# Impact of Exogenous Factors on Amino Acid Digestibility in Non-ruminants

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## ABSTRACT

The nutritional value of an amino acid (AA) is determined by its bioavailability, however concept of digestibility is mostly used in practical situations. Four studies were conducted to test 2 exogenous factors that were hypothesized to affect the AA digestibility in non-ruminant animals. In study 1, broiler chicks were randomly allotted to 4 dietary treatments of control and distillers dried grains with solubles (DDGS, 20%) diets supplemented or not with a novel mixture of carbohydrases. Results indicated the ability of carbohydrase mixture to increase energy utilization of the DDGS diet, with significant improvements in AA digestibility, consequently improving growth performance of broilers.

Study 2 examined effect of the carbohydrase mixture in pigs fed a high DDGS diet. Ileal cannulated growing pigs (n = 8, 64.3 ± 0.5 kg) were allotted to 4 dietary treatments in a replicated 4 × 4 Latin Square design. Control and DDGS (40%) diets were supplemented or not with a mixture of carbohydrases. Numeric increases for AA digestibility, along with a decreased tendency of urinary energy output suggested a possibility for improved nutrient utilization in pigs when carbohydrases were supplemented to 40% corn DDGS diet. Collectively, carbohydrase seems less effective for swine applications due to greater water content and consequently a lower viscosity in pig digesta.

Next, study 3 showed changes in AA digestibility and endogenous AA losses (EAAL) when pigs were challenged orally with *Salmonella enterica* serovar Typhimurium. Nursery pigs (n = 48, 17.9 ± 0.5 kg) were randomly assigned to a 2 × 2 factorial arrangement

consisting of two diets (control or N-free) and inoculation (sterile broth or $9.8 \times 10^9$ CFU of *Salmonella*). Measurements at 24 and 72 h post-inoculation indicated that AA digestibility of pigs is impaired through the initial phase of *Salmonella* infection and gradually restored, but not fully by 72 h.

Finally, study 4 determined the dynamic fluctuations of EAAL and subsequent AA digestibility in response to *Salmonella* Typhimurium measured at multiple time points. Ileal cannulated pigs (n = 8, 76.0 ± 1.4 kg) were randomly assigned to either a control or a N-free diet and challenged orally with $1.3 \times 10^{10}$ CFU of *Salmonella*. Inflammatory diarrhea was associated with reduced AA digestibility and increased EAAL showing respective peak values at 8-16 h post-inoculation. Alterations in AA digestibility and EAAL were gradually recovered to near pre-inoculation values by 56-64 h post-inoculation, but showed impaired digestibility at 72-80 h post-inoculation.

