NUTRITIONAL INTERVENTIONS DURING RECOVERY IN PROTEIN DEFICIENT PIGLETS WITH DEXTRAN SULFATE SODIUM INDUCED COLITIS

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ABSTRACT

Objectives: The main objective of this study was to establish and characterize a recovery model of pediatric IBD using piglets. Secondly, the objective was to characterize and compare the effects of adequate protein (anabolism) and N-acetylcysteine (antioxidant and anti-inflammatory) on growth and disease severity of colitis piglets during early recovery.

Methods: The study conducted over 15 days. 40 female piglets, that were 7 to 10 days old weighing 3.17 to 4.09kg (7 to 9 pounds) underwent surgery for catheter insertion on Day 1. Piglets received a liquid protein deficient diet (15% of National Research Council requirement) infused intragastrically from Day 1 to Day 9. Starting on Day 3 until Day 9, piglets received Dextran Sulfate Sodium (DSS) intragastrically to induce colitis. At the end of Day 9, piglets were randomized into one of 4 groups: Active Control (PD-ACT; n=5), Adequate Protein (PD-AP; n=6), Recovery (PD-PD; n=5) and N-acetylcysteine supplement (PD-NAC; n=5). On Day 10, the PD-ACT group were euthanized, subsequent groups entered into the recovery phase for 5 days with no DSS administered. During the recovery phase, PD-AP group received an adequate protein diet (100% of protein requirement). PD-PD group continued on a protein deficient diet. The PD-NAC group similarly continued on the protein deficient diet, but were supplemented with N-acetylcysteine (NAC). All recovery groups were euthanized on Day 15. Colon tissue samples were collected for histology assessments after piglets were euthanized. Growth was measured by anthropometrical measurements, body composition and nitrogen balance. Weight was obtained daily, whereas chest circumference and snout-to-rump length were measured on Day 1, 9, and 14. Body composition was measured using DXA on Day 1, 9, 14. Nitrogen balance was performed on Day 8, 9, 13 and 14.

Results: In the recovery phase, the average weight gain of PD-AP piglets was 53 ± 5g/(kg·day), which was 2.5 times more than PD-PD (21 ± 6g/(kg·day); $p = 0.0002$) and PD-NAC piglets (20 ± 25g/(kg·day); $p = 0.0003$). Moreover, the rate of weight gain tripled in the

recovery phase compared to the active colitis phase for the PD-AP piglets ($p < 0.0001$) but were not significantly different for PD-PD ($p = 0.5443$) and PD-NAC ($p = 0.4259$) piglets receiving low protein diets. Overall, PD-AP piglets gained more than 40% of their initial weight. Whereas PD-PD and PD-NAC groups gained 25% of their initial weights. Snout-to-rump length and chest circumference were not different among groups for the duration of the study. Overall, lean mass gain was 1301 ± 59g for PD-AP, 744 ± 147g for PD-PD, and 628 ± 107g for PD-NAC piglets. In the recovery phase, lean to fat gain ratio was 3:1 in PD-PD, 41:1 in PD-AP, and 6:1 in PD-NAC piglets. Nitrogen balance in the recovery phase was higher in PD-AP piglets compared to other piglets ($p < 0.0001$), and no difference between PD-PD and PD-NAC ($p = 0.1473$). Surprisingly, total histopathological scores of PD-AP piglets, in both distal and spiral colon, were significantly higher than PD-ACT (active colitis control) piglets (spiral: $p = 0.0342$; distal: $p = 0.0103$). Likewise, despite achieving the greatest growth, histology results of PD-AP piglets showed the most severe damage compared to all groups.

Conclusion: Adequate protein diet was effective at promoting growth, but showed increased disease severity during early recovery stages of colitis in protein deficient piglets. Administration of NAC during the recovery phase, the anti-inflammatory intervention, did not show a greater impact on growth parameters or disease severity, when compared to protein deficient piglets, that did not receive the NAC treatment.

RÉSUMÉ

Objectifs: L'objectif primaire de cette étude fut d'établir un modèle pédiatrique de maladie inflammatoire chronique de l'intestin (MII) en utilisant des porcelets de récupération. L'objectif secondaire fut de comparer les effets de deux régimes spécifiques sur la croissance de ces procelets ainsi que sur la gravité de leur maladie. Le premier régime étudié était conçu pour avoir une quantité de protéine adéquate (où l'anabolisme serait favorisé), et l'autre, était conçu pour créer une déficience en protéines mais un surplus de N-acetylcysteine (NAC), un acide aminé anti-inflammatoire.

Méthodes: L'étude dura 15 jours. Quarante porcelets femelle agées de 7 à 10 jours et pesant 7-9 livres ont subi une intervention chirurgicale pour l'insertion du cathéter le premier jour de l'étude. Les porcelets ont reçu un régime pauvre en protéines liquides (15% de l'éxigence du Conseil National de Recherches du Canada infusées de manière intragastrique) du jour 1 au jour 9. À partir du jour 3 jusqu'au jour 9, les porcelets ont reçu du sulfate de dextran de sodium (DSS) de manière intragastrique pour induire la colite. À la fin du jour 9, les porcelets ont été randomisés dans l'un des quatre groupes: "active control" (PD-ACT; n = 5), "suffisamment de protéines" (PD-AP; n = 6), "récupération" (PD-PD; n = 5) et "supplément de N-acetylcysteine" (PD-NAC; n = 5). Au jour 10, le groupe PD a été euthanasié, et les trois autres groupes ont continué jusqu'à la phase de récupération pour cinq jours sans DSS administré. Pendant la phase de récupération, le groupe PD-AP a reçu un régime alimentaire suffisant en protéines (100% des besoins en protéines). Le groupe PD-PD a continué avec le régime déficient en protéines. Le groupe PD-NAC a également poursuivi le régime déficient en protéines, mais ce groupe a aussi reçu un supplément de N-acetylcysteine (NAC). Tous les groupes de récupération ont été euthanasiés le jour 15. Suivant l'euthanasie, des échantillons de tissu du côlon ont été récoltés pour les évaluations histologiques. La croissance des porcelets fut quantifiée par des mesures anthropométriques, la composition corporelle et l'équilibre d'azote. Le poids a été noté chaque jour, alors que la tour de poitrine et la longueur du museau au croupe ont été mesurées uniquement les jours 1, 9 et 14. La composition

corporelle a été mesurée à l'aide du DXA lors des jours 1, 9, 14. Le bilan de l'azote a été réalisé pour les jours 8, 9, 13 et 14.

Résultats: Dans la phase de récupération, le gain de poids moyen des porcelets PD-AP était de 53 ± 5g/(kg·day), 2.5 fois plus que PD-PD (21 ± 6g/(kg·day); p = 0.0002) et le porcelets PD-NAC (20 ± 25g/(kg·day); p = 0.0003). En outre, le taux de gain de poids a triplé en phase de récupération par rapport à la phase de la colite actif pour les porcelets PD-AP (p <0.0001), mais étaient pas différent pour PD-PD (p = 0.5443) et PD-NAC (p = 0.4259) porcelets qui sont restés sur les régimes alimentaires faibles en protéines. Dans l'ensemble, les porcelets PD-AP a gagné plus de 40% de leur poids initial, alors que PD-PD et PD-NAC ont gagné 25% de leur poids initial. Museau-a-croupion et circonférence de la poitrine n'étaient pas différents chez les groupes à tous les points de temps de l'étude. Globalement le gain de masse maigre était 1301 ± 59g pour PD-AP, 744 ± 147g pour PD-PD, et 628 ± 107g pour les porcelets PD-NAC. Dans la phase de récupération, le ratio de la masse maigre de la masse grasse était 3:1 dans PD-PD, 41:1 dans PD-AP, et 6:1 chez les porcelets PD-NAC. Le bilan azoté en phase de récupération était plus élevée chez les porcelets PD-AP par rapport aux autres porcelets (p <0.0001), et aucune différence entre PD-PD et PD-NAC (p = 0.1473). Étonnamment, le total des scores histopathologies de porcelets PD-AP pour le côlon distal et la spirale étaient significativement plus élevé que PD-ACT (contrôle de la colite actif) porcelets (spirale: p = 0.0342; distale: p = 0.0103). De même, en dépit de la réalisation de la plus forte croissance, des résultats d'histologie de porcelets PD-AP étaient les plus graves endommagé parmi tous les groupes.

Conclusion: La protéine adéquate a été efficace dans la promotion de la croissance, mais plus montré une augmentation gravité de la maladie lors de la récupération anticipée de la colite chez les porcelets protéines déficientes. L'intervention anti-inflammatoire de compléter le régime alimentaire faible en protéines avec le NAC dans la phase de récupération n'a pas eu un grand impact sur les paramètres de croissance ou sur la gravité de la maladie par rapport aux protéines déficientes porcelets qui ne reçoivent pas NAC.

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25OHD: 25-hydroxycholecalciferol

5-ASA: 5-aminosalicylates

AP: adequate protein

APCs: antigen-presenting cells

ATG16L1: autophagy-related protein 16-1

CARD15: caspase recruitment domain-containing protein 15

CD: Crohn's disease

CD4+/8+: cluster of differentiation 4+/8+

CDH1: cadherin-1

CMARC: Comparative Medicine and Animal Resources Centre

DSS: dextran sulfate sodium

DXA: dual-energy x-ray absorptiometry

ECM1: extracellular matrix protein 1

EEN: exclusive enteral nutrition

EN: enteral nutrition

GCS: glutamylcysteine synthetase

GH: growth hormone

GI: gastrointestinal

GNA12: guanine nucleotide-binding protein subunit alpha-12

GSH: glutathione

GSSG: glutathione disulfide

HNF4A: hepatocyte nuclear factor 4 alpha

IBD: inflammatory bowel disease

IBD1: inflammatory bowel disease protein 1

IFN - γ: interferon gamma

IGF -1: insulin-like growth factor-1

IGFBP: insulin-like growth factor binding protein

IL: interleukin

IRGM: immunity-related GTPase family M protein

JAK2: janus kinase 2

LAMB1: laminin subunit beta-1

LRRK: leucine-rich repeat serine/threonine-protein kinase

MHC: major histocompatibility complex

mTOR: mammalian target of rapamycin

NAC: N-acetylcysteine

NADPH: nicotinamide adenine dinucleotide phosphate

NKCC1: Na-K-Cl co-transporter

NOD2: nucleotide-binding oligomerization domain-containing protein 2

NRC: National Research Council

NSAID: nonsteroidal anti-inflammatory drugs

OCTN2: organic cation transporter 2

PD: protein deficient

PTH: parathyroid hormone

RBC: red blood cell

ROS: reactive oxygen species

SOCS: cytokine-inducible signaling

STAT3: signal transducer and activator of transcription 3

Th: T helper

TNF- *α*: tumor necrosis factor-alpha

TNFSF15: tumor necrosis factor superfamily, member 15

TYK2: tyrosine kinase 2

UC: ulcerative colitis

1. INTRODUCTION

Inflammatory bowel disease (IBD) is a term for a group of complex and idiopathic lifelong diseases, characterized by relapsing and remitting chronic inflammation in the gastrointestinal (GI) tract. [1] IBD can occur in healthy adults, as well as in young children. Historically, IBD is more prevalent in developed countries located in high latitudes. However, in recent years IBD has started to emerge in low latitude developed countries and in developing countries in Asia, South America and South Africa. [2] The world-wide increase in IBD incidence and prevalence suggests an upcoming global disease. In addition, Canada has one of the highest reported IBD in the world with approximately 233,000 Canadians diagnosed with IBD, that is about 1 in every 150 Canadians has IBD. [3] Moreover, IBD also affects Canadians financially, with an estimated economic cost of over $11,900 per affected Canadian annually. [3] In 2012, it is estimated that $2.8 billion is spent on IBD in Canada. [3] In general, IBD impacts one's quality of life tremendously; furthermore, lack of disease awareness, inappropriate diagnosis and inadequate treatments including nutrition further exacerbate the disease related health complications.

Crohn's disease (CD) and ulcerative colitis (UC) are the two major diseases of IBD. Although they are both under the IBD umbrella, they have distinct pathologic and clinical characteristics.[4] Main differences include disease location, inflammation distribution, clinical complications, and surgical interventions. CD can occur anywhere in the GI tract, from mouth to distal bowel.[5] It causes patchy transmural inflammation and lesions which can lead to stenosis, microperforation, fistula, and obstruction of intestine via fibrosis.[6] Presence of visual blood in stool or bleeding from rectum during a bowel movement is uncommon in CD, which sometimes delays diagnosis.[3] In addition, currently there is no existing cure for CD. On the other hand, UC occurs in the rectum and usually progresses to other portions of the colon. Its inflammation can be characterized as proximal, continuous, shallow and limited to the mucosal level.[4] In addition, due to ulceration bleedings, visual

blood is common in stool; however, UC can be eliminated through colectomy.[3] Some other differences between CD and UC include gender differences in disease occurrence, degree of nutrition malabsorption, associated genes and intestinal microfloras.[3, 4, 6, 7]

In Canada, the most common onset age of CD and UC is in the twenties, with Nova Scotia and Quebec having the highest incidence, and British Columbia having the lowest. [3] However, IBD can also occur at younger ages, even in children and infants. 20-30% of the current Canadian IBD patients were diagnosed before the age of 20. [3] Meta-analysis of 139 studies from 32 countries reported a statistically significant increase in pediatric IBD incidence globally, in both developed and developing countries. [2] Although adult and children IBD patients share many the same clinical manifestations, growth failure and delay in puberty critical complications present only in children with IBD. [1] Therefore, pediatric IBD research is needed to understand its etiology and formulate treatment strategies to prevent later-life health complications.

Inflammation causes a cascade of immune response involving the release of various cytokines, which increases metabolic demand and inhibits expression of several growth factors. [8, 9] Malnutrition can occur due to reduced caloric intake associated with clinical symptoms of IBD.[1] Malnutrition and inflammation are the main causes of growth failure in pediatric IBD. When children have an acute episode of IBD, their nutrient intake can be low, and often this continues into remitting stage of IBD. The ideal situation is to provide adequate energy and protein for anabolism and growth, protein synthesis and repair during this remitting phase of IBD. However, it is common that a complete balance diet is not possible due to low appetite and abdominal pain. Therefore, an anti-inflammatory approach where N-acetylcysteine (NAC), a precursor of the limiting amino acid to make glutathione, should be considered for the reduction of inflammation and to improve disease severity. Due to the difficulty of studying children, especially sick children, a well-established dextran sulfate sodium (DSS) induced colitis piglet model can be used to study pediatric IBD.[10] The present study characterized a recovery model of colitis. In addition, the impact of nutritional

interventions on growth and disease severity during recovery targeted anabolism through adequate protein, and anti-inflammatory response through supplementing N-acetylcysteine was also investigated.

2. LITERATURE REVIEW

2.1 Inflammatory Bowel Disease (IBD)

2.1.1 Pathophysiology

Although IBD is idiopathic, numerous risk factors have been identified as causes and/or associated with the development of IBD. The major and most convincing factors include immunology, microbial, environmental/external and genetics.

Immune response has been studied for quite some time in the pathogenesis of IBD, and most studies suggest that a disrupted mucosal immune system results in development of IBD. [8] An abnormal mucosal system could be resultant from innate genetic abnormality or external exposures such virus infections, food antigens, bacterial toxins and pharmaceutical drugs, or even stress. [8, 11] A normal and functional mucosal immune system comprise an effective barrier against infectious and toxic pathogens via healthy intestinal epithelium with surface mucus, pattern recognition receptors and ability to secrete protective agents such as neutrophils, macrophages, T-lymphocyte, B-lymphocyte and dendritic cells to initiate cellular immunity response. [8] Alterations of circulation, composition and function of immune cells have been associated with damages in intestinal epithelium and the development of IBD. For examples, abnormal B cell circulation, excess presence of neutrophils and lymphocytes in the lamina propria are found to have a role in the pathogenesis of IBD. [8] Hence, antibody mediated treatments for IBD such as Natalizumab, an antibody targeting alpha-4 beta 7

integrin that is important for transporting lymphocyte to the lamina propria was developed; and has shown positive effects on both CD and UC. [12]

Microbial microflora has been studied repeatedly in recent years not just as it relates to IBD but also in many other areas. Studies using murine models have revealed that microflora is a prerequisite for IBD, as genetically engineered IBD susceptible mice developed IBD in presence of microflora but not in germ-free conditions. [13, 14] Although presence of high concentrations of microbes in the distal ileum and colon is normal in humans, any disruption in intestinal epithelium can results in microbial related inflammation response and further induction of IBD. [11] In some cases, certain microbes can release products that alter the permeability of healthy intestinal epithelium; in some other cases, genetic related epithelium defects can allow bacteria and other pathogens to trigger mucosal immune response. [8, 11] In addition, clinical studies have shown that gut microflora composition differs between healthy individuals, CD patients and UC patients. [6] Nevertheless, some IBD susceptible loci, that regulate immune responses toward microflora have been identified. [15, 16]

Genetics has been the main focus in finding the etiology of IBD, as it is tightly linked to other risk factors of IBD. Approximately 25 percent of IBD patients have close relatives who also have IBD. [17] As mentioned previously, immunological risk factors includeindividuals with genes that affect epithelial related adaptive and innate immune system responses are susceptible for IBD. In addition, the nonspecific clinical manifestation or phenotype of IBD suggests a variety of genes are involved in IBD pathogenesis. [18] Moreover, genetic impact on CD and UC varies. Human twin studies suggested that genetic factors better predict CD than UC. [19] In terms of specific IBD susceptible genes, genome-wide association studies identified over 160 distinct susceptibility loci for IBD. [20] For example, altered IBD 1 gene causes mutation of protein NOD2 (CARD15) and results in disrupted intracellular innate immune pathways. [21] Genes regulating interleukin (IL)-17, IL-23 receptors, IL12B, STAT3, JAK2, and TYK2 are associated with risk of IBD, both CD and UC via adaptive immunity pathway disruption. [15, 22] Although some genes are associated with both CD

and UC, some are specific to either disease. For instance, IL-27 and TNFSF15 that involve in regulating adaptive immunity are only associated with CD; whereas OCTN2, ECM1, CDH1, HNF4A, LAMB1, and GNA12 are associated with UC specifically, through disrupting epithelial functions. [23, 24] Furthermore, when the autophagy pathway involved in recycling intracellular organelles and removing intracellular microorganisms is disrupted, ATG16L1, IRGM and LRRK genes are altered. [25-27] Mutation in the IL-10 receptor gene has been identified as predictor of very early onset of IBD, and is used as an IBD screening tool for infants. [28] Other than specific genes, some more general genetic determinants are also associated with IBD such as race (Caucasian are higher risk than African American and Hispanic), ethnicity (Jewish), and genetic syndromes (Turner, Hermansky-Pudlak). [29-31]

External/environmental factors including lifestyle habits, have also been studied in relation to IBD pathogenesis and prevention. Hypotheses have been made regarding some pharmaceutical drugs and products' ability to induce IBD.

