a commercial game-bird flight conditioner (crude protein <19.0%, crude fat no <2.0%, crude fiber not >12.0%; Purina Mills, St. Louis, Missouri, USA).

Surgical Procedure

Birds were fasted for 24 hours before surgery. Standard surgical procedures were followed during the cecectomies. Inhaled isoflourine (4-5% flow rate) was used to maintain a surgical plane of anesthesia. Birds were placed in a position of dorsal recumbency. Feathers were removed from the abdomen and the area was disinfected with iodine solution. A laparotomy was performed through an incision in the body wall. Each pair of ceca was localized and the two distal ends were carefully detached by hand from the mesentery which joins them to the antimesenteric wall of the ileum. When both ceca were completely excised, each was transected and both cut surfaces sutured, as near as possible to the junctions of the ceca and the intestine. After the ceca were removed, the exposed intestine was returned to the peritoneal cavity, and the incision in the peritoneum and muscular layers closed by sutures. After the operation, solid food was

withheld for 24 hours, but water was supplied *ad libitum*. During the sham procedure a laparotomy was performed and the viscera were manipulated. The ceca were taken out and the two distal ends were carefully detached by hand from the mesentery which joints them to the antimesenteric wall of the ileum, the ceca were reinserted into the peritoneal cavity, and the incision in the peritoneum and muscular layers were closed by sutures.

The surgeries were conducted over four time periods. The first surgeries were conducted between August 14-20, 2000 by staff from the Department of Laboratory Animal Medicine at the University of Missouri, Columbia. With the exception of one bird, all cecectomized birds died as a result of complications from the surgery. All sham operated birds survived the surgery. Two additional birds were cecectomized on October 04, 2000, and both geese died from surgical complications. The remaining cecectomies were performed on November 14-16, 2000 and on March 22, 2001 by staff from the Department of Animal Sciences at the University of Missouri, Columbia. One of the geese died after surgery, but all others survived without complications.

Gut Morphology Analysis

At the conclusion of feeding trials, Canada geese were euthanized through CO2 inhalation on August 2001, weighed (0.1 g), and the carcasses were kept frozen. During the necropsies, we took several external and internal measurements. A series of external measurements were taken with calipers or a meter stick and included *central culmen* (0.01 mm) – from intersection of skin and premaxilla to the tip of the bill nail, *diagonal culmen* (0.01 mm) – from the proximal tip of the posterior lobe of the premaxilla to the bill nail, *bill width* (0.01 mm) - at the widest point of the premaxilla, *bill height* (0.01 mm) - at the posterior terminus of the nares, *skull width* (0.01mm) – immediately

posterior to the eyes, *head length* (1 mm) –from the bill tip to the posterior extremities of the occipital process, *total body length* (1 mm) – from the tip of the premaxilla to the tip of the most distal retrix, *wing chord* (1 mm) – from the wrist on bent wing to the tip of the most distal primary, *wing length* (1 mm) – outstretched perpendicular to the body from the junction with the body to the tip of the most distal primary, tarsus length (0.01 mm) – from the proximal to the lateral condyles of the metatarsus, middle toe length (0.01 mm) – from the base of the nail to the junction with the metatarsus. *Keel length* (0.01 mm), from the tip of the cranial process to the end of the medial caudal process, was measured following removal of the left breast muscle.

The gizzard, heart, and liver were excised, stripped of adhering fat, washed, and patted dry with a paper towel prior to weighing (0.01 g). The digestive tract organs and pancreases were excised, stripped of adhering fat, washed, and patted dry prior to weighing (0.01 g). The digestive tract was dissected into the upper digestive tract (esophagus and proventriculus), gizzard, small intestine, ceca and large intestine. Each organ was emptied and washed prior to weighing. Total digestive tract mass was calculated by summing the masses of the 5 dissected components. Lengths of the upper digestive tract, small intestine, ceca and large intestine were measured to the nearest 1 mm using a meter stick prior to removal of the ingesta. Cecal length was measured as the combined length of both ceca (when present). All measurements were made on unstretched digestive tract components before removal of ingesta to reduce variation of these organs. Gizzard width was measured to the nearest 0.01 mm across the widest point in the mid plane using calipers. Total digestive length was calculated by summing the measures of the 5 dissected components.

Correcting Digestive Body Components for Body Size

Thomas (1984), Kehoe and Ankney (1985) suggested possible variation in digestive organs with body size. In response, several interspecific comparison studies utilized analysis of covariance with body weight as a covariate. For intraspecific comparisons of gut morphology, however, the first principle component score from an analysis of several structural size variables was deemed more appropriate as a covariate because it is independent of the nutritional state of the bird (Alisauskas and Ankney 1987, Kehoe et al. 1988). To account for this variation, we did principal component analyses of the correlation matrix for the eleven structural measurements taken on each bird (PROC PRINCOMP; SAS Institute Inc. 1990). The first principal component (PC_1) described positive correlations in the eleven variables with loadings ranging from 0.05 to 0.42. The corresponding eigenvalue was 4.45 and the first principal component explained 40.49% of the total variance in structural variables. We used PC_1 scores for each bird as a measure of body size. To determine if digestive components and internal organs were related to body size, each variable (gizzard length and weight, heart weight, liver weight, total upper digestive tract length and weight, small intestine length and weight, large intestine length and weight, ceca length and weight, pancreas weight, and total digestive tract mass) was subsequently regressed on PC_1. To eliminate potential bias from the data collected (ceca removal), we conducted separate regressions for intact ($n = 9$) and cecectomized ($n = 9$) birds. Gizzard length and weight, heart weight, liver weight, total upper digestive tract weight, small intestine length and weight, ceca length and weight, large intestine length and weight, pancreas weight, and total digestive tract mass were unrelated to body size in intact birds ($P > 0.05$). However, upper digestive

tract length was related to body size in intact birds. In cecectomized birds, liver, small intestine, and pancreas weight were the only three variables related to body size. The relationships are indicated by the following equations:

