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The Influence of a Dietary Beta-Fructan Supplement on Digestive Functions, Serum Glucose, and Yolk Lipid Content of Laying Hens

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Abstract: When young, rapidly growing broiler birds are fed a diet supplemented with beta-fructans, there is increased activity of pancreatic amylase, but not lipase, and decreased accumulation of fat. The present study compared amylase and lipase activities in pancreatic tissue, rates of glucose and proline absorption by intact small intestine tissues, the amount of digested protein in the jejunum, amounts of abdominal and yolk fat, and serum glucose concentrations of mature laying hens (White Leghorn, 57 weeks of age) fed for 4 weeks a basal diet (control) with birds fed the same diet, but supplemented with either a short chain (oligofructose; Raftifeed PS; 1.0 %) or a long chain (inulin; Raftifeed PE; 1.3%) beta fructans. Higher serum glucose concentrations of birds fed diets with oligofructose and inulin ($P<0.05$) corresponded with increased pancreatic amylase activity ($P<0.05$), but without an increase in glucose transport. The prebiotic supplement increased the amount of digested protein in the jejunum contents ($P<0.05$), but decreased pancreatic lipase activity and the amounts of abdominal and yolk fat ($P<0.05$). These findings indicate supplementing the diet fed to mature laying hens with beta fructan prebiotics (oligofructose and inulin) alters digestion and metabolism.

Key words: Pancreas, amylase, lipase, serum glucose, yolk, abdominal fat, beta-fructan prebiotic

Introduction

There is increasing interest in supplementing the diet of production animals and humans with prebiotics to improve health and nutritional status. Reported benefits include, but are not limited to, greater resistance to infection and disease, increased availability of undigested nutrients by bacterial fermentation, and beneficial alterations in lipid metabolism (Van Loo, 2004).

Only a limited number of studies have been performed with chickens. Yet, similar benefits were demonstrated. For example, feeding diets supplemented with fructooligosaccharides (FOS) to young, rapidly growing chickens (broilers) resulted in lower serum lipids and smaller abdominal fat pads (Ammerman *et al.*, 1989; Yusrizal and Chen, 2003). There is also some evidence that diets supplement with β-fructan prebiotics [oligofructose (FOS) and inulin] alter digestive functions. Specifically, the intestinal contents of broilers fed a diet with FOS had higher activity of amylase, but not lipase, (Xu *et al.*, 2003).

The effects of prebiotics on digestive and metabolic characteristics have not been reported for laying hens. Of particular interest is whether there are changes in digestive functions and lipid metabolism, and if they correspond with serum glucose concentrations and deposition of fat in abdominal pads and egg yolk. Therefore, the present study investigated the effects of supplementing a diet fed to mature laying hens with a short chain (oligofructose) and a long chain (inulin) beta fructans. This was accomplished by measuring serum glucose concentrations, deposition of fat in abdominal pads and egg yolk, the activities of amylase and lipase in pancreatic tissue, rates of carrier-mediated glucose absorption by small intestine tissue, and digested protein in the jejunum.

Materials and Methods

Birds and diets: A total of 60 White Leghorn layers with similar weight, egg laying performance, and in good health condition were obtained at 57 weeks of age and randomly allocated to 3 groups (n=20 each; 2 per cage). A regimen of 16 hr light:8 hr dark/day was provided throughout the study.

A control group was fed a basal diet free of antibiotics and wheat (Table 1). One experimental group was fed the basal diet to which was added 1.0% (w/w) oligofructose (Raftifeed PS, Orafti, Belgium) with an average degree of polymerization (DP) of 4 (range 2 to 8). The diet of the other experimental group was supplemented with 1.3% (w/w) of a semipurified chicory inulin extract (Raftifeed PE, Orafti, Belgium), which provided 1.0% (w/w) inulin with an average DP of 10 (range of 2 to 65). The control diet and the oligofructose and inulin diets were prepared as needed during the 4 week feeding period. The diets were stored at room temperature for less than three days before being fed *ad libitum* to birds. The birds had free access to water.