Antibiotic use has been associated with IBD, even in children. Early prescription of antibiotics to children is associated with childhood IBD. It is hypothesized that antibiotics may alter certain gut microflora that have protective effects on IBD. [32] Isotretinoin, a product for acne has also shown to be associated with IBD, possibly by altering the innate or adaptive immune system. [33] Oral contraceptives and hormone replacement therapy are hypothesized to induce IBD by creating thrombosis on microvasculature. [34] Nonsteroidal anti-inflammatory drugs (NSAID) have disruptive effects on intestinal epithelium, and alter platelet, inflammatory and microvascular responses, which increase the risk of IBD. [35] In terms of lifestyle risk factors, smoking has been shown to increase the risk of CD. However, smoking also appears to have some protective factors; but cessation might even increase the risk of UC. It has been theorized that the nicotine in cigarettes improves UC symptoms via suppressing cytokine production and antioxidant property. [36, 37] Diet can also play a role in inducing IBD. Although food antigens can be expected to involve in causing IBD, currently no specific pathogenic antigens have been identified. High intakes of processed foods, high-fat, refined sugary foods are associated with development of IBD. [38, 39]

Introducing cow's milk to infants with cow's milk protein hypersensitivity can also increase the risk of IBD. [40] On the other hand, high intakes of vegetable & fruits and omega-3 fatty acid, low intakes of omega-6 fatty acid, adequate vitamin D intake, and breastfeeding during nursing may be preventive of IBD development. [41, 42] Moreover, regular physical activity has also been shown to lower the risk of CD. [43]

2.1.2 Inflammatory Response in IBD

As mentioned previously, inflammatory response in IBD is a result of activation of innate and adaptive mucosal immune systems. Inflammation response begins when pattern recognition receptors on epithelial cells bind to potential foreign pathogenic antigens or bacteria, which triggers the migration of neutrophils, macrophages and natural killer cells. The triggered release of these immune boosting cells provide a first hand response towards the exposure, as a response of the innate immune system. Next the adaptive immune system becomes active, which is comprised of T- and B-lymphocytes, and dendritic cells. T-lymphocytes then can be further divided into CD4+, CD8+ helper T-cells and regulatory T-cells. CD4+ helper T-cells are subdivided into Th1, Th2 and Th17 cells. Th1 cells then release interferon gamma (IFN - γ), tumor necrosis factor-alpha (TNF- α), IL-2, and IL -12. Th17 cells regulate inflammatory response by secreting IL-17, IL-6 and G-CSF. Th2 cells release IL-4, IL-5 and IL-13 to regulate B-lymphocyte production; where some B-lymphocyte can make antibodies against antigens, and serve as antigen-presenting cells (APCs). APCs process antigens and present them to T-lymphocytes with association with major histocompatibility complex (MHC). Another example of ACP is the dendritic cell, which is also involves in releases of IL-23, where IL-23 is crucial for Th17 cell generation. All these cytokines participates in inducing cell-mediated inflammation. [15, 44, 45] Furthermore, as T-lymphocyte cells respond to antigens presented by APCs and MHC, different pathogenic antigens or disease etiology results in different T-lymphocyte mediated inflammatory pathways. Since CD and UC etiologies are different, T-lymphocyte responses differs between the two diseases. Previous studies suggested that CD follows the Th1 cell pathway where APCs produced IL-12

stimulates release of IFN- γ, which then mediates the inflammatory response. However, more recent studies have suggested the Th17 cell pathway may also play a role in CD, where IL-23 stimulates IL-17 production, and both are present in high concentrations in CD tissues. [46-48] Although, IFN- γ and IL-17 inhibits each other's production and hence debating the co-existence of both pathways in CD, some studies suggested that the IFN- γ mediated pathway is still the major one; however, the Th17 cell pathway also plays a key role in certain phases of CD inflammatory response. [49] In general, chronic inflammation in IBD can result in numerous clinical manifestations and complications such as fatigue, fever, abdominal pain, intestinal bleeding, diarrhea, arthritis, uveitis and skin disorders. [50]

2.1.3 Pediatric IBD and Growth Failure

Pediatric IBD and adulthood IBD share the same risk factors; such as family history, ethnicity and gender. More CD is seen in children than UC, and CD is more common in boys whereas UC incidence is similar in both girls and boys. [1, 3] Interestingly, as girls age past puberty, the incidence of IBD starts to increase and eventually surpasses the number of males with IBD. [3] This suggests that hormones may play a role in IBD development. More interestingly but troubling, children aged 0-10 years account for most the recent increase in pediatric IBD incidence whereas the rate of increase among age 10 to 17 years is rather stable. [3] It raises the questions of environment changes, infant feeding practices and diet habits' impacts on risk of IBD. As mentioned previously, early introduction to cow's milk and antibiotics poses risks for IBD development in infants. On the other hand, breastfeeding has been shown to provide better gut immune system than formula milk, and hence lower risk of IBD and infections. [51] Moreover, development of IBD in childhood is positively associated with the frequency of diarrheal sickness during infancy. [52] Therefore, extra attention should be made on children 0 to 10 years of age in terms of early detection and intervention. Any common early clinical features of IBD in children should not be ignored. In children age 0 to 10 years with IBD, abdominal pain, weight loss, diarrhea, hematochezia, growth failure and extra-intestinal symptoms are commonly present. [3, 53] In addition, tests

for Celiac disease should also be performed in suspicion of IBD due to similar clinical manifestation of CD and Celiac disease. [54] Early diagnosis and identification of disease location is essential in maintaining normal growth of children with IBD. CD commonly occurs in terminal ileum (~25 percent), colon (20 percent), and both ileum and colon (~45 percent) in diagnosed children. [53]

Limited information regarding the pathophysiology of IBD is known, and risk factors may be difficult to eliminate. Thus, current pediatric IBD interventions heavily focus on minimizing growth failure. Growth failure is defined by height increase not greater than 0.3 standard deviation per year; annual growth velocity less than 5cm; or a drop in annual growth velocity of equal or greater to 2cm. [55] In order to assess for growth failure, growth rate and height-for-age need to be compared with expected growth rates for age; and Z-scores should be used. [56] Studies used these criteria reported more growth failures in children with CD (approximately 30 percent) compare to children with UC (approximately 5 to 10 percent). [57] Studies also found that a decrease in height velocity may occur before display of common clinical symptoms; therefore, plotting children's height and weight on appropriate growth chart may help early detection of IBD. [58]

The pathogenesis of growth failure in children with IBD is complex, and it involves factors like malnutrition, pharmaceutical treatment side effects, and inflammation. Malnutrition in children with IBD is resulted from reduced nutrient intake, malabsorption, maldigestion, increased energy expenditure and enteral protein loss. [1] Moreover, glucocorticoid is used as a pharmaceutical treatment for IBD. However, it can contribute to growth failure by interfering with growth hormone secretion, which negatively affects bone and collagen formation, nitrogen retention, and other growth metabolic processes. [59] Lastly, inflammation is a major mediator of growth failure in children with IBD. Growth hormone (GH) / insulin-like growth factor-1 (IGF -1) axis is very important in maintaining normal growth. GH is secreted by the pituitary gland, which binds to GH receptor to produce IGF-1. IGF-1 then binds to insulin-like growth factor binding protein (IGFBP), which up regulates

the concentrate of IGF-1 at the tissue level. GH also acts on growth plate to stimulate differentiation of chondrocytes. IGF-1 then induces chondrocyte expansion via hypertrophy and results in long bone growth. Moreover, during puberty, GH also plays important role in regulating sex steroids for growth and sex maturity. The elevated pro-inflammatory cytokines in IBD, such as IL-1β, TNF- α and IL-6, would disrupt the normal regulation of the GH/IGF-1 axis and sex steroids. IL-6 can induce suppressor of cytokine-inducible signaling (SOCS) proteins that inhibit growth promoting actions of GH. IL-6 can also increase the break down of IGFBP-3 and reduce the half-life of IGF-1. [9] In addition, TNF- α reduces expression of GH receptors in the liver and results in lower production of IGF-1; and IL-1β is also capable of reducing concentration of IGF-1. Furthermore, IL-1β, TNF- α and IL-6 can inhibit testosterone production in Leydig cells, and IL-1β, TNF- α can inhibit steroid production in ovarian cells, which decrease sex steroids stimulated growth. [9] In general, pro-inflammatory cytokines are largely responsible for growth failure and delayed puberty in IBD.

2.1.4 Nutrition Status

Malnutrition not only affects children with IBD but also heavily impacts adults with IBD. Persistent malnutrition in IBD patients increases both morbidity and mortality. [60] As mentioned previously, the causes of malnutrition in both children and adults with IBD include reduced nutrient intake, malabsorption, maldigestion, enteral nutrition loss, and inflammation. [1] Common clinical outcomes of malnutrition in IBD are growth failure in children, weight loss, risk of developing bone disease, and micronutrient deficiencies. [3, 53] Consequences of malnutrition can further aggravate the disease, and results in a vicious cycle.

The major contributor to malnutrition in IBD patients is usually inadequate food and nutrient intake. Abdominal pain and other discomfort may cause early satiety and decrease in appetite which leads to low food intake. Other factors such as low zinc intake and high levels of TNF-

α can also contribute to lack of appetite in IBD patients. [61] Malabsorption and maldigestion can be resulted from intestinal tissue and enterocyte damages, bacteria overgrowth, mucosal inflammation, insufficient secretions of digestive enzymes, surgically shortened bowel length, and drug interactions. [1, 3] Nutrient loss is common in IBD patients. Diarrhea and blood loss causes mineral loss in minerals such as iron, zinc, potassium and magnesium. Protein loss through capillary leaks in diseased tissue is also common in IBD patients. [62] Therefore, IBD patients must a higher food and nutrient intake than healthy individuals to meet the required increased energy expenditure associated with inflammation responses in IBD patients.

The major consequences of malnutrition in IBD include growth failure, described earlier, weight loss and micronutrient deficiencies. With the increasing awareness of IBD and early diagnosis and interventions, studies reports weight loss in both CD and UC adults has decreased in recent years. [63] However, lean body mass loss still occurs in approximately half of the IBD patients. Losing more than 10 percent of initial lean body mass is associated with increased morbidity in IBD patients. [64] Nevertheless, lean body mass loss in adult IBD patients is primarily resulted from inflammation induced protein breakdown, and glucocorticoid treatments. Inadequate protein intake contributes less significantly in lean body mass loss in adults with IBD, whereas children with adequate protein, macronutrient and energy intakes are crucial for growth. [65] Protein deficiency has been studied in pediatric research using swine models. In protein deficient disease-free piglets, protein synthesis rate decreases significantly in most organs and tissues including the liver, jejuna mucosa and muscles. [66-68] Decreased whole body turnover associated with protein deficiency is likely due to inhibited mammalian target of rapamycin (mTOR). [69] Prolonged protein deficient diet also reduces red blood cell (RBC) glutathione concentration and synthesis in piglets. [70] Glutathione is a tripeptide that can be made endogenously and serves as an important antioxidant in preventing cellular damages by reactive oxygen species (ROS) that is also present in IBD. [71] In children with IBD, protein deficiency and malnutrition in general, coexist with inflammation, and studies have looked at the combined

effect. Studies found that the coexistence of protein deficiency and inflammation further depletes plasma total protein concentration via additional catabolism, primarily of skeletal muscle to increase synthesis of acute-phase proteins for immune response. However, in piglets that have adequate protein intake, both total protein and albumin concentrations increased in response to inflammation. This suggests adequate protein intake is essential for combating catabolic effects of inflammation. [68]

Unlike children with IBD, macronutrient deficiencies are less common in adults. Nonetheless, micronutrient deficiencies are common in IBD across all age populations, particularly in CD. Different micronutrient deficiencies can occur according to specific disease location in the gut, as different nutrients are digested and absorbed in different parts of the GI tract. IBD patients who had small bowel resection are at higher risk of micronutrient deficiencies. [3] Furthermore, common historical water-soluble vitamins deficiencies include folic acid and Vitamin B12. Folic acid deficiency in IBD patients is less common compared to a decade ago, as a result of supplementation and decreased use of sulfasalazine. [63] Vitamin B12 deficiency is a concern for patients who have diseased or surgically removed terminal ileum. It is reported that approximately 20 percent of the CD patients have vitamin B12 deficiency. Serum vitamin B12, homocysteine and methyl malonic acid levels should be monitored regularly in IBD patients who have undergone terminal ileum resection. [72] Fat malabsorption can occur in CD patients that have deficient bile acid or are taking medications that prevent bile acid resorption; and it can result in fat-soluble vitamin deficiencies. [3] Among the fat-soluble vitamins, inadequate vitamin D intake is common in IBD patients. Studies reported that approximately 25 percent of adult CD patients have deficient serum 25-hydroxycholecalciferol (25OHD) concentrations and 6 to 36 percent in children with IBD. The large variability may be related to factors such as genetics, environment, severity of malabsorption and intake.[73, 74] Other studies suggested that IBD can alter normal vitamin D metabolism via pro-inflammatory cytokines suppressing parathyroid hormone (PTH) activity and disrupt conversion of 25OHD to 1,25-dihydroxycholecalciferol. [75] In addition, vitamin D deficiency is one of the contributors for

common bone disease in IBD patients. [76] Although current vitamin D intake recommendation for IBD patients is the same as for healthly individuals, regular monitoring of serum 25OHD and PTH levels should be conducted, and calcium and vitamin D supplementations as needed to prevent deficiencies and metabolic bone disease. [77] In terms of mineral deficiencies in IBD patients, iron deficiency is a major concern. Studies revealed that up to 90 percent of the adults with IBD are iron deficient, and it is the major cause of anemia in IBD patients. [78] It can also cause developmental and cognitive problems in children. [79] Therefore, iron supplement is recommended with intravenous administration being more effective than oral. [80] Moreover, zinc serum concentration is commonly decreased in CD patients; however, deficiencies are not very common. Zinc supplements are recommended when chronic diarrhea is present. [81] Calcium malabsorption occurs in approximately 13 percent of adults with CD. In order to reduce the risk of metabolic bone disease in IBD, adequate calcium intake and supplementation is recommended. [63] Furthermore, some studies suggest deficient intakes of dietary antioxidants may contribute to the high oxidative stress seen in IBD patients. Suggesting adequate intake of foods rich in antioxidants or that provide substrates for endogenous antioxidant production may prevent further damage to the diseased tissues and cells. [82] In general, malnutrition can have detrimental effects on IBD patients; hence, appropriate actions should be made to minimize malnutrition.