Upper Digestive Tract Length = $47.66 + 1.17PC_1$

$F = 19.72$, $P = 0.0030$, $R^2 = 0.74$

Liver Weight = $88.04 + 6.35PC_1$

$F = 8.23$, $P = 0.0241$, $R^2 = 0.54$

Small Intestine Weight = $39.48 + 3.04PC_1$

$F = 8.74$, $P = 0.0212$, $R^2 = 0.56$

Pancreas Weight = $7.80 - 0.44PC_1$

$F = 7.21$, $P = 0.0313$, $R^2 = 0.51$

The residuals from these regression equations were used to derive new size-corrected values (yi) for the upper digestive tract lengths of intact birds, and liver, small intestine, and pancreas weights of cecectomized birds. We used Ankey and Alisauskas (1991) equation to derive the new values:

$$Yi = Y_{obs} - [a + b(PC_1)] + Y_{obs}$$

Where Y_{obs} equals the unadjusted variable for and individual specimen and Y_{obs} equals the mean of the unadjusted variable for all specimens.

To determine if, and which digestive organs varied between intact and cecectomized groups, mean sizes of organs were compared using *t*-tests (PROC TTEST, SAS Institute Inc. 1990).

RESULTS

The ceca regenerated in all but one cecectomized goose (8 out of 9). In three cecectomized geese, the ceca regenerated on only one side, while five of the cecectomized geese had partial cecal tissue regeneration in both the left and the right ceca (Table 1; Fig. 1). The ceca of intact geese were 88% longer than the ceca of cecectomized geese (49.77 ± 1.23 cm *vs* 5.82 ± 1.71 cm, $p < 0.05$; Table 2), and cecal mass of intact geese was greater than the cecectomized geese (6.74 ± 0.45 cm *vs* 0.58 ± 0.15 g, $p < 0.05$; Table 2). Figure 1 illustrates the ceca of an intact goose, while figure 2 illustrates cecal regeneration among one of the cecectomized geese.

Mean pancreas mass of intact geese was 16% greater than the pancreas of cecectomized geese (9.94 ± 0.65 g *vs* 8.31 ± 0.27 g, $p < 0.05$; Table 2). There was no statistical difference in gizzard, liver, large intestine, and total digestive tract mass between intact and cecectomized geese (Table 2). There was also no statistical difference in gizzard, small intestine, and large intestine length between intact and cecectomized geese (Table 2). Although cecectomized geese had heavier hearts, heavier small intestines, and heavier and longer upper digestive tracts, there was no statistical difference (Table 2).

DISCUSSION

Compensatory Growth in Digestive Organs

We hypothesized that if ceca play a role in digestion, their removal would lead to compensatory growth in the remaining digestive organs. Contrary to our expectations, we did not find any evidence to suggest that compensatory growth occurred. There was no significant difference in gizzard, small intestine, large intestine, and liver mass

between cecectomized and intact birds. Chen et al. (2003) found no significant differences in the relative weight (g/100g BW) of gizzard, small intestine, rectum and colon between intact and cecectomized White Roman goslings. The relative length of the colon and the rectum was longer among cecectomized goslings.

We found a 16% reduction in pancreas size between cecectomized and intact geese. Principal pancreatic functions include the secretion of digestive enzymes and insulin. In a study conducted on lesser snow geese (*Chen caerulescens caerulescens*), Ankney (1977) noted that failed-nester males had heavier pancreases than late incubating males. He theorized that reduction in pancreas weight among incubating lesser snow geese reflected a reduced need for digestive enzymes during incubation. We expected that the removal of the ceca would have led to a higher demand for digestive enzymes among remaining organs; therefore, it seems counterintuitive that cecectomized geese had lower pancreatic weights in our study.

Hanson (1962) reported that pancreas weight declined with increased age. It is possible that cecectomized geese were older than intact geese, and age might partially explain the decreased pancreas weight. All of the geese in our intact group were young-of-the-year captured during the flightless period as part of a goose round-up. Due to surgical complications we had to capture additional geese during the fall, and their age was unknown. A more detailed study utilizing known-age geese is necessary to determine the factors responsible for differences in mean pancreas weight.

Another explanation for the lack of compensatory growth in the digestive organs between cecectomized and intact geese could be the maintenance diet fed to them between feeding trials. Canada geese were maintained on a high fiber diet (>12%)

between feeding trials in an attempt to maintain their natural cecal microfauna and –flora. It is possible that their organs were at their maximum size in order to accommodate the high fiber levels. If the maintenance diet contained a much lower fiber content, we might have detected a difference in digestive organ size. For example, the maintenance diets provided by Petrie (1994) and Buckley (1989) between feeding trials had a much lower fiber content (<5%).

Cecal Regeneration

During our necropsies, we unexpectedly discovered that 8 out of 9 cecectomized geese regenerated their ceca. Although we completely removed the cecum, geese had one or both ceca in various stages of regeneration. Cecectomized birds are regularly used in the poultry industry as a method of eliminating the possibility of residual food in the bird's digestive tract beyond the collection period. Clarke et al. (1980) conducted an experiment analyzing the possibility of ceca regeneration in domestic fowl (*Gallus gallus*). They surgically removed varying amounts of cecal tissue (47-100%) and found that birds were capable of regrowth. The remaining cecal tissue grew even when it appeared that one side of the ceca had been removed. However, the average growth rate was dependent on the amount of residual cecal tissue after surgery.