Table 1: Ingredient composition, crude protein and metabolizable energy of the diet (g/kg) used as the control diet

Ingredients	(g/kg)
Corn	676
Soybean meal	190
Dicalcium phosphate	22
Limestone	90
DL Methionine	0.5
Vitamin mixture	3.5
Salt	5
Fat	13
Calculated nutrient content	
Crude protein (g/kg)	150
Metabolizable energy (MJ/kg)	11.99

Eggs were collected during the feeding period. At conclusion of the feeding period (d 28) the birds were killed, weighed, and blood and solid tissues were collected for analysis.

Abdominal fat pad (%): The abdominal fat pad, which included all fat tissues surrounding the gizzard and adjacent abdominal tissues (Kubena *et al*., 1974), was removed and weight recorded. Abdominal fat pad percentage was calculated as the quotient of abdominal fat pad weight divided by live bird weight times 100.

Yolk lipid content: The lipid content of the egg yolk was measured according to method 925:32 of AOAC (AOAC, 1995). Specifically, 2 g of yolk were added to a Mojonnier tube, vigorously mixed with 10 ml 12N HCl, heated in a water bath at 700C, then boiled for 30 minutes with shaking every 5 min). After water was added to nearly fill the lower bulb of Mojonnier tubes, the tubes were cooled to room temperature. Ethyl ether was added and mixed for 1 min. The extracted fat was dissolved in 25 ml of redistilled petroleum ether, and the tube was centrifuged at 600 X g for 20 min. The ether solution was decanted from Mojonnier Flask into a tared Mojonnier fat dish. The remaining liquid was re-extracted two more times in the same manner and the pooled ether on the dish was evaporated (<1000°C) and the dish and fat were dried to a constant weight in a forced air oven at 1000°C for 90 minutes.

Measurement of serum glucose concentrations: Approximately 5 ml of blood were collected into non-anticoagulant centrifuge tubes (Becton Dickinson VACUTAINER Systems, Franklin Lakes, NJ) and allowed to sit for one hour. The tubes were then centrifuged (Lourdes Clinical Centrifuge, Serial #3394, Model CHT, Lourdes Instrument Corp, Brooklyn, NY) at 27000 X g for 30 minutes and the serum was retained for analysis of glucose concentrations (mg per dL serum) using test strips (ONETOUCH Test Strips, LifeScan, Inc., Milpitas, CA) and a glucometer (ONE TOUCH Johnson-Johnson Co., Mi; pitas, CA).

Measurement of digested protein in jejunum contents: The contents of the small intestine (from the distal end of the duodenum to the ileocecal junction) were collected, homogenized, and the amount of partially digested protein was measured using a method described by Chang (1998) that was based on the procedure of Hull (1947). Briefly, 5 ml of the homogenate were mixed with 1 ml of distilled water and 10 ml of 0.72 N trichloroacetic acid to precipitate undigested protein, vortexed for 1 min and allowed to settle for 10 minutes before being filtered through No. 2 filter paper. Aliquots of the filtrate (0.5 ml) were diluted with 4.5 ml of a solution with 75g of anhydrous sodium carbonate and 10g of anhydrous tetra sodium pyrophosphate in 500 ml of water before adding 1.0 ml of the Folin and Ciocalteu phenol reagent (Sigma Chemical Co., St. Louis, MO). Absorbance was read after five minutes at 650 nm and compared with a standard prepared with bovine albumin (BSA) (Sigma Chemical Co., St. Louis, MO).

Measurement of pancreatic amylase and lipase activity: The pancreas was harvested from each bird within 5 min after death, placed in aluminum foil, snap frozen in liquid nitrogen, and stored at -800°C. Pancreatic amylase and lipase activities were measured using methods described by Buddington *et al*. (2003).

Prior to the enzyme assays, frozen pancreas tissue (0.025 to 0.05 g) was homogenized in 0.05mM Tris-Buffer (pH 7.4) with 0.05 mM $CaCl_2$ using a Polytron tissue homogenizer. The homogenates were centrifuged twice (2420 X g for 5 min., then 16,000 X g for 15 min; both at 40°C. After the second centrifugation, the supernatant was divided into aliquots and stored frozen at -800°C. Before an assay, the supernatants were diluted with the Tris buffer so that measured activities of each enzyme would be within the dynamic range of the assay and were comparable to the standards prepared with known activities of amylase and lipase (1000 times for lipase, and 10000 times for amylase).