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## Table of Contents

## List of Tables

### Chapter 2



### Chapter 3



### Chapter 4

**Chapter 5**



**Chapter 6**

## List of Figures

### Chapter 2



### Chapter 6

## List of Abbreviations

AA: Amino acid

ADFI: Average daily feed intake

ADG: Average daily gain

AEM: Apparent energy metabolizability

AID: Apparent ileal amino acid digestibility

aME: Apparent metabolizable energy

ANOVA: Analysis of variance

AWFI: Average weekly feed intake

AWG: Average weekly gain

BGA: Brilliant green agar

BW: Body weight

CDC: Centers for Diseases Control

CFU: Colony forming unit

CP: Crude protein

DDGS: Distillers dried grains with solubles

DE: Digestible energy

DG: Distillers grains

DM: Dry matter

EAAL: Endogenous amino acid losses

GIT: Gastrointestinal tract

G:F: Gain to feed ratio

LPS: Lipopolysaccharide

ME: Metabolizable energy

NFD: Nitrogen free diet

NRC: National Research Council

NSP: Non-starch polysaccharide

RT: Rectal temperature

SBM: Soybean meal

SEM: Standard error of the mean

SID: Standardized ileal amino acid digestibility

TSB: Tryptic soy broth

WDGS: Wet distillers grains with solubles

# CHAPTER 1

## Introduction

Amino acids (AA) are the building blocks for proteins, which are large biomolecules with vast biological functions. Since AA exist primarily in the form of zwitterions, they can react either as acid or base, depending on their medium pH. This unique chemical property, along with their ability to form complex molecular structures, allows AA to play a major role in numerous biological reactions. Unlike carbohydrates and lipids, AA cannot be stored in the body for later use. Moreover, a species-specific limitation in AA biosynthesis creates the need for a continuous exogenous supply of essential AA (e.g., diet). The amount of these AA in the diet, their relative proportions, and bioavailability, in the presence of adequate energy supply, determine protein accretion in productive animals.

However, the amounts of AA present in diet are not fully converted into animal protein accretion. Everything that affects the physical, chemical, and physiological nature of the digestive environment results in alterations to AA digestibility. Factors that affect AA digestibility in animals can be categorized as endogenous or exogenous factors. Endogenous factors are difficult or impossible to control, including: species, breeds or strains, gender, physiological state (e.g., growing, lactation, gestation or egg production, and maintenance), and age among many others. On the other hand, exogenous factors are somewhat controllable, including: dietary AA balance (e.g., excesses, deficiencies, imbalances, and antagonisms), anti-nutritional factors, stress level (e.g., heat stress or infection), and dietary energy density among others.

Of particular interest, feed ingredients used in diets have differences in AA compositions as well as their digestibility. Moreover, some feedstuffs, even at low levels, can be detrimental to the AA digestibility of other feed ingredients by lowering the efficiency of digestive enzymes (e.g., trypsin inhibitors), increasing the amount of endogenous nitrogen losses, or altering the micro-environment within the gastrointestinal tract (GIT). Thus, depending on the type and level of feedstuff being fed, the overall amount of AA digested and absorbed from the GIT to be used for biological functions is likely altered. In many cases, protein supplements or synthetic AA are added to the diet in order to meet the daily requirement for essential AA and/or ameliorate excesses and imbalances of AA. Because such supplements are costly, strategies have been developed to improve the AA digestibility of diet (e.g., particle size and exogenous enzyme supplementation). The application of exogenous enzymes has been proven to be an effective method for improving nutrient utilization, including AA digestibility.

Immunological stress places a heavy burden on the host metabolism, resulting in significant changes throughout the overall physiology, including its digestive system. Stimulation of the immune system by enteric pathogens likely induces fever, growth suppression, anorexia, and stimulates synthesis and release of hepatic acute phase proteins and cytokines in avian and mammalian species. In this state, AA metabolism is shifted into a catabolic state, where the degradation of endogenous protein sources is accelerated in order to meet the demands for biosynthesis of various proteins with immunological functions during anorexia. In order to develop nutritional strategies to cope with such challenging situations, it is important to understand how immunological stress affects AA digestibility in animals.

The studies conducted in this dissertation were designed to test two exogenous factors that were hypothesized to affect AA digestibility of monogastric animals. First, we examined

the effect of a novel mixture of carbohydrases (i.e., mixture of cellulase, hemicellulase, β-glucanase, and xylanase) on energy and AA digestibility in diets containing a high inclusion of distillers dried grains with solubles in poultry (**Chapter 3**) and swine (**Chapter 4**).

Second, we quantified the impact of *Salmonella enteric* serovar Typhimurium (*S*. Typhimurium) infection on apparent ileal AA digestibility and endogenous AA losses in pigs. Furthermore, results were combined to derived standardized ileal AA digestibility coefficients, using both a comparative slaughter technique (**Chapter 5**) and ileal cannulation (**Chapter 6**) in pigs orally inoculated with *S*. Typhimurium.