During Clarke's post-mortem examination of the ceca, they reported that the regenerated ceca appeared normal, and the morphology and histology of the regenerated ceca were similar to the equivalent regions of the non-surgical ceca. In Clarke's et al. (1980) study, they removed only one ceca, whereas in our study we removed both ceca. We did not conduct a histological or a thorough morphological examination of the

regenerated cecal tissue. However, upon dissection, we did not find any digesta in the regenerated ceca, and therefore assumed nonfunctionality.

There are several potential reasons for cecal regeneration. According to Clarke et al. (1980), the surgical technique utilized might influence the subsequent growth and form of the ceca. All of our cecectomies, however, were conducted by the same individual, and we have no reason to believe that the surgical technique might have varied from bird to bird. Age of the bird could play a role in the amount and type of cecal regeneration. There could be differences in the capacity of cecal tissue to regenerate depending on age at surgery. Clarke et al. (1980) conducted their surgeries on 22 day-old birds, and sacrificed them 5 weeks following surgery. In another study conducted by Crompton (unpublished study cited in Clarke et al. 1980), they cecectomized birds at 17 days of age, and found considerable cecal regeneration five months after cecectomy. We had to conduct additional captures during the fall due to the surgical complications with first set of captures. During the fall, we were unable to distinguish between first year geese and older geese. Thus, the differences we observed among our geese might partially be explained by age differences.

Conclusions

The results of this study indicate that digestive organs do not undergo a compensatory size adjustment in response to cecectomy. Other than the ceca, the pancreas was the only organ for which there was a significant difference between cecectomized and intact geese. Contrary to our expectations, the pancreases were smaller among cecectomized geese. We also found that the ceca of Canada geese are capable of regeneration even after complete surgical removal. Complete histological and

morphological examinations are necessary to assess functionality of regenerated ceca. Additional research should be designed to determine the specific relationship between age at surgery, surgical techniques, and the cecum's capacity to regenerate.

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Table 1. Lengths of ceca in cecectomized and intact giant Canada geese.

Bird I.D.	Ceca 1*	Ceca 2*	Total*
Cecectomized:			
356	0.0	2.2	2.2
357	2.4	1.9	4.3
360	6.2	6.1	12.3
361	2.7	0.0	2.7
367	6.6	9.4	16.0
371	4.0	1.2	5.2
372	2.1	4.2	6.3
376	0.0	0.0	0.0
378	0.0	3.4	3.4
Intact:			
330	26.7	25.3	52.0
335	23.7	24.4	48.1
336	23.6	22.8	46.4
337	25.2	24.1	49.3
338	23.4	21.3	44.7
343	27.4	27.2	54.6
345	26.1	26.0	52.1
349	26.3	28.2	54.5
352	22.8	23.4	46.2

*Lengths in centimeters

Table 2. Mean values for digestive organ measurements of intact and cecectomized giant Canada geese.

Organ measurement	Intact Group mean ± SE	95% C1	Cecectomized Group mean ± SE	95% C1	DF	t-value	Pr > |t|
Mass[1]:							
Gizzard	51.06 ± 1.24	48.20, 53.91	48.73 ± 1.40	45.51, 51.96	16	1.24	0.2313
Heart	34.08 ± 2.08	29.30, 38.87	36.92 ± 1.44	33.60, 40.25	16	-1.12	0.2778
Liver	85.63 ± 5.37	73.24, 98.01	80.73 ± 3.73	75.36, 100.72	16	-0.31	0.7574
Upper digestive tract	25.41 ± 0.98	23.14, 27.67	25.98 ± 0.85	24.01, 27.95	16	-0.44	0.6642
Small intestine	38.58 ± 1.43	35.29, 41.87	35.98 ± 1.73	33.49, 45.46	16	-0.30	0.7654
Large intestine	6.03 ± 0.36	5.19, 6.86	5.14 ± 0.36	4.31, 5.96	16	1.75	0.0997
Ceca*	6.74 ± 0.45	5.69, 7.79	0.58 ± 0.15	0.23, 0.93	16	12.89	<0.0001
Pancreas*	9.94 ± 0.65	8.44, 11.45	8.31 ± 0.27	7.13, 8.46	16	3.01	0.0083
Total digestive tract	200.14 ± 8.12	181.43, 218.86	183.49 ± 5.27	171.33, 195.64	16	1.72	0.1045
Lengths[2]:							
Gizzard	80.41 ± 2.24	75.24, 85.59	79.04 ± 1.17	76.35, 81.74	16	0.54	0.5964
Upper digestive tract	48.81 ± 0.40	46.58, 48.68	50.17 ± 1.02	47.81, 52.52	16	-2.26	0.0378
Small intestine	207.68 ± 5.43	195.15, 220.20	196.60 ± 2.32	191.25, 201.95	16	1.88	0.0791
Large intestine	14.62 ± 0.35	13.81, 15.43	14.22 ± 0.58	12.87, 15.57	16	0.59	0.5657
Ceca*	49.77 ± 1.23	46.93, 52.60	5.82 ± 1.71	1.87, 9.77	16	20.84	<0.0001

*Indicates significant difference between treatments ($P < 0.05$), [1]Weight in grams, [2]Length in centimeters.

Figure 1. Cecum of intact Canada goose.