Amylase activity was measured using a diagnostic kit (Stanbio Alpha-Amylase No.577-20, Sigma Chemical Company, St. Louis, MO.) that was adapted for a microplate reader (Spectramax Plus384, Molecular Devices Corporation, Sunnyvale, CA.). Fifty µl of diluted supernatant were placed in a well, and then 150 µl of reagent substrate were added. The plate was placed into a and changes in absorbance were recorded for a period of two minutes at a wavelength of 405 nm. Units of activity were calculated by use of α-amylase Type VI-B as the standard (Sigma Chemical Company, St. Louis, MO).

Lipase activity was measured using a diagnostic kit that contained substrate reagent and diluents, activator

reagent, and standards for the assay (LIPASE-PSTM No.805-A, Sigma Chemical Company, St. Louis, Mo, U.S.A.) according to a method described by Buddington *et al.* (2003). First, 25 µl of sample were placed in a well, and then 150 µl of substrate were added. After incubating for 3 min at 37°C, 50 µl of activator were added. The plate was then placed in the spectrometer (Spectramax Plus384, Molecular Devices Corporation, Sunnyvale, CA.) and changes in absorbance were read for a period of two minutes at a wavelength of 550 nm. Units of lipase activity were calculated from the standard. Activities of amylase and lipase in the pancreas were adjusted on the basis of one mg protein in the supernatant (specific activity). Protein content of the samples was determined by using bovine serum albumin (Sigma Chemical Company, St. Louis, MO) as a standard. Samples (5 µl) were placed in a microtiter plate to which a protein dye reagent (Brastors reagent, BioRad, Herwler, CA.) (200 µl was added. Fifteen minutes later, the plate was placed in a spectrometer and read at 595 nm.

Measurement of glucose and proline absorption: Segments of small intestine from birds fed the control and 1.3% inulin diet were harvested at the time of death from 10 cm distal to the pyloric sphincter (proximal) and 10 cm before the ileocolonic junction (distal). The segments were rinsed of digesta and patches of tissue and secured by ligatures onto the tips of 0.5 cm rods. The tissues were handled and kept in cold (2-40°C) Ringers that was aerated with 95:5 mixture of O_2 and CO_2. Beginning 45 min after death the tissues were transferred to 400°C Ringers for 5 min before they were incubated for 2 min in 400°C Ringers with D-glucose or L-proline. Rates of absorption were quantified by adding tracer concentrations of 14°C D-glucose (0.002 mmol/l) and 3H L-proline (0.0004 mmol/l) to Ringer with 0, 0.2, 1, 5, 25, and 50 mmol/l of unlabeled forms of each nutrient. Osmolarity of the solution was maintained by isosmotic replacement of NaCl. Tracer levels of 3H L-glucose, which is not a substrate for the various glucose transporters, were added to the glucose solutions correct for D-glucose associated with extracellular fluids and absorbed independent of transporters. Tracer quantities of 14°C polyethylene glycol, which is impermeant and functions to label the extracellular fluid, were added to the proline solutions. After the incubation, tissues exposed to the glucose solutions were rinsed for 20 sec in cold Ringers, but not the tissues exposed to the proline solutions. The tissues were removed from the rods, placed in tared vials and after mass was recorded were solubilized (Soluene; Perkin Elmer), scintillant was added (UltimaGold; Perkin Elmer), and radioactivity measured by liquid scintillation counting. Rates of absorption were calculated following Karasov and Diamond (1983). Due to the use of L-glucose, rates of D-glucose absorption represent carrier-mediated uptake whereas rates of proline absorption are the sum of carrier-mediated plus carrier-independent absorption. Rate of absorption were normalized to tissue mass. The kinetics of absorption [maximum rates (Vmas) and apparent affinity constant (Km)] were calculated from rates of absorption measured at the different concentrations using a software package (Enzyfitter; Biosoft, UK).

Statistical analysis: The experiment was conducted using a completely random design (CRD) (Steel and Torrie, 1980) with six or more replications of each treatment. Data were analyzed using analysis of variance (ANOVA) (SAS Institute, Inc., 1993). When a treatment effect was detected, differences between treatments were identified using the Least Significant Difference (LSD) test (Freud and Wilson, 1997). For all comparisons, values of $P \leq 0.05$ were accepted as the critical level of significance.