2.2 IBD Management

Due to the unknown etiology of IBD, the first line treatment intervention for IBD is that it is better to manage the disease by restoring GI tract health, minimize inflammation and ensure adequate nutrition. Common IBD managements include pharmacological interventions, surgeries and nutrition related therapies. [3] In addition, early detection and diagnosis with proper disease classification is important in developing treatment strategies and better manage the disease. [7]

Current pharmacological treatment strategies for CD are based on the severity of the disease. In mild to moderately classified CD patients, normally outpatients, “step-up” or “top-down” oral medication therapy approach is used; with “step-up” usually being the first choice. The “Step-up” approach uses less potent drugs with less side effects, and increases the potency if the first drugs are ineffective. [83] Common first-line drugs used are 5-aminosalicylates (5-ASA) which include sulfasalazine and mesalamine. If 5-ASA does not improve symptoms, antibiotics including ciprofloxacin and metronidazole are prescribed. [84] Glucocorticoids are needed for patients with moderate CD who are unresponsive to 5-ASA and antibiotics. [85] Immunomodulators (eg. azathioprine, 6-mercaptopurine) or biologic therapy (eg. Infliximb, adalimumab) may be used for patients with refractory CD. [85] Surgery may be needed for severe cases of CD. Pharmacological strategies to treatment UC is very similar to CD. [85] In severe cases of UC, colectomy is performed if aggressive drug therapies are ineffective, and uncontrolled hemorrhage and bowel perforation or toxic mega-colon is present. [86]

Nutrition intervention is important for IBD treatment as it has preventive and therapeutic properties. Meeting proper nutrition needs helps prevent further worsening of disease by helping the body fight against it. Nutrition support such as enteral nutrition (EN) is one of the ways to help meet nutrition needs. [5] Although EN is not as effective as glucocorticoids, and there no evidence that diet alone is used as the primary therapy in UC. Exclusive enteral nutrition as primary nutritional therapy in pediatric CD has shown to be effective in inflammation remission and promote GI mucosal recovery. [5, 87, 88] In addition, there is no significant difference in effectiveness between elemental, semi-elemental or polymeric formulas. [5] Another nutritional intervention is nutrition supplementation. Nutritional supplements including antioxidants, omega-3 fatty acids and amino acids such as cysteine have demonstrated in some studies therapeutic effects in improving inflammation and IBD remission; however, conflicting data does exist in other studies regarding the benefits of omega-3 fatty acids and some antioxidants for treating IBD. [89, 90] Moreover, probiotics such as VSL#3 and Align have shown positive effects in preventing intestinal inflammation. Most promising results were observed in pouchitis patients, whereas limited results in other

specific disease settings. [91, 92] In general, current recommendations suggest fast and accurate identification of under nutrition in IBD patients, especially children, is the key in implementing an effective nutritional intervention. Nutrition support is needed for patients who are malnourished, and multivitamin supplementation is recommended for all IBD patients. [63]

2.2.1 Adequate Protein for Recovery

Nutrients especially protein are important for those who are malnourished to promote recovery, as protein and amino acids are the building blocks of the body. One of the most common and effective ways of refeeding is through enteral nutrition support. Formulas providing adequate protein with complete amino acid profiles improves whole body protein turnover and restore tissue damages. Exclusive EN with adequate protein in pediatric CD has shown to improve mucosal healing and induce disease remission. [88] However, the positive effect may be due to a combination of factors and not adequate protein alone. Nevertheless, these results suggest EEN, with adequate protein replenishes nutrients, eliminates food antigen, reduces inflammation via reduction of dietary fat, alters gut microflora, and increases transforming growth factor beta 1 transcription to induce CD remission and promote growth in children. [93, 94]

The impact of protein on animals with infection and inflammation has been studied previously. Given similar energy intake, protein adequate colitis piglets had significantly greater weight gain than protein deficient piglets. [95] More interestingly, after refeeding protein deficient animals with adequate protein diet, animals were able to acquire a significant weight gain similar to controls.[96] Plasma IGF-1, whole body protein turnover and disease severity were improved, and TNF - α production is lowered in protein deficient animals re-fed with adequate protein. [96, 97]

Amino acids are important for cellular proliferation and essential for recovery in IBD. Glutamine is a preferred substrate for enterocytes and immune cells, suggesting clinical benefits in IBD. [98] Studies have shown glutamine not only induces GI epithelial cell growth but also reduces apoptosis. It also stimulates expression of heat shock proteins that protects cellular integrity. [98] In human trials, glutamine increased intestinal expression of hemoxygenase-1, which had potent antioxidant properties. Other amino acids such as glycine, cysteine, histidine and taurine have also been studies to modulate intestinal inflammation. [98] In animal models, glycine reduced proinflammatory cytokines, chemokines production, and improved intestinal lesions. Both histidine and taurine shown to decrease IL-8 secretion, and taurine also has protective properties against oxidative stress. [98]

2.2.2 Cysteine and Glutathione

Cysteine is another amino acid that also been studied in colitis as a prodrug, and demonstrated positive results. [98] Cysteine is sulfur containing amino acid that involves in various protein metabolisms. [89] If cysteine is readily available, 50 to 80 percent of methionine can be spared; and it is also involved in the synthesis of taurine. Both methionine and taurine play an important role in defense against oxidative stress. [89] Cysteine was also reported to protect intestinal epithelial through stimulating colonic mucin synthesis in rats with colitis.[99] Other murine colitis studies also reported decrease of IL-6, TNF-α and IL-1β with use of cysteine containing compounds.[100] In addition, cysteine is the limiting amino acid for glutathione (GSH) synthesis, which is a potent and important intracellular antioxidant. [89] Cysteine has been used in animals to restore and boost immune system. In study by Kim et al. [89] L-cysteine supplement was given to DSS-induced colitis piglets during recovery, where histological characteristics such as crypt depth, improved muscle thickness similar to the level of healthy animals; thus suggesting L-cysteine supplement is effective in restoring gut immune balance of colitis piglets. [89]

GSH is a tripeptide synthesized from glutamate, cysteine and glycine. It involves in many cellular reactions including acting as a scavenger for free radicals and other ROS. When reacting with ROS, GSH is oxidized to glutathione disulfide (GSSG), and GSSG is reduced back to GSH by NADPH dependent glutathione reductase. [101] In addition, adequate GSH concentration is needed for normal proliferation of cells including lymphocytes and intestinal epithelial cells. However, dietary protein deficiency decreases gamma-glutamylcysteine synthetase (GCS) activity, where GCS is one of the key enzymes in GSH synthesis. Therefore, adequate protein or sufficient precursors for GSH synthesis is critical to protect tissues and cells from oxidative stress. [101]

N-acetylcysteine (NAC), a derivative of amino acid L-cysteine, which has been used in clinical practice for the past decade. [102] It has been used for treatment of several disorders including cardiac injury, bronchitis, HIV/AIDS and psychiatric disorders. NAC has diverse biological effects, and most are complex and not at all well understood. Its activities involve cell regulation and apoptosis, gene expression, immune modulation, carcinogenesis and etc. Nevertheless, NAC's main mechanism of action is serving as a precursor of cysteine for GSH synthesis, reduce disulfide bonds, and scavenging ·OH, ·NO2, CO3- and thiyl radicals. [102] Furthermore, in animal colitis studies, NAC was reported to reduce colonic myeloperoxidase, ROS, TNF-α, and IL-1β levels, resulting in better remission of colitis. [103] Moreover, a study looked at supplementing NAC during refeeding of severe edematous malnourished children with infection. Compared to control supplemented with alanine, treatment group with NAC had significant change in GSH concentration and absolute synthesis rate; and the faster restoring of GSH pool was associated with faster resolution of clinical signs and symptoms. [104] NAC has been studied widely in colitis mice models. A couple of studies have shown that with a small dose (40mg/(kg·day)) of NAC, there were no significant positive effect on histological results in DSS and acetic acid induced mice. [105, 106] However, in studies where a larger (>150mg/(kg·day)) dose of NAC was administered, positive effects such as greater weight gain during the recovery period of colitis and improved histological results were observed. [107] These studies also suggested that NAC

acts as a ROS scavenger itself and also increases GSH synthesis and lowers cytokines such as TNF- α and IL-1β. [103, 108]

2.3 Inflammatory Bowel Disease Research

2.3.1 Animal Model Research

Animals have been used in research for centuries, and they play a crucial role in helping scientists understanding disease and in developing effective treatments. [109] Animal research also allows scientists to examine hypotheses that are not feasible to conduct on humans. [110] Currently, most of the animal studies are disease treatment and prevention focused with fulfillment of the "3Rs"; refinement of animal pain and discomfort, reduction of animal used, and replacement of experiment design to non-animal if possible. [109]

For the past couple decades, neonatal piglets have become the primary model for pediatric research, especially in nutrition studies. Besides primates, piglets are the most similar to human infants. Despite the faster growth rate of piglets and some anatomical differences from a human infant, GI and cardiovascular physiology and functions, nutrient metabolisms of macronutrients are very similar between the two species. [111] Respiratory, renal and hematologic systems of piglet are also very similar to human infants'. [112] In addition, piglets are suitable for infant body composition studies as they are sufficient in size to perform invasive procedures such as inserting gastrostomy tubes. [113]

Several different animal models have been developed to study IBD, and the desirability of a model and type of animal depend on the focus of specific research of IBD. Common IBD research involves chemical, bacterial, genetically engineered induced models, and transgenic mouse, mutation knock-in, and adoptive transfer models. [112] Moreover, piglet chemical induced models are commonly used in pediatric IBD research given the developmental and

physiological similarities between piglets and human infants, and the relatively inexpensive and rapid induction of colitis by chemicals such as dextran sulfate sodium (DSS). [10, 112]

2.3.2 DSS Induced Colitis Model

DSS is a polysaccharide that has heparin-like properties. [114] It has been widely used to induce colitis via oral administration or enteral route. The effectiveness of colitis induction depends on molecular weight of DSS, dosage, duration, strain and sex of animal, as well as microbial environment of animals. 5kDa DSS is used for inducing mild colitis, whereas 40 kDa is for severe colitis. Acute colitis can be induced by administrating a 3 to 10 percent dose for 7 to 10 days in piglets. A cyclical regimen of 7 days DSS administration followed by 7 days rest can mimic chronic relapsing colitis. [10, 114] Clinical and histological features of DSS induced colitis include diarrhea with occult blood, anemia, weight loss, and degeneration of cecal, colorectal epithelial and mucosa. It also increases the activation of pro-inflammatory cytokines IL-1, IL-6, IL-8, TNF-α, and up-regulates NF-κB and nitric oxide. In general, DSS is capable of rapid induction with easy adjustments to mimic the characteristics of colitis. [10]

2.4 Investigation Methods

Malnutrition and growth failure are common in children with IBD, since increased morbidity and irreversible short stature are likely if untreated. [115] Common measurements or indicators of growth in children include weight gain, height/length gain, skeletal maturation and body composition, to assess gain in lean body mass. [56, 115] A similar approach will be used to assess growth in piglets. We will determine changes in anthropometry and body composition in piglets during active colitis, as wells as response to diet interventions during recovery. Changes in body composition will be assessed in vivo with repeated measurements.

2.4.1 Anthropometry

Basic anthropometry measures, particularly height and weight, are commonly used to assess health, nutritional status and growth in children. [116] Height, including height-for-age and height velocity (gain over time) is regarded as the most sensitive anthropometry indicator of growth status in children. [115] Snout to rump length and chest circumference are basic anthropometry measures that can be repeated to assess growth rate and/or velocity in piglets.

Body weight can be measured daily in piglets to determine the pattern of weight gain over time or standardized for different body size as g/(kg·d). However, body weight does not give information on body composition or change in lean body mass. The piglets in the present study will be receiving low protein but an adequate energy diet with high fluid intake Interpreation of weight gain data may be complicated by changes in hydration status. Therefore, more precise measures of lean body mass changes are needed.

2.4.2 Dual-energy X-ray Absorptiometry

Dual-energy x-ray absorptiometry (DXA) is widely considered the "gold standard" method in measuring infant body composition. [117, 118] The DXA machine (QDR 11.2 4500A series; Hologic Inc., Waltham, MA, USA) emits two different energy X-rays and by measuring the amount of X-rays absorbed, whole body or regional fat, lean and bone mass can be determined. [119] Infant Whole Body is a software function used in DXA to analyze infant body composition. [120] In addition, Infant Whole Body has been validated using piglet carcasses, and has shown accuracy in measuring infant body composition and bone mass. [117, 118, 120-123]

DXA is a fast, accurate, non-invasive and reliable method of measuring bone mineral content, lean body mass and fat mass in infants and piglets. [117, 122, 123] However, DXA has some limitations. DXA has the best precision for young and healthy subjects, but is less accurate in populations with abnormal hydration statuses such as patients with edema. [124] Moreover,

DXA uses a 2 dimensional test rather than 3 dimensional; therefore positioning of the body and thickness of the body can significantly alter the results. [124, 125] Nevertheless, by following a set of standard operation procedures and having trained operators can minimize scan errors. [125]

Whole body DXA analysis can be performed in vivo with piglet in prone position while anesthetized with Isoflurane. Our previous study revealed that well nourished piglets' average net weight gain was 93% lean mass (221.50g/d), 5% fat mass (17.00g/d) and 2% bone mass (3.75g/d). In contrast, protein deficient piglets had 52% lean (26.67g/d), 46% fat (18.67g/d) and 2% bone mass (0.42g/d) gain. [126] This shows that weight gain maybe due to disproportion gain in fat rather than lean body mass, which does not accurately assess growth. Therefore, the use of DXA to complement weight gain data can better assess growth.

2.4.3 Nitrogen Balance

Nitrogen balance is another approach to assess gain in lean body mass in growing piglets. This is based on the nitrogen content of mixed protein (1g nitrogen in 6.25g of protein, and 31.9g of nitrogen or 200g protein in 1kg of lean body mass). [126-128]

Analysis of nitrogen is straight forward and accurate using Tru-Spec N system (LECO, St Joseph, MI, USA). Tru-Spec N system is a nitrogen analyzer machine based on thermal conductivity measurements of nitrogen, where samples are combusted under 950ºC first, then under 850ºC to separate and remove particles. Product gases are collected and mixed with oxygen in a ballast, then catalyzed to remove oxygen, and N_2 is subsequently measured. [129]

In order to obtain nitrogen balance, urine and fecal collection for at least 48 hours, with total diet intake are needed to determine nitrogen intake and nitrogen excretion. Nitrogen balance is the net difference between nitrogen content in diet and nitrogen excreted. [127] Although

nitrogen analysis is accurate, obtaining complete urine and fecal output is often difficult. We have shown previously that diet nitrogen retention in well-nourished piglets was 78% versus only 46% in protein deficient piglets. [126] Gain in lean body mass calculated from nitrogen retention was 54.77g/(kg·d) in well-nourished piglets and 4.47g/(kg·d) in protein deficient piglets. [126] Conceptually, estimated lean body mass gain by nitrogen retention and DXA should be similar in piglets. The protein gained in previous well-nourished piglets determined by nitrogen retention was 547g and 532g by DXA, which were statistically different. [126] Therefore, nitrogen balance is another important method to determine lean body mass gain and dofr assessing growth rate changes during acute recovery phases in the current study.

3. RATIONALE

IBD is a group of chronic diseases characterized by remission and relapse of inflammation. [1] Due to the unknown etiology of IBD, the primary focus of IBD management is to promote restoring of GI tract health, minimize inflammation and ensure adequate nutrition for growth. Previous and more recent studies of IBD all focused on the active phase of IBD, which resembles an acute disease. Therefore, it is interesting to discover and characterize the intermittent nature of the disease with frequent clinically occurred protein deficiency, and to examine the inflammation and nutrition interaction in terms of growth and clinical outcomes. In addition, with the rising incidence of IBD in young children, pediatric IBD is needed to better explore nutrition interventions for treating IBD.

To mimic acute recovery in colitis and study the impact of nutrition interventions on growth and disease severity during remission, six and a half days of DSS administration was followed by five and a half days of recovery without DSS. 20% concentrated DSS is given at 0.4g/kg/d to ensure successful induced colitis. [114] The length of recovery time is based on diet change related digestive enzyme adaptation, colonic mucosa and small intestine epithelial turnover rates of approximately 3 to 8 days in healthy pigs. [130] Due to the rapid

growth rate of piglets, five and a half days is sufficient to detect metabolic and physiology changes. [89]

Malnutrition is common in children with IBD, and can lead to growth failure if unresolved. In this study, protein deficiency is simulated by providing a severe protein deficient diet that meets 15% of the daily total protein requirement in piglets. The level of deficiency was chosen based on previous studies, where even a moderate protein deficient diet at 50% protein requirement no severe decrease in protein synthesis in piglets was discerned; at 15% protein requirementl, a significant decrease was observed. [67, 68] Moreover, implementation of protein deficiency alone rather than protein-energy malnutrition can isolate the impact of protein alone on inflammation, IBD recovery, and will minimize other potential complex interactions between nutrients and inflammation.

In cases where adequate nutrition cannot be met to promote proper growth in children with IBD due to abdominal pain and decreased appetite, nutrition supplements can be used to minimize further worsening of inflammation. Although nutrient supplementation use in IBD is common, current evidence is not conclusive regarding their beneficial effects on IBD. Antioxidant supplementation of NAC is well studied in several diseases as a potential treatment approach. NAC's anti-inflammatory and antioxidant effects are mediated through replenishing substrates for GSH synthesis, to decrease inflammation and oxidative stress and may promote recovery despite protein deficiency. It is unknown whether GSH synthesis will increase with NAC supplementation despite a protein deficient state, as other substrates such as glycine may not be available for proper GSH synthesis. The dose of NAC in this thesis study was based on studies done on malnourished children (see Methods). [104] NAC with diet will provide a total of 2.5 times the amount of cysteine in a normal nutritious piglet diet. NAC was given at a high level slightly below the toxicity level, to examine for maximal effects on decreasing disease severity during recovery.

In general, this study aimed to develop and characterize early recovery in colitis, and the assessment of growth and disease severity during recovery in response to adequate protein intake and NAC supplementation. Such studies are needed to provide evidence for developing nutritional interventions for pediatric IBD, in order to prevent growth failure in children and minimize intestinal inflammation.

4. OBJECTIVES & HYPOTHESES

4.1 Global Objective I

Malnutrition often co-exists with inflammation in children with IBD. Increased metabolic demand decreases growth rate in children with IBD. Our piglet model of colitis represents children with slowed growth during active colitis. The main objective of this study is to establish and characterize a recovery model of pediatric IBD using piglets.

Specific Objective 1

Characterize growth, body composition and disease severity in protein deficient piglets with active colitis and during recovery.

Hypothesis 1

Within 5 days of discontinuing DSS administration, growth rate will be slightly greater. Histological assessment of disease severity will be less severe in protein deficient (PD) piglets when compared to Active Control PD-ACT piglets, with no recovery intervention.

4.2 Global Objective II

Another objective of this study is to investigate the effects of nutrition on growth and disease severity during recovery. Providing adequate protein, to promote anabolism, will be studied by assessing body composition, anthropometry and nitrogen balance. Controlling inflammation through NAC supplementation to decrease disease severity will be examined by histological assessment.

Specific Objective 1

This study aims to assess anabolic nutritional intervention through characterizing growth, body composition and disease severity in piglets receiving adequate protein versus piglets maintaining low protein diet during recovery.

Hypothesis 1

Protein deficient (PD) piglets receiving adequate protein diet during recovery will improve and demonstrate greater growth, nitrogen balance and lean body mass gain, and less severe histological assessment compared to PD piglets receiving low protein diet during recovery.

Specific Objective 2

To assess anti-inflammatory nutritional intervention strategeies by characterizing growth, body composition and disease severity in piglets maintaining low protein diet with NAC supplementation versus without during recovery.