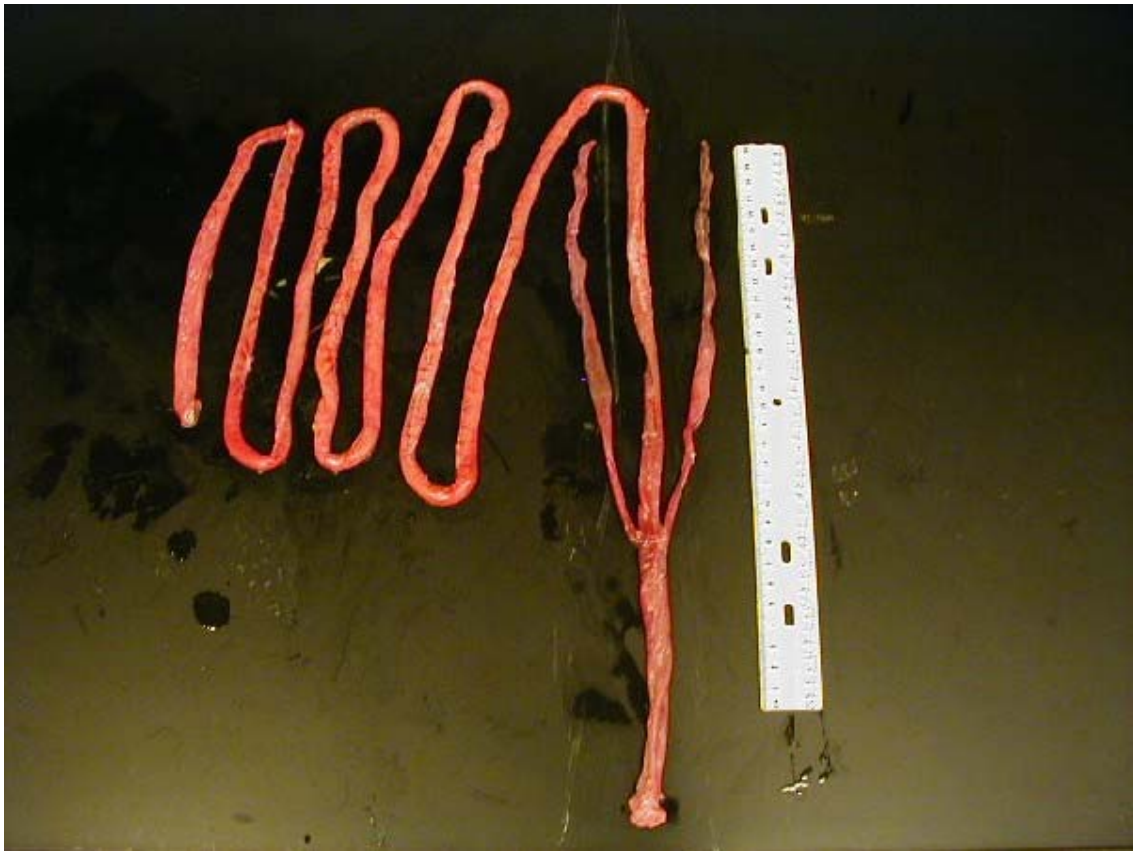
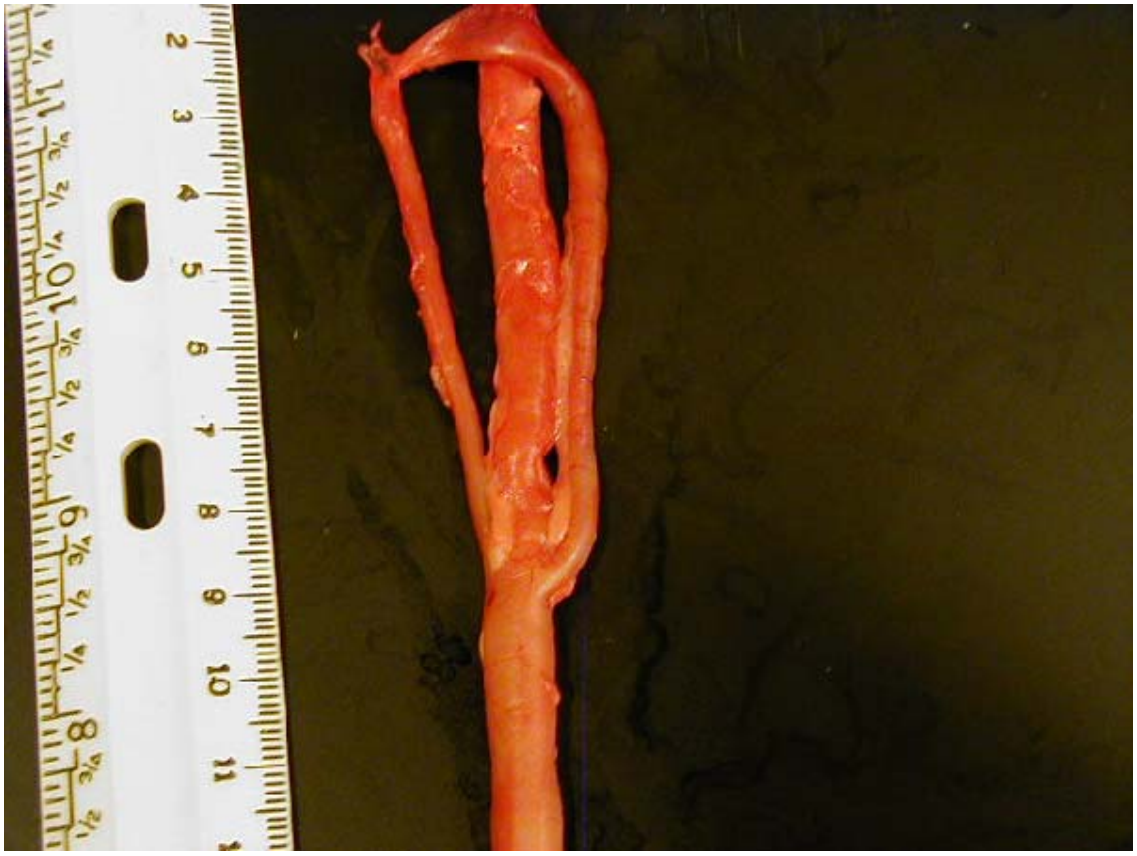


Figure 2. Cecum of cecectomized Canada goose.