Results and Discussion

Feed consumption, body mass, and intestinal length: Feed consumption averaged 99 g/hen-d and did not differ among treatments over the feeding period, and all treatments experience similar declines in body weight between weeks 57 to 61 (6.7 to 8.6% of original body weight). These findings contrast with reports of improved body weight gain when broilers are fed diet supplemented with prebiotics (Ammerman *et al.*, 1989; Yusrizal and Chen, 2003). The discrepancy may be associated with the metabolism difference between broilers and layers. As a consequence, when egg product on was considered, feed conversion for the diets with prebiotics (1% Raftilose: 1.9 kg feed/kg egg; 1.3% Raftifeed: 1.9 kg feed/kg egg) was higher compared to the control diet (2.1kg feed/kg egg; $P<0.05$).

At conclusion of the feeding period small intestine lengths of chickens fed the two diets with prebiotics (157cm for both 1% Raftilose and 1.3% Raftifeed) were longer than those of controls (150cm; $P<0.05$). The two prebiotic diets also resulted in longer large intestines (6.1cm and 6.4cm, respectively, vs 5.3cm; $P<0.05$). Similarly, female broilers fed a diet supplemented with oligofructose have longer gut lengths (Yusrizal and Chen, 2003). The trophic response of the intestine to β-fructans and other prebiotic fibers has been reported for dogs (Buddington *et al.*, 1999).

Abdominal fat pad (%): The abdominal fat pad of layers fed the diets with oligofructose and inulin were similar in weight, but were 19 and 24% lower compared to birds fed the control diet (Fig. 1; $P<0.05$). (Fig 1). These findings are consistent with the small fat pads of broilers fed a diet supplemented with 0.375% fructooligosaccharides (FOS) for the first 47 days after

Table 2: Yolk fat (%) and total yolk fat (mg/egg) of eggs collected from 57-week old layers fed diets with and without 1.0% oligofructose or 1.3% inulin

	Treatment		
	Control	Oligofructose	Inulin
Yolk fat level (%)	30.08[a]	29.71[a]	28.47[b]
Total yolk fat content/egg (mg)	5.27[a]	4.89[b]	4.83[b]

Each value is the mean of 8 measurements. Means in the same row not sharing the same letter are significantly different (P<0.05)

Table 3: Activities of amylase and lipase in pancreatic tissues collected from 57-week old layers fed the control and experimental diets with 1.0% oligofructose or 1.3% inulin

Parameter	Treatment		
	Control	Oligofructose	Inulin
Amylase (x10^4)			
Units/mg protein	1.12[a]	2.93[b]	3.91[b]
Whole pancreas (units)	5.97[a]	13.49[b]	13.54[b]
Lipase			
Units/mg protein	2.84[a]	1.02[b]	0.74[b]
Whole pancreas (units)	15.55[a]	5.97[b]	5.39[b]

Each value is the mean of 6 pancreases. Means in the same row not sharing the same letter are significantly different (P<0.05)

hatch (Ammerman *et al.*, 1989), whereas others have noted a lack of change in the size of the fat pad after a similar period of feeding (49 days; Waldroup *et al.*, 1993). Although the fat content of the liver was not determined, the smaller fat pads of broilers fed the β-fructans is comparable to the decreased accumulation of lipid in the liver of rats (Daubioul *et al.*, 2002).

Yolk fat content: The concentration of fat in the yolk was lower when the birds were fed the diet with oligofructose and inulin. Correspondingly, total yolk fat was 7.2 and 8.4% lower compared with the eggs produced by birds fed the control diet (Table 2). Supplementing diets fed to humans and animal models with prebiotic β-fructans decrease concentrations of serum triglycerides and phospholipids (Delzenne *et al.*, 1993; Fiordaliso *et al.*, 1995; Roberfroid and Delzenne, 1998; Roberfroid, 2000), with a similar response reported for chickens fed a diet with soluble fiber (Martinez *et al.*, 1992). Apparently, the lower serum lipid levels are caused by inhibition of lipogenic enzymes, leading to a decrease in fatty acid synthesis (Delzenne and Kok, 2001).