Hypothesis 2

Protein deficient (PD) piglets receiving NAC supplement during recovery will have a less severe histological assessment, but similar growth compared to PD piglets receiving low protein diet but no NAC supplement during recovery.

Specific Objective 3

Assess differences in growth, body composition and disease severity in piglets receiving adequate protein versus piglets maintaining low protein diets, receiving NAC supplement.

Hypothesis 3

Anti-inflammatory NAC supplement will decrease disease severity, similar to anabolic adequate protein diet, but will not improve growth and body composition to the same extent as adequate protein.

4.3 Specific Measures of Objectives

Assessment of Disease Severity:

Fecal occult blood test (Hemoccult Sensa ®)
Histology grading of colon tissue slides with validated grading scales by licensed pathologist (**See Table 8**.)

Assessment of Growth:

Anthropometry (body weight, chest circumference, snout-to-rump length)
Nitrogen balance and nitrogen retention
Body composition via DXA (total lean, fat mass and percentage, bone mineral density)

5. METHODOLOGY

5.1 Methods

5.1.1 Study Design

21 female piglets (7 to 10 days old, 7 to 9 pounds or 3.2 to 4.1kg; Les Porcheries Chanca Inc.; St-Louis de Gonzague, QC, Canada) underwent surgery for catheter insertion, on Day 1 and received a liquid protein deficient diet (15% of National Research Council requirement) infused intragastrically from Day 1 to Day 9. [131] Starting on Day 3 until Day 9, piglets received Dextran Sulfate Sodium (20% solution, 40,000MW; ICN Biomedicals Inc., OH, USA) intragastrically to induce colitis. On the end of Day 9, DSS was discontinued and 21 piglets were randomized to 1 of 4 groups (n = 5 each, except n = 6 for PD-AP). One active control group and 3 recovery groups. Recovery groups received either:

a) A liquid diet supplying 100% of protein requirement.

b) Continued low protein diet with an additional supplement of N-acetylcysteine.

c) Continued the low protein diet.

After 5 days of recovery, on Day 5, piglets were euthanized with Euthanasol (750mg sodium pentobarbital IV; Schering Plough Canada Inc, Pointe Claire, QC, CA), and colon issue samples were collected for histology assessments. The active control group of piglets were euthanized on Day 10 with no recovery phase.

Growth was measured by anthropometry measurements, body composition and nitrogen balance. Weight was obtained daily, whereas chest circumference and snout-to-rump length was measured on Day 1, 9, 14 and 15. Piglets underwent DXA scan (QDR 11.2 4500A series; Hologic Inc., Waltham, MA, USA) on Day 1, 9, 14; and nitrogen balance on Day 8, 9, 13 and

14. This study was approved by McGill University Animal Care Committee in accordance with the Canadian Council on Animal Care Guidelines.

The study flow-chart and summary chart of a 15-day trial see **Figure 1** and **Figure 2**.

5.1.2 Anesthesia and Surgery

Surgery was performed by three trained personnel, two surgeons and one anesthetist. Anesthesia was induced with 5% isoflurane (Baxter Healthcare Co., Richmond Hill, ON, Canada) by mask. Anesthesia was maintained at 2% isoflurane by mask throughout the surgery, with continuous monitoring and recording of vital signs by the anesthetist. Catheters (Silastic, Dow Corning Corporation, MI, USA) were inserted under aseptic conditions into the femoral vein for infusions, into the jugular vein for blood sampling, and into the stomach for diet and DSS administration. Catheters were tunneled subcutaneously to exit on the side of the chest. Piglets were fitted with a jacket to house the catheters in a pocket and to connect to a tether and swivel system to allow the piglet freedom while connected with feeding tubing. Buprenorphine (10μg/kg body weight) were administered immediately after surgery and again 12 hours later.

Note: For detailed anesthesia and surgery procedures and equipment please see study Standard Operation Procedures Part I and II.

5.1.3 DSS Administration and Daily Piglet Care

Piglets were housed individually in metabolic cages, and kept warm with heating lamps. A typical day of piglet care involved weighing, DSS administration, monitoring disease activity, making and refilling diet, cleaning piglet room, and documenting progress and events.

A DSS solution (0.4g/kg/d split between 2 doses) was administered via gastric catheter starting in the morning of Day 3 until the morning of Day 9.

Disease activity was determined by stool consistency and fecal occult blood test. All observations and results were documented. In terms of documentation, daily weight, activity, diet pump readings, stool description and catheter conditions were recorded.

5.1.4 Diet

Liquid diets were designed to deliver energy and micronutrient requirements of piglet according to National Research Council (NRC) Nutrient Requirement of Swine 10th and 11th Edition. [131, 132] The diets differed in protein content: to be severely deficient in protein (to deliver 15% of requirement) or to supply adequate protein for healthy growing piglets.

5.1.4.1 Macronutrients

(**See Table 2, 3, 4 & 5**)

The macronutrient distribution of AP diet was 26.9% protein, 36.6% carbohydrate and 36.6% fat; and PD diet was 4.1% protein, 48% carbohydrate and 48% fat. The equal distribution of energy from carbohydrate and fat was to provide balance in ingredients to avoid diarrhea caused by carbohydrate related high osmolarity and fat related steatorrhea. In addition, energy requirement was calculated to be 214kcal/kg/d based on the Nutrient Requirement of Swine. [131] Considering a typical swine liquid feed was composed of 20-30% dry ingredients, and having 250ml/kg/d liquid feeding was not excessive and meets the daily recommended fluid intake, AP diet's concentration was calculated to be 19.58%, and PD diet's is 17.98%. [133]

Whey protein (Quadra, Vaudreuil, QC, Canada) and egg white solid (Harlan Laboratories Inc., Millstone, NJ, USA) contributed equally as source of protein. As whey protein was limited in phenylalanine and tyrosine, and egg whites causes foam bubbles, having a

balanced contribution of source protein provided a better amino acid profile and a better consistency of the final diet. [95, 134] Amino acid profile of each diet (**Table 5**) and intake (**Table 2**) were shown. The AP diet supplied each amino acid at 119-189% of NRC requirements, whereas the PD diet was deficient in all amino acids. The most limiting amino acids were histidine and cysteine, 18 and 19% of requirement respectively.

Lactose (Quadra, Vaudreuil, QC, Canada) and maltodextrin (Harlan Laboratories Inc., Millstone, NJ, USA) were the sources of carbohydrate for the piglet diet. Both contribute equally in AP diet, where lactose contributed more in PD diet to make up part of the energy deficit from low protein content. The reason being that high maltodextrin levels were associated with a higher risk of enterocolitis in piglets. [135]

Corn oil and canola oil were used as the source of fat, giving a n-6 to n-3 fatty acid ratio of 7.59 to 1, which was within the recommended range of 4:1 to 16:1 for infant formulas. [136] To make the PD diet, additional carbohydrate and fat (in equal % of total calories) was added to maintain diets with consistent energy levels.

5.1.4.2 Micronutrients

(**See Table 6 & 7**)

Custom made vitamin and mineral mixes (Harlan Laboratories Inc., Millstone, NJ, USA) were used to provide micronutrient needs. Micronutrient requirements of piglets receiving milk-based versus corn and soy diets are not well known. Due to limited available references, mineral content of the diets was calculated to meet approximately 120% of the requirements and vitamins were calculated to meet approximately 200% of the requirements. [131, 132] Moreover, vitamin D content of 142.08 IU/kg/d was determined to maintain normal serum vitamin D levels of 75nM. As previous studies demonstrated, low serum vitamin D levels occurred when diets only contained 100% of vitamin D requirement. [95] Therefore, a ten times higher than recommended vitamin D content was used to target serum vitamin D level

of 75nM. Nevertheless, current vitamin D content was below the upper toxicity level of 248000 IU/d. [131]

Furthermore, on average, 0.05% and 0.8% of protein presented in egg white was avidin and riboflavin binding protein (RFBP). [137, 138] Avidin affects bioavailability of biotin with 1mg avidin binds to 15μg biotin, whereas 1mg RFBP binds to 15μg riboflavin. [139, 140] In order to compensate the potential decrease in biotin and riboflavin bioavailability, biotin and riboflavin contents in both AP and PD diets were adjusted to meet biotin and riboflavin requirements.

5.1.4.3 NAC Supplement

The NAC supplement (N-acetyl-(L)-cysteine; Fisher Scientific, Fair Lawn, NJ, USA) dosage was determined based on studies in malnourished children recovering from protein energy malnutrition. [104] In the study conducted by Jahoor et al. [104] a 3g protein per kg body weight per day dose was adminstered to promote rapid growth recovery in severely malnourished children, where the protein intake was 2.5 times the normal recommendation. [104] For the purpose of this study, we similarly adapted the 2.5 factor when formulating the NAC supplement for piglets. In piglets under 5kg, normal protein requirement is 14.4g/(kg·day). [131] The protein mix used in the piglet diet provides an average of 0.311 g/(kg·day). Since supplementing NAC for recovery and anti-inflammatory response was the focus, a total intake of cysteine was extrapolated and calculated based on protein and cysteine levels described in Jahoor's study. [104] Therefore, a total cysteine intake for recovery and anti-inflammatory response was calculated by multiplying normal cysteine intake by the 2.5 factor obtained from Jahoor's study, which gives 0.778 g/(kg·day). By subtracting this total cysteine intake by the amount cysteine in the diet, the amount of cysteine supplement was calculated to be 0.731 g/(kg·day), which is equivalent to 0.984 g/(kg·day) NAC, based on the molecular weights of cysteine and NAC. NAC was made into a solution and infused with a separate pump from the PD diet group. 7.872g of NAC was used to make 500mL NAC

solution (concentration: 1.57%). The fluid content of the PD diet used in NAC group was adjusted to account for the fluid in the NAC supplement.

5.1.4.4 Diet Regimen

All diets including NAC supplement were infused using enteral pumps (Compat Enteral Delivery System with Dose Limit, Enteral Delivery Pump Set with Inline "Y" Adaptor and 1000mL Vinyl Bag; Nestlé Healthcare Nutrition Inc., Minnetonka, MN, USA). Diet total daily volume was calculated based each day's weight of the piglets. Diet was made fresh, and was not kept for more than 2 days. Due to the use of continuous feeding, total daily diet volume was divided by two and diet was added in the morning and the afternoon to minimize bacteria growth. Diet feeding sets were washed or changed daily. In addition, both total volume of diet added and pump readings were recorded to accurately calculate total nutrient intake. Enteral feeding pumps were observed to provide inaccurate readings in previous studies.

Post-surgery diet adaptation was performed by giving a rating of 60% of targeted rate on Day 1, followed by rate of 80% of targeted rate on Day 2, and reaching 100% target rate on morning of Day 3. AP diet change adaptation was done by gradually switching (25% increase per 12 hours) PD diet to AP diet spanning 2 days.

5.1.5 Growth

5.1.5.1 Anthropometry

Piglets were weighed to the nearest 5g every morning at approximately the same time using an electronic balance (Toploading Balance XI-30K; Denver Instrument, Arvada, CO, USA) with the piglet either lying down or all four feet on the balance. Snout to rump measurements and chest circumference measurements were taken with a flexible measuring tape on Day 1, 9

and 14 when piglet was anesthetized. Ideally length measurements should be done by the same investigator with the same measuring tape to minimize errors. Any signs of edema, stomach distension and constipation were recorded, as these ailments affect true body weight. Body weights were plotted graphically in each piglet chart to track general growth trend.

5.1.5.2 Body Composition

Body composition measurements were done on Day 1, 9 and 14 using DXA machine (QDR 11.2 4500A series; Hologic Inc., Waltham, MA, USA). Infant Whole Body is a software function that analyzes DXA scans to output lean body mass, fat mass bone mineral content and bone mineral density. (Hologic Discovery Software; Hologic Inc., Waltham, MA, USA) Piglets under isoflurane anesthesia were scanned in the prone position with four feet naturally extended outwards. If the piglet moved during a scan, the scan was repeated. In addition, Quality Control scans using Spine Phantom (3 times) and Step Phantom were performed before actual scans to ensure the machine was in excellent working condition and was able to provide an accurate and stable coefficient of variation (CV). Quality control reports indicated CV values were all under 5% for Bone area, Bone Mineral Content and Bone Mineral Density.

5.1.5.3 Nitrogen Balance

Nitrogen balance was performed over 48 hours in the active phase on Day 8 - 9 and in the recovery phase Day 13 - 14 for each piglet. Nitrogen balance started when the piglet was transferred to a clean cage. Diet infusion volumes were recorded and feces and urine were collected for the next 48 hours. Stool was collected by scraping feces off the piglet cage walls and screen. Fifty ml plastic tube(s) were used to store feces and then refrigerate under -4ºC. Uncollectable feces were estimated by their apparent volume and/or weight and included in the total volume or weight.

Urine was collected in urine drains from piglet cage through a screen into a 3L bottle containing 5ml of 3.52M H_2SO_4 on ice. Total urine output volume were recorded and 50ml was aliquot and kept at -80ºC.

Oven dried fecal samples and freeze dried urine samples were weighed and analyzed in duplicate by Tru-Spc N System (LECO, St Joseph, MI, USA) to obtain nitrogen content (%; g nitrogen / g sample). Nitrogen balance and nitrogen retention was calculated using following equations:

$$\text{Urine Freeze-dry Factor (UFF), g/ml} = \frac{\text{freeze dried urine weight (g)}}{\text{volume of urine to be freeze dried (ml)}}$$

$$\text{Urine Nitrogen, g/(kg·d)} = \frac{\text{urine nitrogen content (\%)} \times [\text{total urine volume (ml)} \times \text{UFF} \left(\frac{\text{g}}{\text{ml}}\right)]}{\text{average weight of Day 8 \&9 } or\ Day 13 \text{ \& } 14 \text{ (kg)} \times 2 \text{ (day)}}$$

$$\text{Fecal Nitrogen, g/(kg·d)} = \frac{\text{fecal nitrogen content (\%)} \times \text{total dried fecal weight (g)}}{\text{average weight of Day 8 \&9 } or\ Day 13 \text{ \& } 14 \text{ (kg)} \times 2 \text{ (day)}}$$

$$\text{Nitrogen Excretion, g/(kg·d)} = \text{urine nitrogen} + \text{fecal nitrogen}$$

$$\text{Nitrogen Balance, g/(kg·d)} = \text{Nitrogen Intake} - \text{Nitrogen Excretion}$$

$$\text{Nitrogen Retention (\% per day)} = \frac{\text{Nitrogen Balance}}{\text{Nitrogen Intake}} \times 100$$

5.1.6 Tissue Sampling

Piglets were euthanized with Euthanasol (750mg sodium pentobarbital IV; Schering Plough Canada Inc, Pointe Claire, QC, CA) on Day 15. Approximately 2cm of distal colon and 5cm of spiral colon was cut, cleaned in ice saline and stored in 10% phosphate buffered formalin for histology. For specific necropsy preparation and tissue sampling procedures, please see the study Standard Operation Procedure part VIII.

5.1.7 Histology

Spiral colon and distal colon tissues were embedded in paraffin. Approximately 8μm of each sample was sliced and prepared on to slides. Slides were stained with hematoxylin and eosin. Slides were graded by a McGill Animal Resources Centre Pathologist blinded to group assignment. Grading scales were provided by the pathologist. Scores characterized the degree and extent of inflammation, epithelial cell and goblet cell damage as well as gland loss. See (**Table 8**) for complete grading scale chart.

6. STATISTICAL ANALYSIS

A sample size of 10 per group was determined by pervious albumin FSR standard deviation, to detect a 30% difference of plasma albumin fractional synthesis rates between two groups. However, due to limited resources, only 21 piglets performed in the study. In addition, due to severe fluid retention and/or loss, data from 5 piglets was not included in data analysis. Mixed model repeated measurement and multiple comparisons analyses were done using SAS 9.4 (Statistical Analysis System Institute Inc. Cary, North Carolina, US). Homogeneity of variances was tested using Bayesian Criteria. Normality was tested using PROC UNIVARIATE function and Shapiro-Wilk's Test. Data was transformed if it was not normally distributed. Repeated measurements were done using Scheffe's test. Multiple comparisons were completed using Bonferroni's test. Analyses were done using 5% probability level with statistical significance level corrected according to specific hypothesis for Bonferroni's test.