CHAPTER 5. USE OF CMCASE ASSAY TO EVALUATE PRESENCE OF CELLULOSE DIGESTING MICROORGANISMS IN THE CECUM OF CANADA GEESE

INTRODUCTION

Canada geese (*Branta canadensis*) are primarily herbivorous (Owen 1980), and can spend as much as 90% of daylight hours feeding (Owen 1972). Previous digestive studies have suggested that they spend a considerable amount of time feeding due to an inefficient digestive system (Owen 1972, 1975, Sibly 1981). Food can pass through a goose's digestive system in as little as 30 minutes, and as a result, much of the food is unassimilated (Owen 1980).

The fast passage rates and Mattocks' (1971) inability to find cellulose-splitting bacteria in goose ceca led researchers to assume that geese were incapable of digesting cellulose. In fact, researchers would use cellulose as a digestive marker because they assumed that energy in crude fiber was unavailable to waterfowl (Krapu and Reinecke 1992). Recent studies, however, have demonstrated that herbivorous waterfowl can metabolize 25-74% of hemicellulose (Krapu and Reinecke 1992). Buchsbaum et al. (1986) found that captive Canada geese and free-ranging brant (Branta bernicla) were capable of digesting 18-33% of the cellulose in plants.

These studies confirmed cellulose digestion capabilities in geese; however, the mechanism of fiber digestion is not completely understood. The response of waterfowl ceca to dietary and seasonal changes have led researchers to speculate about their role in fiber digestion (Miller 1975, Ankney 1977, Drobney 1984). Miller (1975) demonstrated that the gizzard, cecum, and small and large intestines of captive mallards (*Anas platyrhynchos*) respond to increased fiber levels in the diet by increasing in size. These morphological changes were also noted in free living birds by Ankney (1977) and Paulus

(1982), for breeding and wintering birds respectively. In wood ducks (*Aix sponsa*), Drobney (1984) noted that changes in their digestive organs reflected adaptations to changes in diet quality, metabolism, and food intake.

Mattocks (1971) might not have found cellulose splitting bacteria in goose ceca because his subjects were domestic geese. Domestication, captivity, and diet are known to influence the avian cecum (Moss 1972, Miller 1975, Ankney 1977, Drobney 1984). As a result of genetic changes due to domestication and the use of nutritionally complete feeds, the cecum of domestic birds have lost most or all of their natural microflora and –fauna and potential physiological capabilities (Thomas 1987). This is also true among captive bred game species, and even those captured from the wild. Mean cecal lengths and cecal microanatomy and biology are known to differ between wild and captive birds (Clench and Mathias 1995). Moss (1972) found that after 4 years of being raised in captivity, the cecal length of red grouse (*Lagopus lagopus scoticus*) was 54% shorter than those of wild birds. Hanssen (1979) found the cecal microbiology of captive willow grouse (*L. l. lagopus*) to be different than that of wild birds.

Approach

Carboxymethyl cellulose (CMCase) is the enzyme involved in the conversion of cellulose into glucose. CMCase assays are regularly used in nutritional studies to measure the amount of cellulose digestion that can be expected for a particular feed. If the cecum of Canada geese is involved in cellulose digestion, then we would expect to find cellulose digesting microorganisms and CMCase within the contents of their ceca. The objective of this study was to determine the presence of cellulose digesting bacteria in Canada goose ceca.

METHODS

Experiment Birds

Geese were captured on 22 June 2000 during the flightless period at Stephens Lake and the Missouri Country Club in Boone County, Missouri. Upon capture, geese were sexed by cloacal examination. We retained 17 and 13 female geese from the Missouri Country Club and the Stephens College Lake respectively. We conducted two additional captures on September 03, 2000 at the City of Columbia Wetlands (4 geese) in Boone County, Missouri, and on December 08, 2000 at the University of Missouri South Farms (17 geese) in Boone County. Rocket nets aimed over a baited cracked corn site were used to capture geese on the latter two occasions. After capture, we transported the geese to the Charles Green Wildlife Area in Boone County, Missouri. They were weighed to the nearest gram using a spring scale and a cotton pillow case, then marked with numbered metal leg bands, and randomly assigned to one of the two following groups: 1) cecectomized (treatment), 2) intact (sham operated).

Husbandry

The geese were housed in 8' x 8' x 8' cages in an indoor facility equipped with windows to provide a natural photoperiod at the Charles Green Wildlife Area. Fresh water and grit (granite) were available to the birds at all times. Geese were initially maintained on a mixed diet consisting of 25% Purina Meat Builder (crude protein not less than 20.0%, crude fat not less than 3.0%, crude fiber not more than 5.0%) and 75% ground rabbit pellets (PMI Feeds, crude protein not less than 14.0%, crude fat not less than 1.5%, crude fiber not more than 25%) to ensure that their digestive system was operating in high fiber mode. After a month, we switched the geese to a commercial

game-bird flight conditioner (crude protein <19.0%, crude fat no <2.0%, crude fiber not >12.0%; Purina Mills, St. Louis, Missouri, USA).