# CHAPTER 2

## Review of literature

### 2.1 Introduction

Factors that affect the amino acid (AA) digestibility of animals can be categorized as endogenous or exogenous factors. On the one hand, endogenous factors are difficult or impossible to control including: species, breeds or strains, gender, physiological state (e.g., growing, lactation, gestation or egg production, and maintenance), and age among many others. On the other hand, exogenous factors are somewhat controllable, including: dietary AA balance (e.g., excesses, deficiencies, imbalances, and antagonisms), anti-nutritional factors, and dietary energy density, stress, and health status among others. The present review of literatures focuses two exogenous factors affecting AA digestibility in monogastric animals: multicarbohydrase supplementation and enteric *Salmonella* infection.

### 2.2 Multicarbohydrase supplementation and amino acid digestibility

The price of feed ingredients has been ever rising. Considering that feed accounts for approximately 60-65% of the total cost of livestock production and about 75-80% of the variable cost (Gillespie et al., 2004), the increasing price of feed ingredients is one of, if not the most, sensitive factor that affects industry profitability. Corn and soybean meal (SBM) mixes encompass the majority of monogastric livestock diets and their prices have the most impact on total feed cost. Indeed, identification and evaluation of alternative feedstuffs for corn and SBM

continues to be a major field of investigation for animal nutritionists. Considering that AA (along with energy and phosphorus) are among the most expensive nutrients, extensive research has been dedicated to maximize their digestibility in various feed ingredients. The application of exogenous enzymes has proven to be an effective method for improving nutrient utilization, including the AA digestibility.

### 2.2.1 Distillers dried grains with solubles

Distillers grains (DG) are the co-product of cereal distillation to yield alcoholic beverages and fuel-grade ethanol. With the rapid expansion of the fuel ethanol industry in North America during the last few decades, production of DG has increased. Currently in the U.S., most of the DG is corn derived compared with other grains (e.g., sorghum, barley, wheat, malt, rice) because it is market-selected for fuel ethanol production. It was obvious that the livestock feed industry was attracted to the increasing production of DG. Since the first published scientific study on DG use as feed ingredient in the U.S. (Lindsey, 1907), numerous studies have been conducted resulting in the higher quality forms of DG available today.

Distillers dried grains with solubles (DDGS) are one such end-product. A general scheme of DDGS production is presented in **Figure 2.1** (Vaage, 2008). Corn is processed to yield fermentable carbohydrates and mixed with yeast that converts the starch into ethanol and carbon dioxide. The ethanol is recovered by distillation and the remaining liquid is centrifuged to remove some water. This residue is called wet distillers grains and usually is 30-35% in dry matter (DM) and contains most of the fiber, fat, protein, and minerals found in the original grain. The liquid removed by centrifuging is usually partially dried and becomes condensed distillers solubles. Condensed solubles are a good source of protein, energy, and vitamins but have the