Figure 1. Study flow-chart of the 15 days study including active colitis phase and recovery phase

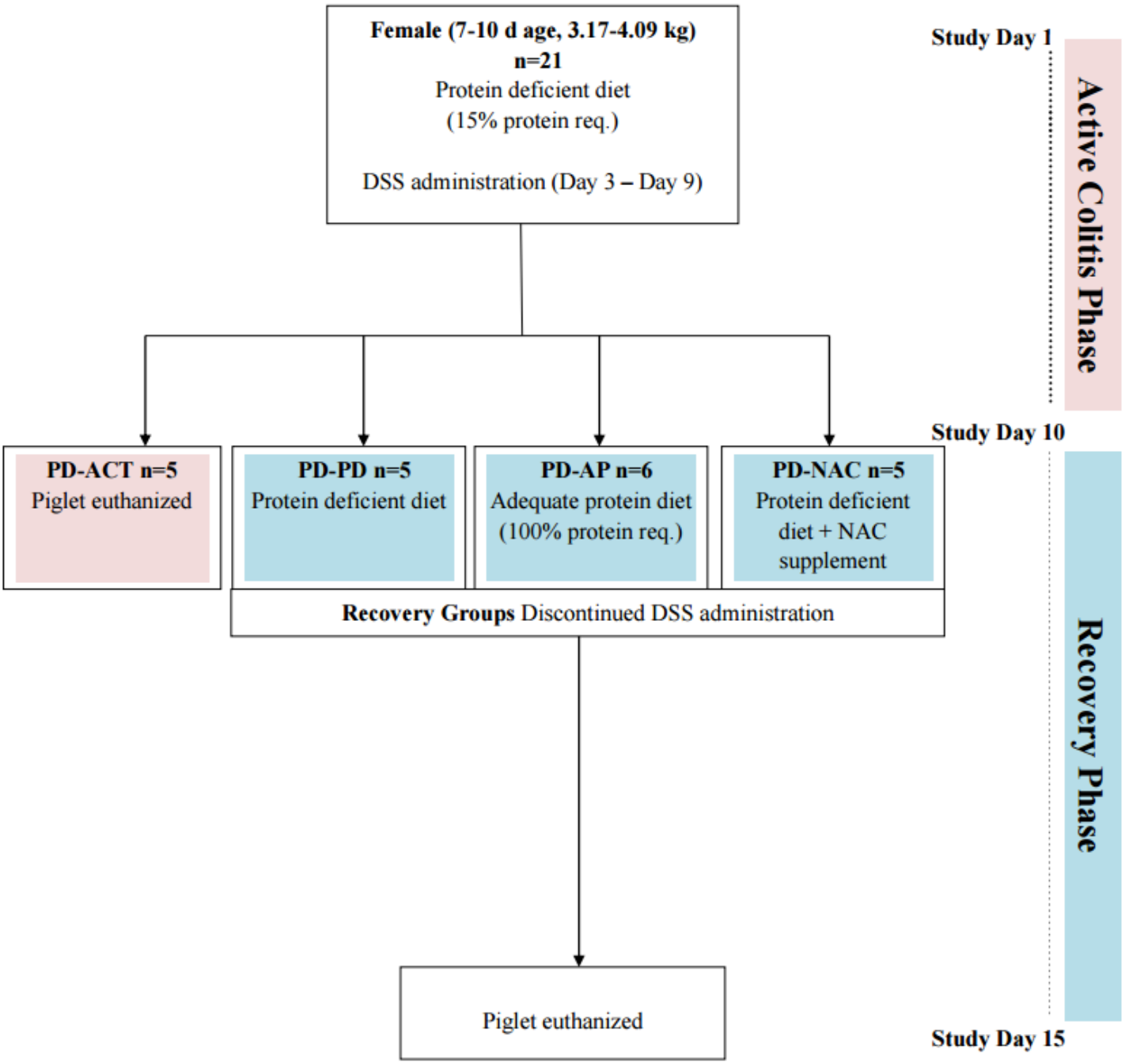
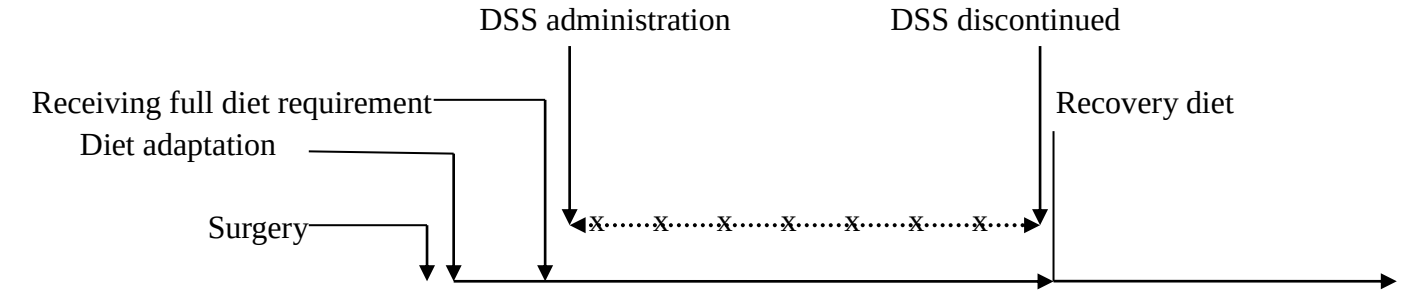


Figure 2: Study Protocol of the 15-day study including surgery, diet, DSS administration, stable isotope infusion, various experimental measures



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Body weight	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Linear growth	x								x					x	
Body composition (DXA)	x								x					x	
Disease activity[1]						x	x	x	x	x	x	x	x	x	x
Nitrogen balance								x	x				x	x	
Blood sampling[2]			x			x				x			x		x
Histological grading (post mortem)										x[4]					x

Infusion Study

L-[ring-$^{2}H_5$]phenylalanine: 35 µmol/kg/h

[^{15}N, 1,2-$^{13}C_2$]glycine: 75 µmol/kg/h

Hour	-2	-1	0	1	2	3	4	5	6
Feeding	x	x	x	x	x	x	x	x	x
Blood sampling[2]			x			x	x	x	x
Tissue sampling[3] (post mortem)									x

1. Fecal biomarker S100A12 and Disease Activity Index without Weight (DAINWT) with parameters including stool consistency and fecal occult blood test (Hemoccult Sensa®).
2. Blood samples are used for measuring the synthesis and concentrations of protein and GSH and concentrations of inflammatory mediators.
3. Tissue samples for protein and GSH synthesis and concentration, myeloperoxidase (MPO) activity, and other inflammatory mediators concentrations and their mRNA expression.
4. Active Control group (PD-ACT, n=5) only.

Table 1: Diet in each study group of piglets with DSS-induced colitis

	PD-ACT	PD-PD	PD-AP	PD-NAC
Colitis Induction Phase	PD diet*	PD diet	PD diet	PD diet
Recovery Phase	N/A***	PD diet	AP diet**	PD diet + NAC

* PD diet: 15% protein deficient diet

** AP diet: adequate protein diet

*** Active Control group was euthanized at the end of colitis induction phase

Table 2: Nutrient component and energy percentage of liquid diets administered to piglets with DSS-induced colitis

	Diet					
	PD			AP		
	g/(kg·d)	kcal/(kg·d)	kcal%	g/(kg·d)	kcal/(kg·d)	kcal%
Protein	2.2	8.8	4.1	14.4	57.6	26.9
Carbohydrate	26.0	103.9	48.0	19.6	78.3	36.6
Lipids	11.5	103.9	48.0	8.7	78.3	36.6
Minerals	3.4	0	0	2.7	0	0
Vitamins	0.1	0	0	0.1	0	0
Total Energy		216.5	100		214.2	100

Table 3: Liquid diet composition administered to piglets with DSS-induced colitis

	Diet	
Components in liquid diet as fed	**PD**	**AP**
Egg White g/L[1]	5.34	34.95
Whey Protein Concentrate (WPC) g/L[2]	5.50	36.00
Lactose g/L[2]	86.63	56.73
Maltodextrin g/L[1]	22.15	22.15
Corn Oil g/L[3]	24.52	18.49
Canola Oil g/L[3]	21.64	16.31
Mineral Mix g/L[1]	13.70	10.80
Vitamin mix g/L[1]	0.38	0.38

1-Obtained from Harlan Laboratories in Indianapolis USA

2-Product from Quadra Ingredients in Vaudreuil-Dorion, CA

3-Product of Compliments in CA

Table 4: Lipid components and n-6 to n-3 fatty acids ratio of liquid diet administered to piglets with DSS-induced colitis

Lipid	**n-6 %**	**n-3 %**	**g/L**	**n-6 (g)**	**n-3 (g)**	**n-6:n-3 ratio**
Canola Oil	16.67%	8.89%	4.08	0.680	0.363	
Corn Oil	55.56%	1.11%	4.62	2.567	0.051	
Total			8.7	3.247	0.414	7.842

Canola oil and corn oil are both from Compliments, Sobeys Inc., Canada

Table 5: Amino acid composition in g/(kg.d) in relation to NRC requirement of the protein deficient and adequate protein diets

Amino Acids		Diet			
		PD		AP	
	Req't g/(kg.d)	Intake g/(kg.d)	% Req't	Intake g/(kg.d)	% Req't
Arginine	0.34	0.093	27	0.621	183
Histidine	0.26	0.046	18	0.309	119
Isoleucine	0.44	0.121	27	0.805	183
Leucine	0.83	0.201	24	1.337	161
Lysine	0.82	0.163	20	1.089	133
Cysteine/Cystine*	0.25	0.047	19	0.311	124
Methionine	0.22	0.062	28	0.416	189
Methionine + cysteine	0.46	0.109	24	0.727	158
Tyrosine**	0.28	0.071	26	0.476	170
Phenylalanine	0.48	0.104	22	0.691	144
Phenylalanine + tyrosine	0.77	0.175	23	1.167	152
Threonine	0.51	0.104	20	0.696	137
Tryptophan	0.15	0.040	26	0.265	177
Valine	0.55	0.135	25	0.901	164
Alanine	n/a	0.117	n/a	0.781	n/a
Aspartic acid	n/a	0.205	n/a	1.369	n/a
Glutamic acid	n/a	0.352	n/a	2.345	n/a
Glycine	n/a	0.060	n/a	0.399	n/a
Proline	n/a	0.108	n/a	0.720	n/a
Serine	n/a	0.130	n/a	0.868	n/a

*: Values calculated by subtracting methionine from methionine + cysteine

**: Values calculated by subtracting phenylalanine from phenylalanine + tyrosine

Amino acid intake composition is based on a 50:50 mixture of spray dried egg white and whey protein concentrate.

Amino acid requirement (g/(kg.d)) is based on the average body weight requirement for growing piglets of 3-5kg and 5-10kg provided by NRC (1998)

Table 6: Mineral intake in liquid diets as fed in relation to NRC requirement as a percentage

Minerals		PD		AP	
	Req't Amt/(kg.d)	Intake Amt/(kg.d)	% Req't	Intake Amt/(kg.d)	% Req't
Calcium (g)	0.55	0.66	120	0.66	120
Phosphorus (g)	0.44	0.52	120	0.52	120
Sodium (g)*	0.21	0.25	120	0.25	120
Chloride (g)**	0.27	0.40	≥120	0.34	≥120
Magnesium (g)	0.03	0.03	120	0.03	120
Potassium (g)	0.19	0.22	120	0.22	120
Copper (mg)	0.39	0.47	120	0.47	120
Iodine (mg)	0.01	0.01	120	0.01	120
Iron (mg)	6.46	7.75	120	7.75	120
Manganese (mg)	0.26	0.31	120	0.31	120
Selenium (mg)	0.02	0.02	120	0.02	120
Zinc (mg)	6.46	7.75	120	7.75	120

Mineral requirement (g/(kg.d)) is based on the average body weight requirement for growing piglets of 3-5kg and 5-10kg provided by NRC (1998).

*: Na and Cl requirement is based on the average body weight requirement for growing piglets of 5-7kg provided by NRC (2012)

**: Cl requirement is based on the average body weight requirement for growing piglets of 5-7kg provided by NRC (2012) and is slightly higher than 120% of requirement because of the presence of Chloride from spray dried egg white solids that is not provided by the company or indicated in CNF.

Table 7: Vitamins intake in liquid diets as fed in relation to NRC requirement as a percentage

Vitamins		PD		AP	
	Req't g/(kg.d)	Intake g/(kg.d)	% Req't	Intake g/(kg.d)	% Req't
Vitamin A (IU)	142.08	284.17	200	284.17	200
Vitamin D (IU)	14.21	142.08	1000	142.08	1000
Vitamin E (IU)	1.03	2.07	200	2.07	200
Vitamin K (mg)	0.03	0.07	200	0.07	200
Biotin (mg)	0.005	0.02	400	0.06	1422
Choline (g)	0.04	0.07	200	0.07	200
Folacin (mg)	0.02	0.04	200	0.04	200
Niacin (mg)*	1.60	3.19	200	3.19	200
Pantothenic acid (mg)	0.71	1.42	200	1.42	200
Riboflavin (mg)	0.24	0.61	254	1.35	558
Thiamin (mg)	0.08	0.16	200	0.16	200
Vitamin B6 (mg)*	0.37	0.74	200	0.74	200
Vitamin B12 (µg)	1.21	2.42	200	2.42	200

Vitamin requirement (g/(kg.d)) is based on the average body weight requirement for growing piglets of 3-5kg and 5-10kg provided by NRC (1998).
*Niacin and Vitamin B6 requirement is based on the average body weight requirement for growing piglets of 5-7kg provided by NRC (2012)

Table 8: Histological Grading Scale used to assess DSS-induced colitis (Provided by McGill Animal Resources Centre Pathologist, Jean-Martin Lapointe)

ID	Inflammatory cell infiltration	Inflammatory cell type	Extent of inflammation	Inflammation score	Inflammatory cell localization	Luminal inflammation	Surface epithelial damage	Gland epithelial degeneration	Goblet cell / enterocyte ratio	Gland loss	Total (0-30)
				0							0

Inflammatory cell infiltration:

0 Occasional resident inflammatory cells in lamina propria (predominantly lymphocytes, plasma cells)
1 Minimal increase in inflammatory cells
2 Mild increase in inflammatory cells
3 Moderate increase in inflammatory cells
4 Marked increase in inflammatory cells

Inflammatory cell type

E Significant presence of eosinophils
L Significant presence of lymphocytes and plasma cells
M Significant presence of macrophages
N Significant presence of neutrophils

Inflammatory cell localization

0 No significant inflammatory infiltration
1 Inflammatory infiltration predominantly in the lamina propria
2 Inflammatory infiltration extending significantly into the submucosa
3 Inflammatory infiltration extending significantly into the muscularis

Extent of inflammation

0 No significant inflammatory infiltration
1 Significant inflammation over less than a 1/3 of colon length
2 Significant inflammation between 1/3 to 2/3 of colon length
3 Significant inflammation from 2/3 to all of colon length

Inflammation Score

Inflammatory cell infiltration x Extent of inflammation

Luminal inflammation

0 No significant luminal inflammation
1 Presence of rare inflammatory cells over mucosal surface
2 Presence of occasional inflammatory cells and/or small fibrin deposits over mucosal surface
3 Presence of frequent inflammatory cells and large fibrin deposits over mucosal surface and into lumen

Surface epithelial changes
0 Normal surface epithelium
1 Rare to occasional areas of epithelial flattening, degeneration or exfoliation
2 Frequent areas of epithelial flattening/degeneration/exfoliation, or rare areas of epithelial ulceration
3 Frequent or extensive areas of epithelial ulceration

Gland loss
0 Normal density of glands
1 Rare foci of gland loss, over small areas
2 Occasional small foci of gland loss, or rare but wider foci
3 Frequent small foci of gland loss, or occasional wide foci

Gland epithelium degeneration
0 Normal gland epithelium
1 Rare glands with epithelium degeneration and/or necrotic debris in lumen
2 Occasional glands with epithelium degeneration and/or necrotic debris in lumen
3 Frequent glands with epithelium degeneration and/or necrotic debris in lumen

Goblet cell/enterocyte ratio
0 Normal goblet cell/enterocyte ratio (marked predominance of goblet cells, except in base of glands)
1 Decrease in goblet cells affecting few glands
2 Decrease in goblet cells affecting occasional glands
3 Decrease in goblet cells affecting frequent glands

7. RESULTS

7.1 General Records of Study

Most animals were healthy upon arrival. However, 2 piglets had diarrhea; and piglets in block 2 appeared to be less healthy upon arrival as suggested by their light weights (< 3kg) and dull hair compared to other piglets. All surgeries were well performed. Piglets were fully active approximately 2 hours post-surgery; only 2 piglets experienced minor breathing complications. All piglets adapted to target feeding rates by study Day 3. Diet changes at study Day 10 were successful. Several feeding pump blockages occurred; nevertheless, most lost intakes were caught up by adjusting feeding rates. Energy intake was not different among groups. Over the entire study, piglets received 89% of the targeted energy intake of 217kcal/(kg·day). Feces were positive for occult blood by Hemoccult Sensa test after study Day 8 for most piglets, which was later than expected, based on previous studies.[126] Fecal output was low in the active colitis phase while piglets were on the low protein diet, as expected.[126] However, almost all piglets developed a viscous dark green-black diarrhea with strong distinct smell approximately 1 day after diet changes; with PD-AP piglets having the most output and the strongest smell. In addition, only 2 piglets (different groups) had negative fecal occult blood by the end of the study. Furthermore, some clinical symptoms and signs were observed throughout the duration of the study. Expected clinical symptoms included increasing abdominal discomfort during active colitis phase; whitening of hair, suggesting protein deficiency; and slow weight gain. Unexpected clinical symptoms included decreased level of activity during recovery phase in almost all piglets; vomiting in some piglets; fluid retention in 5 piglets (different groups); sneezing, throat-clearing coughs, and blockage of nostrils with a hard mucus, observed in 4 out 5 experiment blocks; 3 of 5 blocks of piglets developed light yellow watery diarrhea at different times in the study, with no specific time pattern but appeared to be contagious; only 1 block of piglets resolved the diarrhea. The yellow watery diarrhea was associated with excessive fluid loss and led to

drastic weight changes, and resulted in a wasting appearance in 3 piglets. Fecal occult blood tests completed on the yellow watery diarrhea indicated a severe presence of blood. Fecal analysis results from McGill Comparative Medicine and Animal Resources Centre (CMARC), revealed the presence of enteropathogenic E. coli, in piglets displaying yellow watery diarrhea. All piglets were euthanized without pain. Necropsy revealed abnormal lung tissue. Four piglets showed purple-pink nodules or were fibrous, and had 1 piglet with abnormal edematous kidneys. In general, the study were carried out successfully; however, several unexpected clinical problems occurred that were unlikely to be related to study model.