Surgical Procedure

Birds were fasted for 24 hours before surgery. Standard surgical procedures were followed during the cecectomies. Inhaled isoflourine (4-5% flow rate) was used to maintain a surgical plane of anesthesia. Birds were placed in a position of dorsal recumbency. Feathers were removed from the abdomen and the area was disinfected with iodine solution. A laparotomy was performed through an incision in the body wall. Each ceca was localized and the two distal ends were carefully detached by hand from the mesentery which joins them to the antimesenteric wall of the ileum. When both ceca were completely excised, each was transected and both cut surfaces sutured, as near as possible to the junctions of the ceca and the intestine. After the ceca were removed, the exposed intestine was returned to the peritoneal cavity, and the incision in the peritoneum and muscular layers closed by sutures. The ceca were placed in Whirlpack bags, labeled, and immediately placed on ice. The Whirlpack bags were placed inside of gallon-sized plastic freezer bags and frozen at –20°C. After the operation, solid food was withheld for 24 hours, but water was supplied *ad libitum*. During the sham procedure a laparotomy was performed and the viscera were manipulated. The ceca were taken out and the two distal ends were carefully detached by hand from the mesentery which join them to the antimesenteric wall of the ileum, the ceca were reinserted into the peritoneal cavity, and the incision in the peritoneum and muscular layers were closed by sutures.

The surgeries were conducted over four time periods. The first surgeries were conducted between August 14-20, 2000 by staff from the Department of Laboratory

Animal Medicine at the University of Missouri, Columbia. With the exception of one bird, all cecectomized birds died as a result of complications from the surgery. All sham operated birds survived the surgery. Two additional birds were cecectomized on October 04, 2000, and both geese died from surgical complications. The remaining cecectomies were performed on November 14-16, 2000 and on March 22, 2001 by staff from the Department of Animal Sciences at the University of Missouri, Columbia. One of the geese died after surgery, but all others survived without complications.

Extraction of enzyme activity

The approach used was to conduct carboxymethyl cellulase assays on Canada goose cecal contents. The ceca were removed from the freezer and allowed to thaw at room temperature. Once thawed, we transferred their contents into individual 50 ml in vitro tubes. One gram of wet residue was weighed into tared aluminum dishes. The samples were dried for 8 hours at 105°C to determine dry matter.

We added 20 ml 10mM-sodium phosphate (pH 6.8) buffer to the in vitro tubes and treated with 2.5 ml carbon tetrachloride and 20 μg lysozyme/ml. The in vitro tubes were vortexed at low speed and incubated in a 37°C water bath for 3 hours. During the 3 hour incubation period, the samples were vortexed at low speed each hour to mix the carbon tetrachloride and buffer layers. After the 3 hour incubation, the in vitro tubes were centrifuged at 29,000xg (17,000 rpm on JA-17 rotor) at 4°C for 15 minutes. The resulting supernatant was drawn with a Pasteur pipet into disposable 12x75 mm polypropylene culture tubes with snap cap, and frozen.

Subsequently, we thawed the tubes and drew 1 ml of supernatant in triplicate into marked glass culture tubes. We prepared D-Glucose standards of 10, 20, 30, 40, 50 and

60 mg/ml, and delivered 1ml of each standard into glass culture tubes. We placed the culture tubes in a 39°C water bath and added and 1.5 ml of a warmed (39°C) 2% carboxymethylcellulose solution to each incubation tube and to each standard on the rack. The tubes were allowed to incubate for 30 minutes (timed from the first tube). After 30 minutes, we stopped the reaction by adding 3.0 ml of 3,5-dinitrosalicyclic acid (DNS) color reagent to each tube. While the "30 minute" tubes were incubating, we processed the 0-time tubes. We added 1.5 ml of to each tube immediately followed by 3 ml of 3,5-dinitrosalicyclic acid (DNS) reagent.

We placed the test tubes in boiling water for 5 minutes to develop color. After color development, the tubes were cooled by immersion in tap water for 5 minutes. Absorbance was read at 560 nm using D-Glucose as the standard. CMCase activity was expressed as μmoles glucose released/ min/ g DM.

RESULTS

We collected 28 pairs of ceca from the cecectomies for this analysis. Three of the samples became contaminated with water during the incubation process and were discarded. We completed the CMCase assays on the remaining 25 pairs. Eighteen of the ceca originated from cecectomies we conducted in August and October 2000. The remaining ceca were harvested from the March 2001 cecectomies.

We observed CMCase activity in all 25 samples (Table 1). Mean CMCase activity was 34.16 ± 4.83 μmoles glucose released/ min/ g DM, but there was considerable variation in the amount of activity measured between samples. CMCase activity ranged from 3.78 to 86.26 μmoles glucose released/ min/ g DM. There were no significant differences in CMCase activity between ceca harvested in August/October

2000 and those harvested in March 2001 (Table 2); however, mean CMCase activity was higher among ceca harvested during August/October 2000 (35.57 ± 5.98 vs 30.53 ± 8.36 μmoles glucose released/ min/ g DM).

DISCUSSION

Mattock's (1971) inability to find cellulytic bacteria within the ceca of domestic geese led researchers to believe that geese were incapable of cellulose digestion. In this study we utilized CMCase assays to look for evidence of cellulose digesting bacteria within the contents of Canada geese ceca. CMCase activity is restricted to microorganisms intimately involved in fiber degradation, and is an indication that enzymes capable of cellulose hydrolysis are present within a sample (Silva et al. 1987). We detected CMCase activity in all 25 cecal samples, which lead us to conclude that bacteria capable of fiber digestion were present within the ceca of Canada geese. This finding supports our results from Chapter 2 in which we found that cecectomized geese were not as efficient in extracting energy from high fiber foods. It also lends support to the hypothesis that waterfowl ceca do play a role in fiber digestion.