Serum glucose concentrations: The glucose concentrations measured in the chickens are comparable to the range of 190 to 220 mg/dl at 4-6 weeks after hatch (Hazelwood, 1986). Supplementing diets with oligofructose increased (P<0.05) serum glucose levels when compared with the control group, with the response more pronounced for inulin (Fig. 2). The highest (P<0.05) serum glucose level was observed in inulin group, followed by oligofructose group and control group. These findings contrast sharply with the hypoglycemic response and lower serum insulin levels of mammals fed diets supplemented with β-fructans (Kaur and Gupta, 2002; Kok *et al.*, 1998), which are consistent with the decreased serum lipids. The different serum glucose responses to β-fructans for mammals versus broilers are interesting, but not yet explained.

Amount of digested protein in jejunum contents: The jejunum contents of chickens fed the two diets with beta fructan prebiotics had higher levels of digested protein (Fig. 3; P<0.05). Since feed consumption, protein intake, was comparable among the three treatments, the most likely explanation for the difference would be increased hydrolysis of dietary protein. This is consistent with the improved feed efficiency, when egg production is considered, but needs to be confirmed.

Activities of pancreatic amylase and lipase: Pancreatic tissue of layers fed the diets with the prebiotic supplements had higher amylase activity (P<0.05) compared with layers fed the control diet (Table 3). A similar response, but for amylase activity in the digesta, has been reported for broilers fed diets supplemented with 2 to 4 g/kg fructooligosaccharides (Xu *et al.*, 2003). The higher activities of amylase would improve availability of dietary carbohydrate and corresponds with the improved growth performance and feed efficiency when fructooligosaccharides are added to diets fed to broilers (Fukata, 1999; Wu *et al.*, 1999).

Why β-fructans, and possibly other prebiotics, increase amylase has not been explained, but may be related to diet-induced changes in the assemblages of bacteria in the intestine. For example, administering adherent *Lactobacillus* cultures to chickens, either as a single

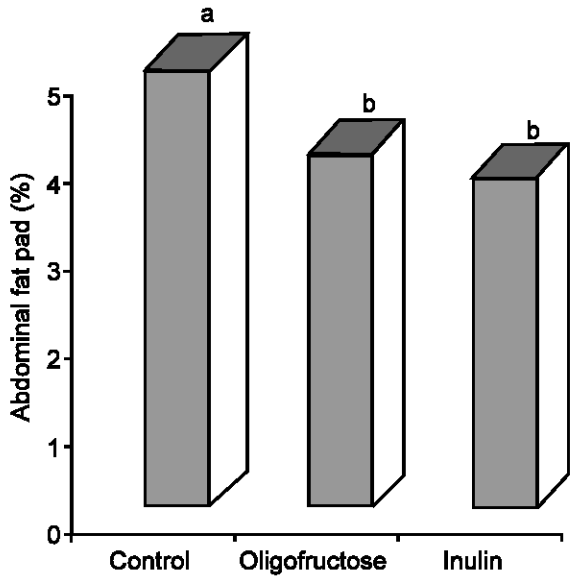

Fig. 1: The size of the abdominal fat pad as a percentage of body weight when mature layers (57-week old) were fed diets with and without 1.0% oligofructose or 1.3% inulin

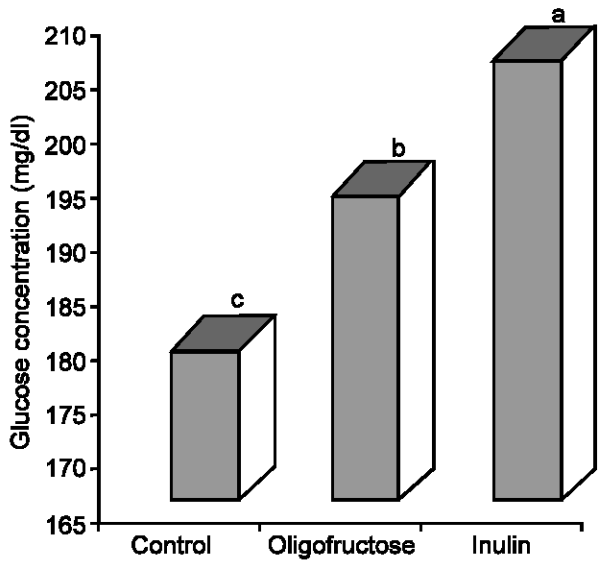

Fig. 2: Serum glucose content of layers (57-week old) (mg/dl) fed diets with and without 1.0% oligofructose or 1.3% inulin.