7.2 Weight Gain and Growth

Body weight, snout-to-rump, chest circumference data was analyzed using mixed model repeated measures. Normality and homogeneity of variances were verified using Shapiro-Wilk's Test and Bayesian Information Criterion (Appendix 1). Transformation (either log or square root) of data was performed if the results were not normally distributed. No heterogeneous variance were present. Piglets weighed an average of 3.29kg upon arrival, and gained 15% of their body weight during the active colitis phase while receiving the low protein diet (**Figure 3**). In the recovery phase, average weight gain of PD-AP piglets was 2.5 times more than PD-PD ($p = 0.0002$) and PD-NAC piglets ($p = 0.0003$) (**Table 11**). Moreover, the rate of weight gain tripled in the recovery phase compared to the active colitis phase for the PD-AP piglets ($p < 0.0001$) but not for the piglets on the low protein diet (**Table 9**). Overall, PD-AP piglets gained more than 40% of their initial weight, whereas both PD-PD and PD-NAC gained 25% of their initial weights (**Table 12**). Snout-to-rump and chest circumference were not different among groups at any time during the study (**Table 13**). Piglets overall gained 13 to 18% of their initial length, and about 8% in chest circumference. The PD-ACT group (control) had more snout-to-rump growth during the active colitis phase than PD-PD group ($p = 0.017$). In addition, snout-to-rump growth rate (cm/day) during the

recovery phase almost doubled compared to active colitis phase in all groups (PD-PD: p = 0.0066; PD-AP: p = 0.0481; PD-NAC: p = 0.0049).

7.3 Body Composition

All DXA scans were successfully performed. Body composition was determined with average CV values of 2%, 12%, 1% and 0.2% for bone mass, fat mass, lean mass and total mass, respectively. Total mass by DXA was 129 ± 46g higher than scale weight; however it was not statistically different. Initial body composition of piglets was 93.5% lean, 4.5% fat and 2% bone, with no significant changes throughout the study (**Figure 4**). In the active colitis phase, piglets gained on average 402g lean fat, 157g fat and 5g bone mass, despite receiving low a protein diet (**Table 10**). In the recovery phase, lean mass gain in PD-AP piglets was 2 times more than PD-PD piglets (p = 0.0019), and 3 times more than PD-NAC (p = 0.0022) (**Table 12**). In the recovery phase, lean to fat gain ratio was 3:1 in PD-PD, 41:1 in PD-AP, and 6:1 in PD-NAC piglets (**Figure 5**). Compared to the active colitis phase, the recovery phase lean to fat gain ratio increased 14 times in PD-AP, 2 times in PD-NAC, and did not change in PD-PD piglets. Overall, PD-AP piglets had greater lean mass than piglets on low protein diet, which attributed to the highest total mass among groups (**Table 10 & 12**).

7.4 Nitrogen Balance

Diet intake and urinary output were recorded and collected without losses. Due to the strong adhesiveness of dried diarrhea of piglets, as well as presence of vomit in some piglets, fecal collection in the recovery phase was difficult; some estimations were used. Nitrogen corresponded with the calculated value. The NAC supplement in the PD-NAC group contributed 20% of the nitrogen intake. Nitrogen balance was positive in all piglets, and retention was 74% of intake in the the active colitis phase with no differences among groups (**Table 14**). In the recovery phase, nitrogen intake was the highest in AP diet, followed by

NAC, then the PD diet, as hypothesized. Despite higher urinary nitrogen excretion, nitrogen balance in the recovery phase was higher in PD-AP piglets compared to other piglets ($p < 0.0001$). Due to diet changes in the recovery phase, urinary and fecal nitrogen were higher in PD-AP and PD-NAC groups when compared to the active colitis phase. Nitrogen balance, in PD-AP piglets during the recovery phase, increased 6 times, compared to the active colitis phase. No changes were observed in other groups. Nitrogen retention decreased significantly in the PD-NAC group, during the recovery phase ($p = 0.0366$), whereas other groups remained constant. Nitrogen balance results were converted to lean mass using a factor of 31.9g nitrogen per 1kg of lean mass. This was completed to verify DXA lean mass gain results.[127] Average lean mass gain calculated from nitrogen balance was 7g/(kg·day) for all groups during the active colitis phase. However, this lean mass gain was half of the value obtained by DXA. Moreover, average lean mass gain during the recovery phase, calculated from nitrogen balance, was 7 g/(kg·day) for PD-PD, 42 g/(kg·day) for PD-AP, and 8 g/(kg·day) for PD-NAC piglets. For both PD-PD and PD-NAC, DXA results were two times higher than the value obtained from nitrogen balance. ; For the PD-AP group, the DXA result was 10% less than the result calculated from nitrogen balance.

7.5 Histology Assessment

Tissue sampling was successfully performed in all piglets. The scores of most individual characteristics were low and not different among groups. PD-ACT (control) piglets scored the lowest Total and Injury scores in both regions of the colon at the end of the active colitis phase. Contrary to our hypothesis, scores in the PD-AP piglets were highest, and scores in both PD-PD and PD-NAC piglets were intermediate in distal colon regions (**Table 15**). There was no difference in scores between the spiral and the distal colon, except for PD-AP group. PD-AP piglets had greater scores in the distal colon than in spiral colon ($p = 0.0360$).

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC Ψ
Day 1					
	Scale Weight, *kg*	3.309 ± 0.174	3.406 ± 0.197	3.326 ± 0.125	3.132 ± 0.193
	DXA Weight, *kg*	3.411 ± 0.166	3.546 ± 0.214	3.437 ± 0.136	3.243 ± 0.181
	Bone Mass, *g*	61 ± 3	63 ± 5	60 ± 3	60 ± 5
	Fat Mass, *g*	188 ± 24	156 ± 8	160 ± 19	125 ± 6
	Lean Mas, *g*	3162 ± 150	3327 ± 208	3217 ± 122	3058 ± 173
Day 9					
	Scale Weight, *kg*	3.743 ± 0.266	3.934 ± 0.358	3.934 ± 0.171	3.575 ± 0.280
	DXA Weight, *kg*	3.898 ± 0.283	4.083 ± 0.352	4.082 ± 0.169	3.727 ± 0.295
	Bone Mass, *g*	68 ± 3	66 ± 6	65 ± 2	65 ± 5
	Fat Mass, *g*	316 ± 36	316 ± 52	354 ± 44	255 ± 34
	Lean Mass, *g*	3514 ± 256	3701 ± 317	3663 ± 129	3407 ± 270
Day 14					
	Scale Weight, *kg*		4.293 ± 0.399^{ab}	4.773 ± 0.110^{a}	3.875 ± 0.274^{b}
	DXA Weight, *kg*		4.505 ± 0.428^{ab}	4.959 ± 0.132^{a}	4.055 ± 0.298^{b}
	Bone Mass, *g*		71 ± 9	66 ± 3	66 ± 3
	Fat Mass, *g*		418 ± 97	375 ± 51	303 ± 77
	Lean Mass, *g*		4016 ± 367^{ab}	4518 ± 136^{a}	3686 ± 278^{b}

Table 9: Study Day 1, 9 and 14 weights of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean + SEM, n = 4 per group; ϕ PD-AP, n = 5; Ψ PD-NAC, n = 3. Means in the same row without a common superscript differ, $p<0.05$.

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC Ψ
Weight Gains					
Day 1 to 9					
	Scale Weight, *kg*	494 ± 119	581 ± 161	608 ± 135	443 ± 169
	DXA Weight, *kg*	536 ± 133	591 ± 142	645 ± 124	483 ± 193
	Bone Mass, *g*	6 ± 3	4 ± 1	5 ± 2	4 ± 3
	Fat Mass, *g*	147 ± 19	158 ± 42	193 ± 36	130 ± 39
	Lean Mass, *g*	383 ± 116	429 ± 101	446 ± 94	349 ± 158
Day 9 to 14					
	Scale Weight, *kg*		359 ± 95^{a}	839 ± 92^{b}	300 ± 91^{a}
	DXA Weight, *kg*		423 ± 101^{a}	877 ± 95^{b}	328 ± 89^{a}
	Bone Mass, *g*		5 ± 3	1 ± 1	1 ± 2
	Fat Mass, *g*		102 ± 60	21 ± 43	48 ± 44
	Lean Mass, *g*		316 ± 118^{a}	855 ± 97^{b}	279 ± 132^{a}
Day 1 to 14					
	Scale Weight, *kg*		940 ± 182^{a}	1447 ± 86^{b}	743 ± 107^{a}
	DXA Weight, *kg*		1013 ± 191^{a}	1522 ± 80^{b}	811 ± 148^{a}
	Bone Mass, *g*		9 ± 2	6 ± 2	6 ± 3
	Fat Mass, *g*		259 ± 87	214 ± 47	178 ± 82
	Lean Mass, *g*		744 ± 147^{a}	1301 ± 59^{b}	628 ± 107^{a}

Table 10: Study Day 1 to 9, Day 9 to 14 and Day 1 to 14 weight gains of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean + SEM, n = 4 per group; ϕ PD-AP, n = 5; Ψ PD-NAC, n = 3. Means in the same row without a common superscript differ, $p<0.05$.

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC Ψ
Day 1 to 10	Average Weight Gain, g/(*kg·day)*	18 ± 4	17 ± 3	18 ± 2	14 ± 3
Day 10 to 15	Average Weight Gain, g/(*kg·day)*		21 ± 6[a]	53 ± 5[b]	20 ± 2[a]
Day 1 to 15	Average Weight Gain, g/(*kg·day)*		19 ± 2[a]	31 ± 2[b]	17 ± 2[a]

Table 11: Study Day 1 to 10, Day 10 to 15 and total average weight gains of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean + SEM, n = 4 per group; ϕ PD-AP, n = 5; Ψ PD-NAC, n = 3. Means in the same row without a common superscript differ, $p<0.05$.

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC Ψ
Mass Gains, %					
Day 1 to 9					
	Scale Weight Gain, %	13 ± 3	15 ± 4	18 ± 4	14 ± 5
	DXA Weight Gain, %	16 ± 4	17 ± 3	19 ± 4	15 ± 6
	Bone Mass Gain, %	9 ± 5	6 ± 2	9 ± 3	7 ± 5
	Fat Mass Gain, %	78 ± 8	101 ± 18	121 ± 25	104 ± 35
	Lean Mass Gain, %	12 ± 3	13 ± 3	14 ± 3	11 ± 5
Day 9 to 14					
	Scale Weight Gain, %		9 ± 2^{a}	21 ± 3^{b}	8 ± 3^{a}
	DXA Weight Gain, %		10 ± 2^{a}	21 ± 3^{b}	9 ± 2^{a}
	Bone Mass Gain, %		8 ± 4	1 ± 2	2 ± 3
	Fat Mass Gain, %		32 ± 19	6 ± 15	19 ± 15
	Lean Mass Gain, %		9 ± 3^{a}	23 ± 3^{b}	8 ± 4^{a}
Day 1 to 14					
	Scale Weight Gain, %		26 ± 4^{a}	44 ± 4^{b}	24 ± 3^{a}
	DXA Weight Gain, %		29 ± 4^{a}	44 ± 3^{b}	25 ± 4^{a}
	Bone Mass Gain, %		15 ± 3	10 ± 3	9 ± 6
	Fat Mass Gain, %		166 ± 45	134 ± 34	143 ± 71
	Lean Mass Gain, %		22 ± 3^{a}	40 ± 2^{b}	21 ± 3^{a}

Table 12: Study Day 1 to 9, Day 9 to 14 and Day 1 to 14 % weight gains of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean + SEM, n = 4 per group; ϕ PD-AP, n = 5; Ψ PD-NAC, n = 3. Means in the same row without a common superscript differ, $p<0.05$.

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC
Day	***Snout-to-rump***				
1	Length, *cm*	45 ± 1	46 ± 0	46 ± 1	47 ± 1
9	Length, *cm*	50 ± 1	48 ± 1	49 ± 0	49 ± 2
14	Length, *cm*		51 ± 1	53 ± 0	53 ± 1
1 to 9	Gain, *cm*	5 ± 1[a]	2 ± 1[b]	4 ± 1[ab]	3 ± 1[ab]
9 to 14	Gain, *cm*		4 ± 1	4 ± 0	4 ± 1
1 to 14	Gain, *cm*		5 ± 1	7 ± 0	7 ± 1
1 to 9	Gain, *%*	11 ± 2[a]	4 ± 2[b]	8 ± 2[ab]	5 ± 2[ab]
9 to 14	Gain, *%*		7 ± 1	8 ± 1	8 ± 2
1 to 14	Gain, *%*		11 ± 2	16 ± 1	14 ± 1

Table 13: Snout-to-rump length of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean ± SEM, n = 5 per group; ϕ PD-AP, n = 6. Means in the same row without a common superscript differ, $p<0.05$.

		PD-ACT	PD-PD	PD-AP ϕ	PD-NAC Ψ
Nitrogen Profile					
Day 8 & 9					
	N Intake, g/(*kg·day)*	0.305 ± 0.006	0.305 ± 0.004	0.311 ± 0.007	0.314 ± 0.005
	Fecal N Output, g/(*kg·day)*	0.007 ± 0.003	0.009 ± 0.003	0.010 ± 0.003	0.006 ± 0.001
	Urinary N Output, g/(*kg·day)*	0.068 ± 0.005	0.068 ± 0.006	0.095 ± 0.030	0.055 ± 0.004
	Nitrogen Balance, g/(*kg·day)*	0.230 ± 0.011	0.228 ± 0.004	0.206 ± 0.038	0.253 ± 0.001
	Nitrogen Retention, *%*	75 ± 2	75 ± 2	65 ± 12	81 ± 1
Day 13 & 14					
	N Intake, g/(*kg·day)*		0.308 ± 0.015^{a}	1.964 ± 0.090^{c}	0.402 ± 0.006^{b}
	Fecal N Output, g/(*kg·day)*		0.023 ± 0.013^{a}	0.067 ± 0.023^{b}	0.006 ± 0.001^{a}
	Urinary N Output, g/(*kg·day)*		0.060 ± 0.013^{a}	0.560 ± 0.106^{c}	0.139 ± 0.016^{b}
	Nitrogen Balance, g/(*kg·day)*		0.225 ± 0.034^{a}	1.338 ± 0.171^{b}	0.257 ± 0.014^{a}
	Nitrogen Retention, *%*		72 ± 9	67 ± 6	64 ± 4

Table 14: Nitrogen balance, intake and excretion of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean + SEM, n = 4 per group; ϕ PD-AP, n = 5; Ψ PD-NAC, n = 3. Means in the same row without a common superscript differ, $p<0.05$.

	PD-ACT	PD-PD	PD-AP ϕ	PD-NAC
Spiral Colon				
Inflammatory Cell Infiltration	1 ± 0.2	2 ± 0.5	2 ± 0.3	1 ± 0.2
Inflammatory Cell Localization	2 ± 0.2	2 ± 0.2	2 ± 0.0	2 ± 0.2
Inflammation Score	2 ± 0.4	3 ± 1.1	3 ± 0.6	2 ± 0.5
Mucosal Edema	0 ± 0.2	0 ± 0.2	0 ± 0.2	0 ± 0.2
Submucosal Edema	1 ± 0.3	1 ± 0.3	1 ± 0.4	1 ± 0.4
Surface Epithelial Loss	0 ± 0.0	1 ± 0.7	1 ± 0.5	0 ± 0.2
Gland Mucus Dilation	0 ± 0.4	0 ± 0.2	1 ± 0.5	0 ± 0.2
Increased Goblet Cell Number/Size	1 ± 0.2	0 ± 0.2	0 ± 0.2	2 ± 0.5
Crypt Abscesses / Herniation	1 ± 0.2	2 ± 0.3	2 ± 0.3	1 ± 0.3
Goblet Cell / Enterocyte Ratio Decrease	0 ± 0.4	0 ± 0.0	1 ± 0.5	0 ± 0.0
Gland Loss	0 ± 0.0	1 ± 0.7	1 ± 0.5	1 ± 0.4
Mucosal hyperplasia	0 ± 0.2	0 ± 0.2	1 ± 0.4	0 ± 0.4
Total Score (0-52)	6 ± 1.0^{a}	9 ± 3.0^{ab}	10 ± 2.5^{b}	7 ± 1.9^{a}
Injury Score (0-24)	2 ± 0.4^{a}	5 ± 2.5^{ab}	5 ± 1.2^{b}	3 ± 1.0^{ab}
Distal Colon				
Inflammatory Cell Infiltration	2 ± 0.3	2 ± 0.4	3 ± 0.4	2 ± 0.5
Inflammatory Cell Localization	2 ± 0.2	2 ± 0.0	2 ± 0.0	2 ± 0.0
Inflammation Score	3 ± 0.9	4 ± 0.8	5 ± 0.8	5 ± 0.9
Mucosal Edema	1 ± 0.4	1 ± 0.5	1 ± 0.3	1 ± 0.4
Submucosal Edema	1 ± 0.2	1 ± 0.2	0 ± 0.2	1 ± 0.2
Surface Epithelial Loss	0 ± 0.4	1 ± 0.5	2± 0.6	1 ± 0.6
Gland Mucus Dilation	0 ± 0.4	0 ± 0.0	1 ± 0.3	1 ± 0.4
Increased Goblet Cell Number/Size	0 ± 0.2	1 ± 0.4	0 ± 0.2	1 ± 0.5
Crypt Abscesses / Herniation	1 ± 0.2	2 ± 0.2	2 ± 0.4	1 ± 0.3
Goblet Cell / Enterocyte Ratio Decrease	0 ± 0.2	2 ± 0.6	2 ± 0.5	1 ± 0.7
Gland Loss	1 ± 0.4	2 ± 0.7	2 ± 0.5	2 ± 0.5
Mucosal hyperplasia	0 ± 0.4	1 ± 0.5	2 ± 0.7	1 ± 0.4
Total Score (0-52)	8 ± 2.5^{a}	13 ± 3.4^{ab}	17 ± 2.2^{b}	14 ± 2.9^{ab}
Injury Score (0-24)	4 ± 1.5^{a}	7 ± 2.0^{ab}	9 ± 1.8^{b}	7 ± 1.9^{ab}

Table 15: Histopathological colitis scores of piglets with DSS induced colitis consuming a protein deficient diet from study Day 1 to Day 10 (PD-ACT), and then either continue PD diet (PD-PD), or consuming adequate protein diet (PD-AP), or continue PD diet but supplemented with N-acetylcysteine (PD-NAC) from Day 10 to Day 15. Values are mean ± SEM, n = 5 per group; ϕ PD-AP, n = 6. Means in the same row without a common superscript differ, $p<0.05$.