CMCase Variability

CMCase activity measured varied between samples. The lowest CMCase activity detected was 3.78 μmoles glucose released/ min/ g DM, and the highest was 86.26 μmoles glucose released/ min/ g DM. The key factor in fiber degradation is not the number of bacteria present, but rather the number of bacteria capable of carboxymethylcellulase production (Silva et al. 1987). Thus the variation in CMCase activity between ceca suggests that the microbial species composition within Canada geese ceca is not static.

Information on the factors influencing the number and species composition of microorganisms in the avian cecum is limited, but they are known to vary with age (Mead 1989) and diet (Clench and Mathias 1995). Barnes et al. (1972) found that cecal microbial populations in adult chickens took up to about 6 weeks of age to become fully established. In the rumen of sheep, the number and species composition of microorganisms are influenced by the nature of the ration and the time interval since feeding (Warner 1962b). The Canada geese in this study were maintained on the same diet, which was provided ad libitum, so we had no control over when or how much individual birds ate. Food was removed 24 hours prior to surgery, but we did not have any observational notes on their feeding patterns prior to food removal. So it is possible that the "fasting period" varied between birds.

Differences in CMCase activity between samples might also be due to naturally occurring variability in cecal microbial populations between individual geese. Warner (1962b) found that despite being on the same dietary ration and regime, the species composition and number of microorganisms in sheep rumen varied between animals. In the rumen, most (50-70%) microorganisms attach to particulate matter to prevent from being washed out of the rumen (Warner 1962a). This microbial population is closely associated with food particles, and they digest insoluble polysaccharides, such as starch and fiber. The amount of microorganism biomass within the rumen at any one time is highly dependent on the amount of digesta within it (Warner 1962a). The amount of digesta in the Canada geese ceca varied despite the 24 hour fasting period. If microorganisms in the ceca also attach to food particles, then the amount of digesta within the ceca could have influenced CMCase activity.

Seasonal Variation in CMCase Activity

We were able to compare CMCase activity between two different time periods (fall vs late winter) because our cecectomies were conducted several months apart. We found no seasonal differences in CMCase activity between our captive Canada geese. This was not surprising considering that the geese were on the same maintenance diet year round, and diet is known to influence microbial abundance and composition within the ceca (Clench and Mathias 1995**)**. The diet of free ranging geese, however, is likely to vary seasonally; and had we looked at CMCase activity among free ranging wild geese we might have detected differences. In north-central Missouri the food habits of migrating and wintering geese are influenced by food availability, weather, farming conditions, and hunting pressure (Humburg et al. 1985). Eggeman et al. (1989) found that geese relied primarily on native foods during the fall, but as winter progressed they shifted to agricultural row crops, which are usually lower in fiber. Thus, the CMCase activity of free ranging wild Canada geese during the fall might be higher than that of wintering geese.

Conclusions

Unfortunately, we were unable to make inferences about our results as they relate to other studies because there are no published studies that have utilized CMCase assays to investigate cellulose digestion in the avian ceca. In order to fully understand the relationship between CMCase assays and cellulose digestion in the avian ceca, additional research needs to be conducted. Our study, however, suggests that Canada geese ceca are capable of cellulose digestion; however, additional research is needed to ascertain how much cellulose digestion takes place in the Canada goose cecum.

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Table 1. Enzyme extraction from microorganisms found within Canada geese ceca. Carboxymethylcellulase (CMCase) is expressed in µmol glucose released /g DM* per minute.

Bird ID #	Harvest Date	CMCase
328	Aug. 2000	22.74
329	Aug. 2000	86.26
331	Aug. 2000	84.55
332	Aug. 2000	68.79
333	Aug. 2000	38.79
334	Aug. 2000	19.32
341	Aug. 2000	46.82
344	Aug. 2000	23.29
347	Aug. 2000	26.05
348	Aug. 2000	58.43
351	Aug. 2000	38.21
353	Aug. 2000	47.29
356	Aug. 2000	9.07
358	Aug. 2000	7.75
360	Aug. 2000	31.09
361	Aug. 2000	6.23
339	Oct. 2000	10.58
346	Oct. 2000	15.05
365	Mar. 2001	64.87
367	Mar. 2001	3.78
371	Mar. 2001	52.30
372	Mar. 2001	12.58
376	Mar. 2001	21.81
377	Mar. 2001	20.01
378	Mar. 2001	38.35
Mean ± SE**		34.16 ± 4.83

*DM = Dry matter
**SE = Standard error

Table 2. Enzyme extraction from microorganisms found within Canada geese ceca by harvest date. Carboxymethylcellulase (CMCase) is expressed in µmol glucose released /g DM* per minute.

Harvest Date	Mean ± SE**
August/October 2000	35.57 ± 5.98 (n=18)
March 2001	30.53 ± 8.36 (n = 7)

Source of variation	SS	df	MS	F	P-value	F-crit
Between Groups	128.30	1	128.30	0.2124	0.6491	4.2793
Within Groups	13887.08	23	603.78			
Total	14015.38	24				

*DM = Dry matter
**SE = Standard error

CHAPTER 6. SUMMARY AND FUTURE RESEARCH NEEDS

Our study indicates that the cecum plays a major role in nutrient digestion when high fiber, low energy foods are available to Giant Canada geese. We discovered that 1) ceca have the capabilities to extract energy from high fiber foods, 2) the TAAD bioassay was not an appropriate test for amino acid digestibility in this study, 3) ceca have regenerative capabilities, and 4) ceca contain cellulose-digesting bacteria necessary for fiber digestion.