strain of *L. acidophilus* or as a mixture of 12 *Lactobacillus* strains, increases amylase activity in the small intestine (Jin *et al.*, 2000). This is partly due to the expression of amylase by various species of lactobacilli (Talamond *et al.*, 2002). Diets with β-fructan prebiotics, specifically fructooligosaccharides, alter the assemblages and interactions of bacteria in the intestine of chickens (Fukata *et al.*, 1999; Bailey *et al.*,

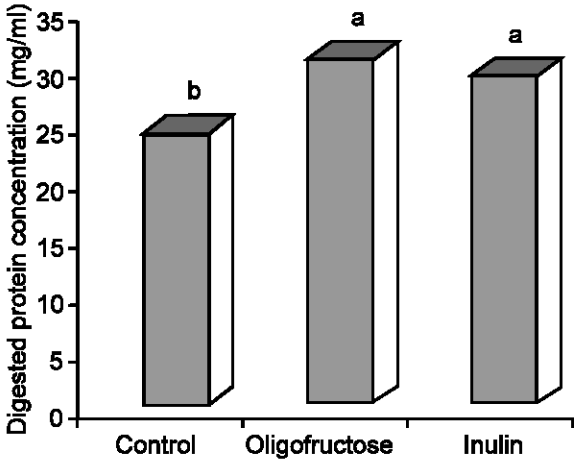

Fig. 3: The amount of digested protein in the jejunum (mg/ml) of mature layers (57-week old) fed diets with and without 1.0% oligofructose or 1.3% inulin

1991), and lead to increased densities of lactobacilli in humans (Hold *et al.*, 2003) and other species (Manning and Gibson, 2004). Although this would contribute to increased amylase activity in the lumenal contents, it does not explain the higher activity measured in pancreatic tissue of layers fed diets with β-fructans.

The lower lipase activity in pancreatic tissue after feeding the diets with β-fructans ($P<0.05$) is interesting and suggests the supplemented diets decrease fat digestion and absorption. This is consistent with the lower amount of fat in egg yok and the smaller abdominal fat pad. There is a need to better understand how prebiotics influence pancreatic lipase activity, particularly in light of how dietary supplements of lipase decrease food consumption by chickens, despite increasing lipid digestion (Al-Marzooqi and Leeson, 1999).

Glucose and proline absorption: Rates of carrier-mediated glucose absorption did not differ between layers fed the control and 1.3% inulin diet at any concentration of glucose and for both regions. As a consequence, the two groups had similar maximum rates of glucose transport in the proximal (control: 0.69 ± 0.06; inulin: 0.65 ± 0.05) and distal (control: 0.58 ± 0.02; inulin: 0.47 ± 0.04; $P<0.10$). Apparent affinity constants were also comparable and were consistent with the presence of a high affinity transporter, with SGLT-1 the most likely candidate.

A significant inulin effect was not detected at any concentration of proline. However, at 50 mmol/l the birds fed the diet with inulin tended to have higher rates of proline absorption in the proximal (control: 0.80 ± 0.14; inulin: 1.35 ± 0.27; $P = 0.07$) and distal (control: 1.31 ± 0.24; inulin: 1.97 ± 0.22; $P = 0.06$) segments. The 50

mmol/l should have been sufficient to saturate the carriers. The higher rates of absorption in the two intestinal regions of chickens fed the diet with inulin could have been caused by increased carrier-dependent or carrier-independent components of absorption, or a combination of both. Ratios for rates of tracer absorption in the presence and absence of 50 mmol/l unlabeled proline did not exceed a value of 1 for the control and inulin treatments, indicating there was little competition for transporters. Hence, only a low proportion of absorption was via a saturable mechanism, preventing attempts to define kinetics for the carrier-mediated component of proline absorption.

When rates of absorption were integrated with intestinal dimensions, the values suggested the larger intestines of layers fed the diet with inulin would have a greater capacity to absorb the higher concentrations of digested protein in the jejunal contents. In contrast, the higher amylase activity measured in layers fed the diet with inulin did not correspond with increased glucose transport capacities.

Conclusions: Supplementing the diet fed to White leghorn hens (57-week old) with 1.0% oligofructose or 1.3% inulin increased pancreatic amylase, serum glucose concentration, and abilities to absorb the higher amounts of digested protein in the jejunum, but decreased pancreatic lipase, serum and egg yolk lipids, and the size of the abdominal fat pad.

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