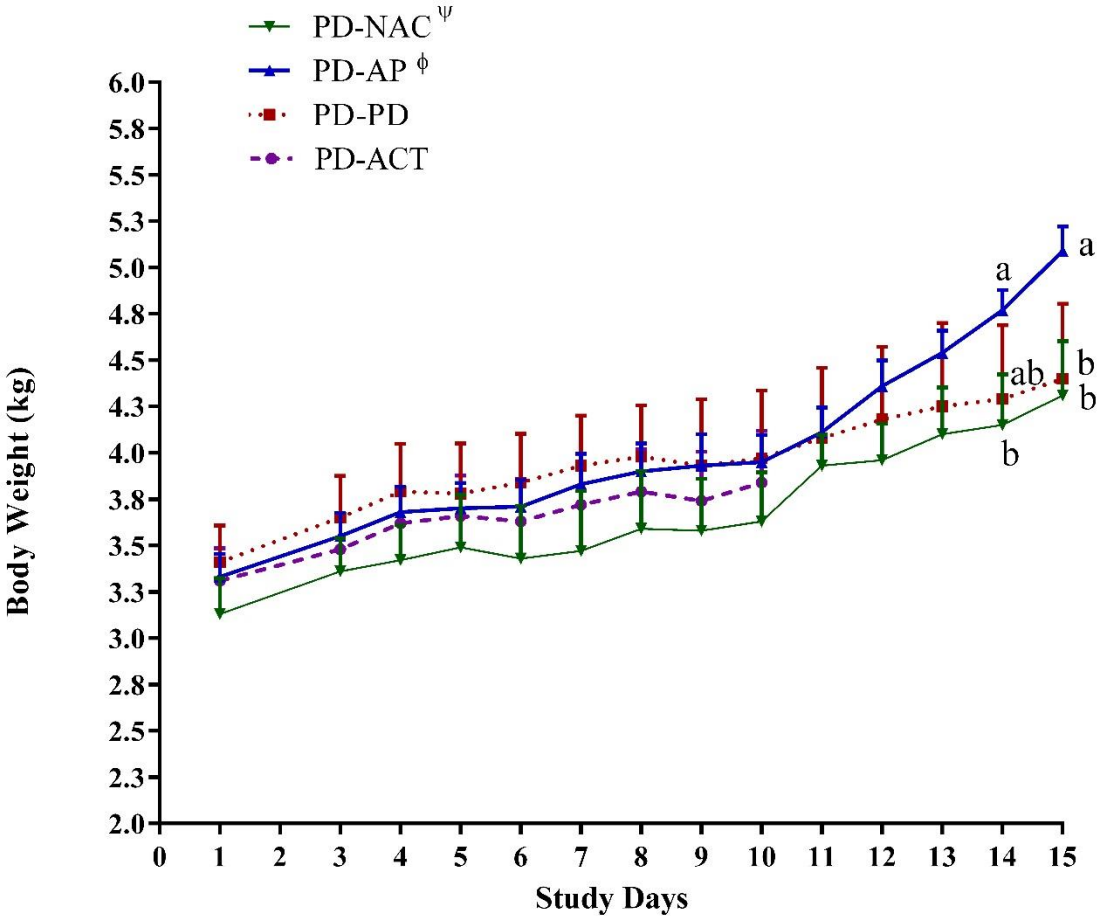


Figure 3: Daily weight of piglets with DSS induced colitis consuming a protein deficient diet from study day 1 to day 10 (PD-ACT), and then either continue on PD diet (PD-PD), or switching to an adequate protein diet (PD-AP), or continue on PD diet but supplemented with N-acetylcysteine (PD-NAC) from day 10 to day 15. Values are mean + SEM, n = 4 per group; $^{\phi}$ PD-AP, n = 5; $^{\psi}$ PD-NAC, n = 3. Means without a common superscript differ, $p<0.05$.

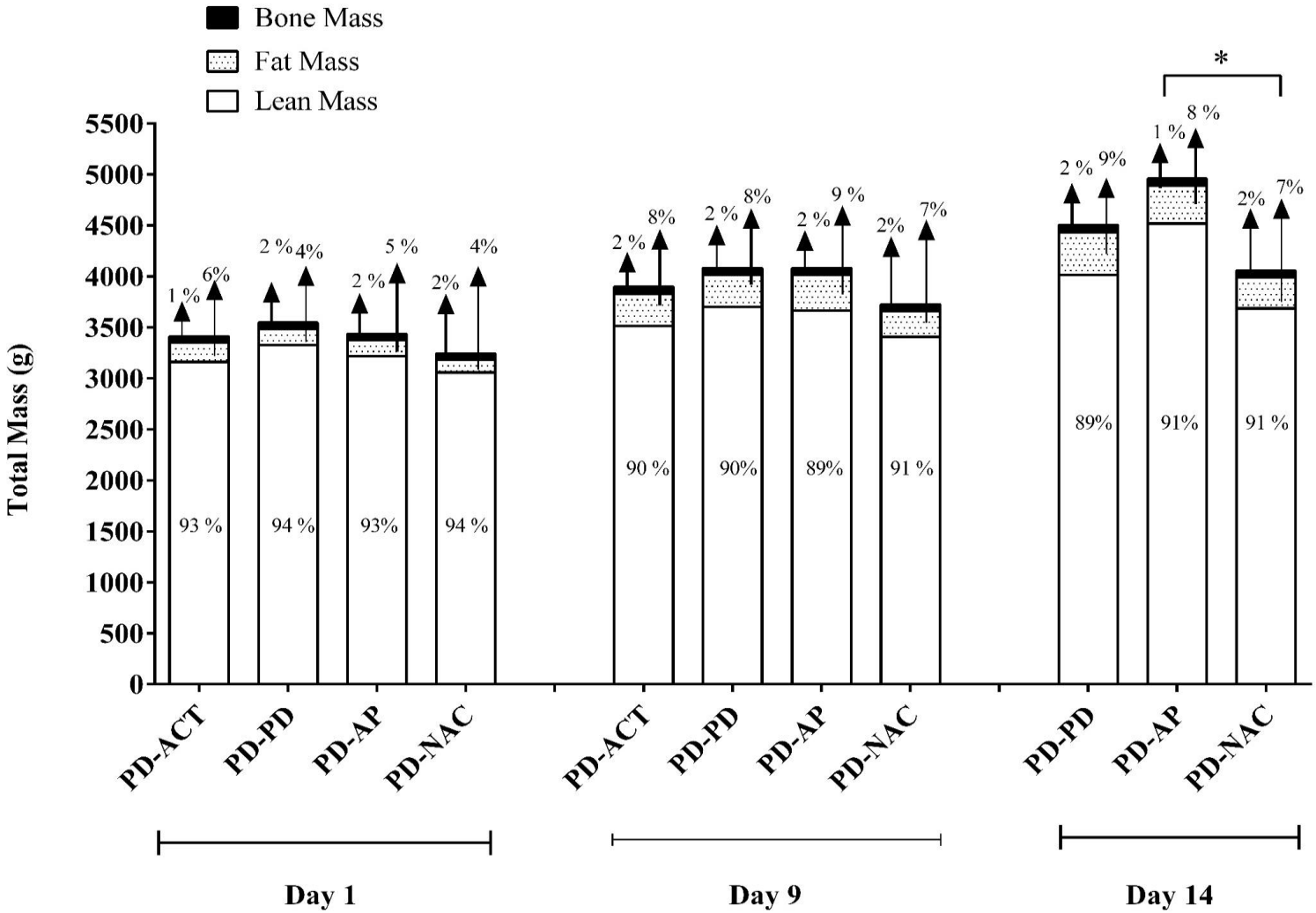


Figure 4: Study day 1, 9 and 14 total mass, and body composition of pigelts with DSS induced colitis consuming a protein deficient diet from study day 1 to day 10 (PD-ACT), and then either continue on PD diet (PD-PD), or switching to an adequate protein diet (PD-AP), or continue on PD diet but supplemented with N-acetylcysteine (PD-NAC) from day 10 to day 15. * indicates total masses are statistical significantly different, $p<0.05$.

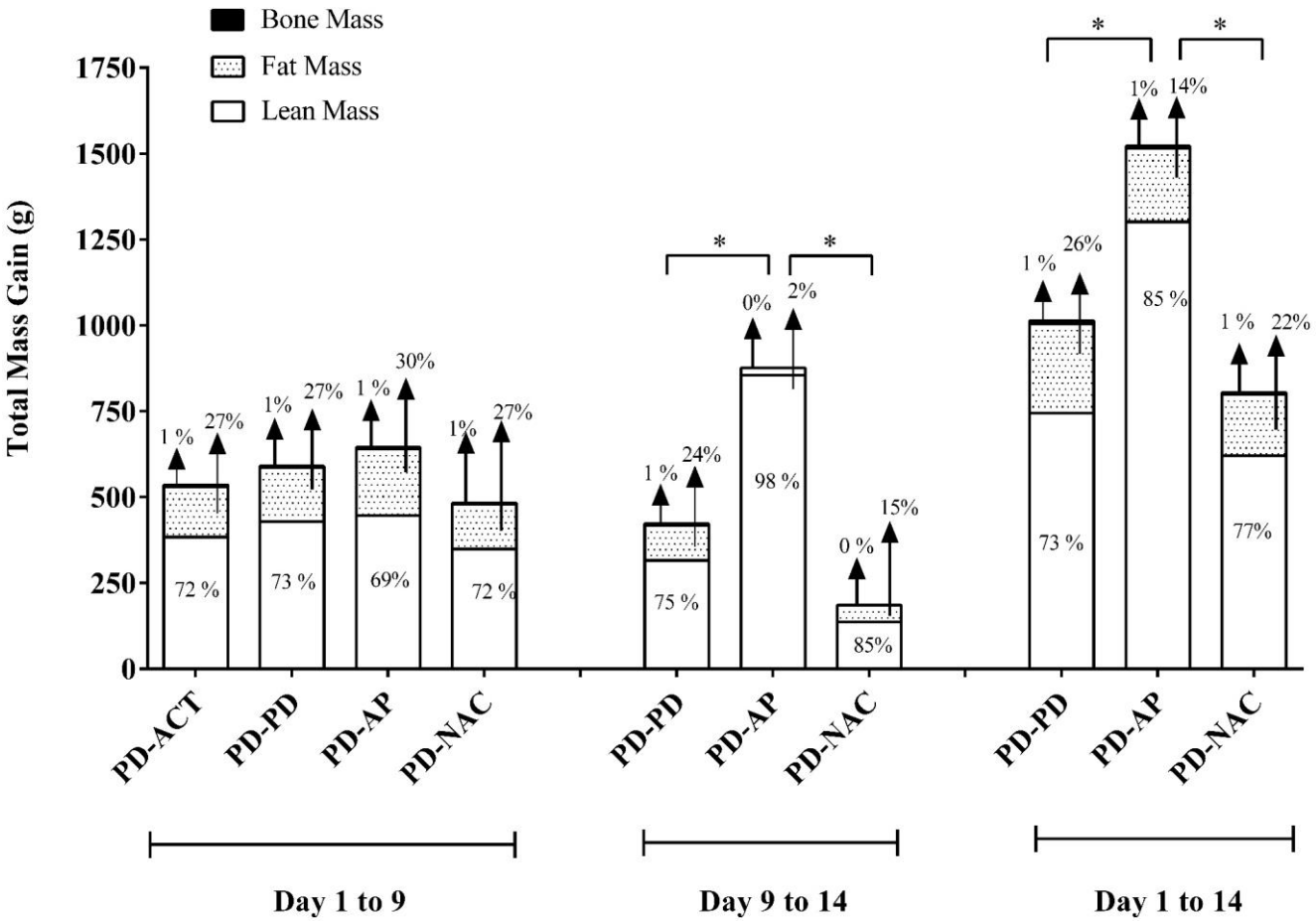


Figure 5: Study day 1 to 9, day 9 to day 14, and net mass gain of pigelts with DSS induced colitis consuming a protein deficient diet from study day 1 to day 10 (PD-ACT), and then either continue on PD diet (PD-PD), or switching to an adequate protein diet (PD-AP), or continue on PD diet but supplemented with N-acetylcysteine (PD-NAC) from day 10 to day 15. * indicates total mass gains are statistical significantly different, $p<0.05$.

8. DISCUSSION

8.1 General

The rapid growth potential of young piglets and sensitivity to dietary interventions makes them useful as a model for the growing child with IBD to study the interaction of malnutrition and the inflammation of colitis. Our previous studies, in this piglet model of DSS-induced colitis, focused on the active phase when reduced food intake in children is common. The early phase of recovery, from a flare-up presents an opportunity for nutritional intervention clinically. We aimed to study this early period for the first time using this piglet model. The first specific aim of the study was to characterize a recovery model of colitis. Our goal was to characterize growth and intestinal pathology in protein deficient piglets while receiving DSS and again 5 days after discontinuing DSS. Despite the co-existence of severe protein deficiency and inflammation, piglets continued to grow throughout the active phase albeit slowly, with a positive nitrogen balance and accretion of lean mass evident by DXA. During the five days after discontinuing DSS, the rate of linear growth was faster and the rate of weight gain tended to be greater than during the DSS phase, however neither nitrogen balance nor the accretion of lean mass were different. Surprisingly, scores of histology in both sections of the colon revealed more severe damage. The second goal was to examine the impact of two nutritional interventions in the recovery phase. The anabolic intervention of increasing protein intake resulted in the greatest rate of weight gain, lean mass gain nitrogen balance compared to other recovery groups. Surprisingly, despite achieving the greatest growth, histology results of the piglets receiving adequate protein diets were the most severe among all groups. The anti-inflammatory intervention of supplementing NAC with the low protein diet in the recovery phase, did not have an impact on growth parameters or on disease severity compared to piglets with active colitis. In summary, the nutritional interventions affected growth parameters as expected, but the histological scores revealed unexpected and intriguing results.

8.2 Active Colitis Phase

During active colitis, children often experience abdominal pain and low appetite, which can lead to decreased intake and malnutrition. [1] Co-existence of malnutrition and inflammation further increase the disease severity and cause growth failure in children with colitis. [1] Although in recent years the cases of malnutrition in children diagnosed with colitis have been low, a problem with late diagnosis still exists, and growth failure can occur even before diagnosis. [58] Due to the difficulties in studying children directly, a piglet model is an appropriate model to study children given their physiological and metabolic similarities. [111, 112] A piglet model also provided flexibility in limiting nutrient intakes. Severe protein deficiency was used in our study, as it was shown to have great impact on growth in previous studies. [95, 126] An active colitis with protein deficiency resembles a case of late or undiagnosed IBD where protein accretion is low. This protein deficient group of piglets served as a control and initial state for the recovery phase, in order to characterize growth under different scenarios. In general, piglets successfully developed colitis and severe protein deficiency by the end of the active phase. Greater lean mass gain and a higher nitrogen balance was seen as compared to previous work, but the same rate of average weight gain and low histology scores relative to previous studies indicated a mild degree of colitis was achieved at the end of the active phase. [126] A mild colitis was designed in the present study to create a smoother transition into the recovery phase.

8.3 Recovery Phase

In children with IBD, meeting nutrient requirements, especially energy and protein, are important due to the high metabolic demands for growth and inflammation. [65] Low protein intake will limit growth and lean mass accretion as more protein will be used to make positive acute phase proteins to participate in immune responses trigged by inflammation. [9]

The remitting nature of IBD is associated with less metabolic catabolism, and is a potential period to promote optimal growth and recovery. The ideal situation is to achieve adequate nutrition, which in our study is presented by PD-AP group. However, it is often difficult to meet optimal nutrient intake in children due to IBD-associated complications mentioned above. NAC was administered to the piglets to examine its effect on reducing inflammation by increasing GSH synthesis and ultimately lead to improved disease severity despite protein deficiency.