In Chapter 2, we examined the role of the ceca in food metabolizability by comparing TME_N values between cecectomized and intact geese. The TME_N values (Chapter 2, Table 4) for five of the six foods did not significantly differ between cecectomized and intact geese. However, intact geese extracted twice as much energy from buttonbush, which had the highest fiber content. Six months prior to the feeding trials, geese were maintained on a high-fiber maintenance diet, which might have masked any treatment effects for all other foods. The fiber content of buttonbush was 5.8 times higher than that of the maintenance diet. From a nutritional perspective, buttonbush is considered a poor quality food, but the cecum plays a key role in enhancing its nutritional value which might make a difference when higher quality food in unavailable.

In order to understand, the role of bacterial activity and amino acid digestibility within the cecum, we collected excreta during the feeding trials to conduct a TAAD bioassay (Chapter 3). Green et al. (1987) suggested that bacteria within the avian cecum synthesize amino acids, or consume undigested amino acids without benefit to the bird (Green et al. 1987). Nutritional studies focusing on amino acid digestibility use cecectomized chickens (Payne et al. 1971, Sibbald 1979, Austic 1983, Parsons 1984,

Raharjo and Farrell 1984, Johns et al. 1986, Green et al. 1987) to eliminate the possibility of bacterial action within the cecum, which could affect nutritional results. Our results indicated no differences in amino acid digestibility between cecectomized and intact geese for five of the six foods tested. Differences between groups were evident only for the true digestibility of methionine, histidine and tryptophan in buttonbush (Chapter 3, Table 12). The TAAD bioassay utilized in this study might not have been the appropriate approach for determining amino acid digestibility of foods fed to Canada geese. Problems with regurgitation precluded us from feeding the minimum amount (40 g) recommended for accurate results. Additionally, the removal of the ceca might have influenced degradation of dietary amino acids by microbes in the gastrointestinal tract anterior to the ceca (Sakata 1987).

Various researchers have reported that digestive organs respond to changes in diet quality and quantity, reproductive state, and food habits by increasing in length and/or weight (Miller 1975, Korschgen 1976, Ankney 1977, Paulus 1982, Drobney 1984). In Chapter 4, we examined the morphological response of digestive organs to ceca removal. Given the plasticity inherit to digestive tissue, we hypothesized that after ceca removal the remaining digestive organs undertake some of the functions normally delegated to the ceca, and changes in organ size and/or weight (compensatory growth) reflect that undertaking.

Contrary to our expectations, we did not find any evidence to suggest that compensatory growth occurred. There was no significant difference in gizzard, small intestine, large intestine, and liver mass between cecectomized and intact birds. However, we did find a 16% reduction in pancreas size between cecectomized and intact

geese. The pancreas of intact geese were heavier than those of cecectomized geese, which is contrary to what one would expect given the pancreas' role in the production of digestive enzymes. There were age discrepancies between the groups however, and Hanson (1962) reported that pancreas weight decrease with age.

During the necropsies, it was surprising to discover that the ceca were at some stage of regeneration in 8 out of the 9 cecectomized geese. We speculate that cecal function(s) are so unique that the remaining digestive organs cannot adapt or compensate for cecal function. However, the high fiber maintenance diet might have also preconditioned digestive organs to their maximum size, thereby restricting compensatory growth.

In Chapter 5, we examined the presence/absence of cellulose-digesting bacteria in the ceca. Results from the in-vitro analysis confirmed the presence of cellulose-digesting bacteria within the cecal contents of Canada geese. CMCase activity was detected among all cecal samples, indicating that the microorganisms capable of fiber digestion are present within the ceca. This finding supports our results from Chapter 2 in which we found that cecectomized geese were not as efficient in extracting energy from high fiber foods. It also lends support to the hypothesis that waterfowl ceca do play a role in fiber digestion. Unfortunately, we were unable to compare our findings to other studies because we were unable to find studies that had utilized CMCase assays to investigate cellulose digestion in the avian ceca.

Future Research

The results obtained during the course of this study were both aided and hampered by suggestions incorporated from previous studies. For example, the

maintenance diet provided was selected in light of previous research which suggested that birds be maintained on as natural food as possible to prevent the loss of digestive function due to changes in digestive flora and fauna. It was suggested that the changes occur as a result of birds being maintained on a low fiber diet. Given our interest in evaluating the role of ceca as it related to fiber digestion, we selected a high-fiber maintenance diet which we hoped would at least maintain a healthy colony of cellulose-digesting bacterium within the goose cecum.

By providing a high-fiber maintenance diet, however, we might have preconditioned the digestive organs to achieve their maximum size which could have masked some of our results. For example, if the maintenance diet had a lower fiber content, we could have obtained more statistically significant results in TME_N between cecectomized and intact geese. Had it not been for buttonbush whose fiber content was beyond that provided in the maintenance diet, we might not have detected any evidence of fiber digestion taking place within the cecum. Similarly, our compensatory growth results might have been different.

Bacterial populations in the digestive system are known to be highly dependent on diet. In an ideal situation we should have maintained the geese on a completely natural diet. Due to budgetary and time constraints, however, we were unable to do so. Thus, the number and species composition of bacteria within the ceca of captive geese might have differed from free-ranging geese. Additional research is needed to ascertain what differences if any, exist between the cecal composition and number of bacteria within the ceca of captive and free-ranging geese.

Finally, the TAAD bioassay utilized in this study was not appropriate for our situation. TheTAAD bioassay requires that birds be fed a minimum of 40g. However, problems with regurgitation precluded us from feeding the minimum amount required for accurate TAAD results. Food was fed as collected or bought, grinding it might have reduced regurgitation by decreasing the amount of irritation. If regurgitation can be decreased and more food fed, then the TAAD assay might provide better results. Additionally, cecal removal might have influenced degradation of dietary amino acids by microbes in the gastrointestinal tract anterior to the ceca. An in vitro study of bacteria within the Canada goose cecum, could elucidate the bacterial capabilities of amino acid breakdown.

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