Anthropometry and body composition results indicated significant growth in AP piglets in the recovery phase but only a trend for more rapid growth of piglets continuing on the low protein diet (PD diet: $P = 0.5443$; PD diet with NAC: $P = 0.4259$). This trend could have been significant if the sample size had been larger. By using the standard deviation of the average rate of weight gain in the recovery phase, a minimum sample size of 16 piglets per group is needed to reduce within group variance and obtain a significant difference of average rate of weight gain between the two phases. [141] Moreover, although there was a slight increase in anthropometrical growth, unchanged nitrogen balance in the recovery phase, suggested that inflammation-related metabolic demand did not decrease in PD recovery piglets. This was further confirmed by histology results, where recovery PD piglets scored higher than active control PD piglets. This unexpected outcome was consistent across all recovery groups, and its causes were uncertain. The design of our recovery phase was based on the length of recovery and turnover rate of colonic mucosa and small intestine epithelial in healthy pigs, as well as a successful recovery in DSS-induced colitis piglets demonstrated in a previous study. [89, 130] In this recovery study by Kim et al., colitis was induced in piglets with 5 days of DSS administration, followed by 5 days of discontinuing DSS as the recovery phase. [89] During the recovery phase piglets were supplemented with L-cysteine. [89]L-cysteine supplementation in DSS-induced colitis piglets was able to achieve total histology scores similar to healthy animals, as well as attenuated weight loss, and reduced expressions of few pro-inflammatory cytokines. [89] In addition, adequate

nutrition was provided to piglets. [89] Given that piglets in Kim's study were not protein deficient, it suggested that perhaps protein deficiency and low protein accretion made tissue repair difficult or was inefficient. The possible delayed DSS adminstration could have also led to more severe histology scores after 5 days of recovery in the present study. Furthermore, in the DSS-induced (5 days) colitis mice model, BALB/c mice resolved colitis after 4 weeks of recovery period (no DSS), while C57BL/6 mice developed chronic colitis. [142] In addition, 1 week into recovery, inflammation scores, histopathological changes and macrophage infiltrations remained high in both strains of mice. [142] Therefore, DSS might have different effects on different strain of piglets, which led to unexpected chronic colitis even after discontinuing DSS. The current study used a new strain of piglets (Naima), whereas previous studies used Yorkshire piglets. Another cause of the unexpected histology results could be attributed to the short duration of the recovery phase. Given all recovery piglets were protein deficient at the start, 5 days of recovery may not have be enough for intestine epithelial turnover to occur, as 3 to 8 days is required in healthy pigs. [10, 130]

8.4 N-Acetylcysteine

NAC, a derivative of cysteine, has been used clinically to treat various diseases. [102] In this study, NAC was given to increase synthesis of GSH as an anti-inflammatory strategy to reduce disease severity. Due to severe body weight shifts and unseen weight loss in 2 piglets receiving NAC in the recovery phase, true body composition and weights were difficult to determine. With a small sample size of 5 piglets, including data from these 2 piglets drastically affected the means of variables in the NAC group; therefore, they were not included in data analysis. A general pattern of excessive fluid loss was observed approximately 1 day after introduction of NAC. This was an interesting finding because it suggested that NAC caused fluid excretion for a possible role in fluid retention clearance. Due to severe protein deficiency and high volume intake (~230ml/(kg·day)) during the active phase (9 days), fluid retention or edema was possible. In addition, puffy appearances were

recorded in most piglets at the beginning of the recovery phase. Subsequently, NAC has been shown to be effective in resolving edema in severely malnourished children. [104] In Wistar rats, NAC was also shown to prevent edema via upregulating Na-K-Cl co-transporter (NKCC1) expression, which is involved in transporting sodium in and out of the cell and is important for maintaining cell volume. [143] Hence, the excessive fluid loss after the NAC treatment may be a sign of resolution of edema, however, it complicates assessment of growth. Weight gain, nitrogen balance and assessment of lean mass by DXA all rely on consistent hydration status. [124]

In general, NAC supplementation did not improve disease severity or growth. The NAC dose used was based on a pediatric study by Jahoor et al.[104] A 3g protein per kg body weight per day was used to promote rapid growth recovery in severe malnourished edematous children, where the protein intake was 2.5 times the normal recommendation. [104] This factor of 2.5 was extrapolated to formulate the dosage of NAC supplement for piglets. The cysteine supplementation was used for recovery and anti-inflammatory purposes; hence protein and cysteine levels that promote rapid growth and recovery described in Jahoor's study was used. Therefore, a total cysteine intake (including the amount of cysteine supplied in the diet) was calculated by multiplying the normal piglet cysteine diet recommendation amount by a factor of 2.5. Lastly, the total cysteine supplementation intake was used to calculate its equivalent in NAC. Although the final NAC dosage was high, it was under the toxic level. By supplementing NAC, the nitrogen intake of the piglets was increased by 30%. Nitrogen balance did not change in the recovery phase despite the increase in dietary nitrogen. Most of the extra nitrogen intake in recovery was balanced by increased urinary nitrogen output. Urinary nitrogen of NAC piglets was more than doubled that of the unsupplemented piglets. This suggested that up to 84% of the infused NAC was excreted in the urine, indicating that NAC was not effectively metabolized. No studies of piglets being treated with NAC were found and therefore the possible physiological reason of the high percentage of NAC excreted is not known. However, in mice models, high doses (>150mg/(kg·day)) of NAC

were associated with positive histology and growth outcomes in most studies. [103, 107, 108] However, in a piglet study done by Hou et al. [144] moderate PD (50% NRC protein requirement) piglets were able to retain 31% of sulfur from administered sulfur amino acids as non-protein forms such as GSH, which was greater than AP piglets. [144] This suggested that the high urinary nitrogen excretion might not be all NAC, since NAC was more likely to be metabolized and stored as GSH. Nevertheless, in order to verify the amount of NAC excreted, urinary NAC should be measured in the future. NAC was given as an anti-inflammatory strategy to increase GSH synthesis and lead to improved disease severity in the present study. However, possibilities of inadequate glutamate and/or glycine accretion, due to protein deficiency, could lead to low GSH synthesis; despite adequate availability of cysteine given that all three amino acids are needed for GSH synthesis. [101] Hence, the constant low GSH synthesis rate led to the unimproved disease severity in the recovery phase. As a result, tissue GSH synthesis should be measured in the future to indicate whether NAC was effective in increasing GSH synthesis.

8.5 Adequate Protein

Similar to previous studies, adequate protein was able to achieve greater weight gain, but also greater lean mass gain than an isoenergetic low protein diet, which emphasized the importance of protein in growth and recovery. By providing adequate protein with discontinuing DSS administration, growth was quickly adjusted. Rate of growth almost tripled in just 5 days. Approximately 98% of the weight gain was lean tissue, whereas fat gain contribution was higher in piglets staying on the low protein diet. Furthermore, comparing the average weight gain of AP piglets during the recovery phase to the average weight gain of the well-nourished piglets with similar initial body weights, age, DSS dose, as well as protein and energy intake from previous study, the impact of the AP diet on recovery can be characterized. The main differences between well-nourished piglets from previous study and the present AP piglets, are that the well-nourished piglets were not protein deficient at the

beginning and DSS was given throughout the whole study, whereas the AP piglets in the present study were protein deficient at the start and DSS was discontinued at the introduction of AP diet. [126] In addition, AP piglets had a 2-day adaptation period where the AP diet was gradually introduced. Despite the absence of DSS, AP piglets had lower average rate of weight gain than the well-nourished piglets. [126] This might be because the period of receiving adequate protein was shorter in the AP piglets. Another possible reason for the lower average rate of weight gain could explained by the pre-existing protein deficiency of AP piglets. AP piglets experienced lengthened the tissue repair and displayed slower growth compared to well-nourished piglets. Therefore, early diagnosis and nutrition intervention are crucial in promoting optimal growth in pediatric IBD. Moreover, although greater growth was achieved with AP diet, percentage of nitrogen retention remained constant throughout the present study. This suggested that metabolic demand remained high during the recovery phase in AP piglets, which was also confirmed by the unexpected high histology scores.

8.6 Inflammation

In the study, inflammation was induced by 6.5 days of DSS administration. Severity of colitis was assessed by a veterinarian pathologist blinded to group assignment using a grading scale designed by the pathologist specifically for colitis. Low histology scores at the end of the active phase indicated mild colitis induction. Low inflammation was designed to create a smoother transition into recovery phase; however, it appeared to be too low indicated by the histology results. By comparing to a previous study, there was only slight difference (0.1g/(kg·day) in DSS dosage, but nitrogen balance and body composition results indicated greater growth and less severe colitis in piglets of the current study. [126] However, the length of DSS exposure was longer in the previous study, and the strain of piglets used was different. [126] Perhaps a higher dose of DSS was needed to create the desired level of inflammation in the current strain of piglets. Moreover, unexpected histology results at the end of the recovery phase were obtained as colon tissues of AP piglets were more severely

damaged than the active control piglets despite absence of DSS. Also, all recovery piglets had more severe diseased colonic tissues than the active control piglets. A possible reason could be residuals of DSS inside the piglets, which caused delayed and prolonged inflammation. DSS-induced colitis involves the high levels of swollen macrophages in the colonic wall, causing inflammation by upregulating pro-inflammatory cytokines, chemokines and nitric oxide synthase. [10, 114] Macrophage engulfs DSS via phagocytosis, and perhaps macrophages were unable to digest DSS and exocytosis of DSS did not occur, which led to more DSS remaining in the piglets to cause inflammation even after DSS administration was discontinued. [114] In a mouse model of DSS-induced colitis over 5 days, the level of macrophages was still elevated one week into recovery phase, but colitis was resolved after a 4 week of recovery period. [142] Although piglets have a fast growth rate, with the pre-existing protein deficiency, 5 days of recovery period may not have been enough to see a positive change in disease severity. Nevertheless, cytokine activities should be measured in future studies to acquire further information about level of colitis related inflammation in piglets. A strong distinct strong smell was noticed in pilot piglets that were given the AP diet during the recovery phase, which was undocumented in previous studies. AP piglets' highest histology scores led to the concern of intolerance towards both AP diet and diet change. Results from pilot piglet studies indicated that the strong distinct smell of fecal matter can occur 1 to 2 days after full feeding rate was reached, regardless the time of introduction and rate of diet adaptation. Although it was uncertain the cause of the smell, it could be speculated that the action of fermentation and microflora were involved. The effectiveness of DSS and severity of colitis both depend on the colonic microbial environment of animals. [10] Thus, high level of stress and malnutrition might have caused the commonly present E. coli identified in some of piglets to act pathogenically. In the recovery phase, where nutrition was provided, it not only provided nutrients for growth but also provided energy for the bacteria, which may have further increased the pathogenic activity of the bacteria and lead to more severe damage in the colon. Another possibility of highest inflammation in AP piglets

could be the higher protein intake supported better pro-inflammatory effects due to protein availability for the positive acute phase response.

8.7 Body Composition

Body composition was measured by DXA. The sensitivity and repeatability were acceptable in all measurements except for fat mass. Large variability in fat mass between repeated scans were obtained. This inaccuracy was present throughout the study with end of active phase measurements being the most variable. Limitations of DXA include hydration status and positioning of subject. [124, 125] Since piglets were anesthetized during scans, positioning was controlled. The primary measurement is lean mass, which depended on hydration status. Fat mass is calculated as the difference between two large components i.e. total and lean mass; therefore, the small compartments of fat are more highly variable and also dependent on variability in hydration status. DXA is not capable of identifying fluid retention, and its logistics could have accounted the extra fluid as either lean mass or fat mass. Nevertheless, body composition analysis was adjusted by minimizing the discrepancy between total mass measured by DXA and scale balance. Lean body mass was the main interest in measuring body composition, as it is a good indicator of growth. Lean body mass could also be extrapolated from nitrogen balance. [127, 128] The lean body mass gain at the end of the active phase obtained from nitrogen balance was half of the lean body mass measured by DXA. In the recovery phase, lean mass gain of piglets remained on the PD diet calculated from nitrogen balance was again half of measured by DXA; however, in AP piglets, the lean mass gain was similar in both methods. This suggested that PD piglets were more likely to have fluid retention than AP piglets, and DXA measurements were less accurate when there is fluid retention. This pattern was also present in a previous study. [126]

9. CONCLUSION

9.1 Summary of Findings

The present thesis includes results that characterize growth and disease severity of protein deficient piglets during recovery from colitis, as well as the separate impacts of an anabolic adequate protein diet and of an anti-inflammatory NAC supplementation on growth and disease severity during recovery. The piglet model used was an appropriate and successful model to study colitis in growing children as positive growth was present during the active colitis phase despite protein deficiency. Body composition, nitrogen balance and histology scores indicated a mild colitis was developed at the end of active colitis phase. In comparison to the active colitis phase, growth during the recovery phase was significantly increased with AP, and a general trend of better growth was also observed in PD piglets with and without NAC supplement. Regarding disease severity, unexpected high histology results during the recovery phase suggest DSS has prolonged inflammatory properties, and longer than 5 days of recovery is needed in piglets with pre-existing protein deficiency. Moreover, NAC was ineffective in achieving significant growth or improving disease severity in PD piglets during recovery. Other studies using NAC supplementation in colitis have reported that NAC has appeared to be effective in promoting better growth and reducing disease severity when adequate nutrition was also present; thus suggesting protein deficiency might lead to low glycine and/or glutamate accretion that is also needed for synthesis of GSH. [89, 101, 103, 104, 107, 108] In general, current results displayed the importance of adequate dietary protein in promoting growth in a protein deficiency and inflammation co-existing situation; hence, early diagnosis and adequate nutrition is key in preventing growth failure in children with IBD.

Anthropometry, body composition and nitrogen balance were used to determine growth in the present study. Current findings revealed DXA's limitation in measuring fat mass and lean mass in piglets with fluid retention; suggesting nitrogen balance is a more appropriate method

to determine lean mass gain in edematous piglets given that collection of nitrogen excretion is accurate.

9.2 Study Limitations

Several limitations of the study should be considered while interpreting the results, and improvements are needed for future studies. First of all, although there is physiological and metabolic similarity between piglets and children, the characteristics of colitis still varies between the two. Since IBD is still idiopathic, and DSS is a toxic agent that induces intestinal inflammation; a development of a gene-disrupted model of spontaneously occurring colitis may be of value in studying colitis in children. The present study's design was limited without the presence of a positive control group; a piglet group without colitis. Without a positive control group histology grading was difficult due to lack of reference. Moreover, non-design related limitations include the pre-existing health condition of the piglets which complicated the variability in responses to stress. Due to transportation, surgery, and new environment, piglets were under high level of stress, which made infections and/or bacterial outbreak easy. McGill CMARC veterinarian indicated that enteropathogenic E. coli is commonly present in pigs, and does not normally cause yellow diarrhea as seen in the present study. In addition, other health problems revealed at necropsy, such as pulmonary infection and edematous kidneys. This health issues indicated potential confounding factors for inflammation. Furthermore, the low dosage of DSS given during active colitis phase, and short period may have complicated interpretation.

9.3 Future Analysis and Research

This thesis focused on characterizing, comparing and contrasting the growth of DSS-induced colitis in piglets using anthropometry, body composition and nitrogen balance. To study protein metabolism in protein deficient DSS-induced colitis piglets in the active colitis phase

and the recovery phase, the rate of protein synthesis in organs and tissues was analyzed. By comparing protein synthesis between the 2 phases, it will help to characterize the impact of recovery in colitis in the future. Whole body protein turnover analysis should also be performed to determine the impact of adequate protein and discontinuing DSS on whole body protein kinetics. In order to better study the recovery of colitis in piglets, a longer recovery period is needed. However, due to the length of catheters and fast growth rate of piglets, any study longer than 15 days is unlikely. Alternatives could involve abandoning isotope infusion studies as infusion catheters are not required, or provide piglets adequate nutrition throughout the study to promote fast recovery within the same period of time.

Although no significant results were found with NAC supplementation, measuring urinary NAC would help identify the fate of NAC in protein deficient colitis piglets. Moreover, by measuring rate of GSH synthesis, the action and metabolism of NAC can be examined. It would be crucial to understand if NAC alone can still provide an anti-inflammatory effect under protein deficient state. There is the possibility of studying the combined effect of adequate nutrition with NAC or providing NAC, glycine and/or glutamate supplement to PD colitis piglets in promoting growth and reduce disease severity.

DSS-induced colonic inflammation was graded and analyzed using histological severity of colon tissues. In order the study the mechanism of inflammation in colitis and the impact of AP as well as NAC on molecular level of inflammation, pro-inflammatory cytokines and mediators should be measured. For example, the elevated pro-inflammatory cytokines in IBD such as IL-1β, TNF- α and IL-6 would disrupt the normal regulation of the GH/IGF-1 axis and sex steroids. [9] In addition, TNF- α induces anorexia and triggers acute phase response and lead to muscle catabolism. [61, 145] In general, pro-inflammatory cytokines are largely responsible for the growth failure and delayed puberty in IBD.

9.4 Implications

This study of colitis in growing but undernourished piglets provided a model of IBD in growing children. This study investigated the recovery phase of colitis, and tested how growth, changes in body composition and nitrogen balance are different in the early the recovery phase compared to the active phase. Malnutrition due to abdominal pain and low appetite decreased food intake is common in children with IBD. In addition, persistent malnutrition in children leads to growth failure. Nutritional therapeutic strategies to either specifically control inflammation (NAC) or to provide an adequate and complete protein diet to target anabolic response were investigated. Results provided basis for nutrition strategies to meet protein requirement in children with IBD will prevent growth failure and promote disease recovery. Moreover, in situations where an adequate protein diet is difficult to achieve,; NAC alone appear to be ineffective at reducing inflammation in the colon. A combination of adequate protein intake in conjunction with an NAC supplement may serve as a possible potent treatment strategy for IBD treatment, providing that both have important roles in reducing inflammatory response. In conclusion, developing and characterizing a recovery model in piglets with colitis is a novel approach towards pediatric IBD research to develop nutritional interventions to optimize growth and decrease disease severity in children with IBD.

APPENDIX

Appendix 1: Results of Verification of Normality (Shapiro-Wilks) and Homogeneity of Variance (Bayesian Information Criterion)

	Shapiro-Wilk's Test	Bayesian Information Criterion (Smaller the Better)	
Variables	P Value Pr < W	Homogenous Fit Statistics	Heterogeneous Fit Statistics
Scale Weight	0.6553	-49.6	-48.8
Snout-to-rump Length	0.1368	212.7	219.7
Chest Circumference	0.0777	230.2	235.1
DXA total mass	0.7293	523.4	549.5
Bone Mass	0.7240	237.9	245.5
Fat Mass	0.6522	433.4	441.7
Lean Mass	0.1611	513.8	541.4
Nitrogen Intake (log)	0.6656	-105.1	-98.7
Urinary Nitrogen (log)	0.1687	5.1	9.0
Fecal Nitrogen (log)	0.6465	26.8	31.3
Nitrogen Balance (log)	0.6339	-24.3	-24.3
Nitrogen Retention (sqrt)	0.6477	99.2	102.1
Spiral Colon Total Score (sqrt)	0.1020	46.6	54.9
Spiral Colon Injury Score (sqrt)	0.3584	39.1	49.6
Distal Colon Total Score	0.7640	120.5	123.8
Distal Colon Injury Score	0.7564	104.2	110.6

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