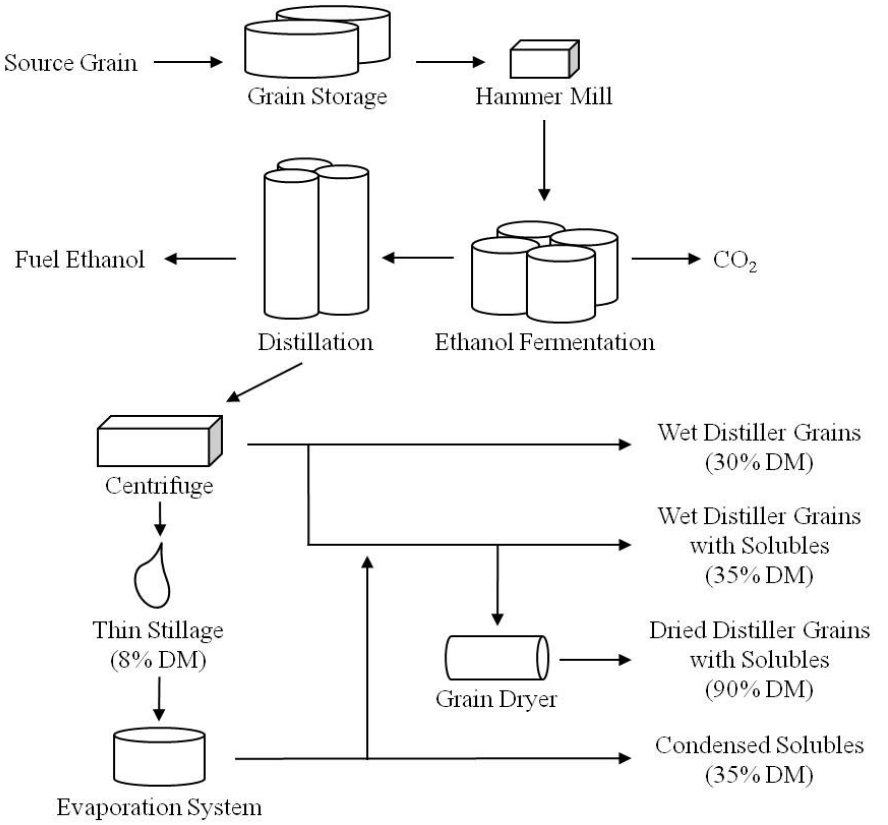


Figure 2.1. A general scheme of dried distillers grains with solubles (DDGS) production. Source grain is processed to yield fermentable carbohydrates and mixed with yeast that converts the starch into ethanol and carbon dioxide. Remaining liquid is centrifuged to remove some water producing wet distillers grains and thin stillage. Thin stillage is partially dried and becomes condensed distillers solubles. The condensed solubles are added back to the wet distillers grains making wet distillers grains with solubles. Wet distillers grains with solubles are heat-dried into DDGS.

(Source: Vaage, 2008)

consistency of molasses, making it difficult to feed. Most distilleries add the condensed solubles back to the wet distillers grains making wet distillers grains with solubles (WDGS). The wet products can be fed as-is, but because WDGS are approximately 65% water content, it results in higher transportation and storage costs. Thus, WDGS are heat-dried into DDGS with the cost of drying added to the price (Weiss et al., 2007).

Table 2.1. Estimated US distillers dried grains with solubles (DDGS) production and use[1]

| Year[2] | 2007-2008 | 2008-2009 | 2009-2010 | 2010-2011 | 2011-2012 | 2012-2013[3] |
|---|---|---|---|---|---|---|
| DDGS production | 23.51 | 28.60 | 35.23 | 38.71 | 38.56 | 39.72 |
| Estimated DDGS usage[4] | | | | | | |
| Swine | 1.19 | 1.40 | 1.67 | 2.07 | 2.12 | 2.21 |
| Poultry | 1.19 | 1.40 | 1.67 | 1.68 | 1.56 | 1.59 |
| Estimated DDGS substituted for corn | | | | | | |
| Swine | 1.09 | 1.28 | 1.52 | 1.77 | 1.85 | 1.99 |
| Poultry | 0.71 | 0.82 | 0.98 | 1.06 | 0.98 | 0.95 |
| Estimated DDGS substituted for SBM[5] | | | | | | |
| Swine | 0.13 | 0.18 | 0.23 | 0.24 | 0.24 | 0.24 |
| Poultry | 0.29 | 0.34 | 0.34 | 0.33 | 0.33 | 0.33 |

[1] All units are million metric tons.
[2] Marketing year starts at September 1 of the production year and ends August 31 of the following year.
[3] Medium projected values
[4] Domestic usage fed to swine or poultry
[5] Soybean meal

(adapted from Wisner, 2008)

Domestic DDGS production and usage by the U.S. swine and poultry industries continues to increase (**Table 2.1**). The production of DDGS has increased by 69% during the past 6 years. It is projected that by 2012-2013, approximately 5.6% and 4.0% of DDGS will be consumed by the U.S. swine and poultry industries, respectively. Thus, it is estimated that DDGS will substitute 2.9 and 0.6 million metric tons of corn and SBM used by the U.S. swine and poultry industries, respectively (Wisner, 2008).

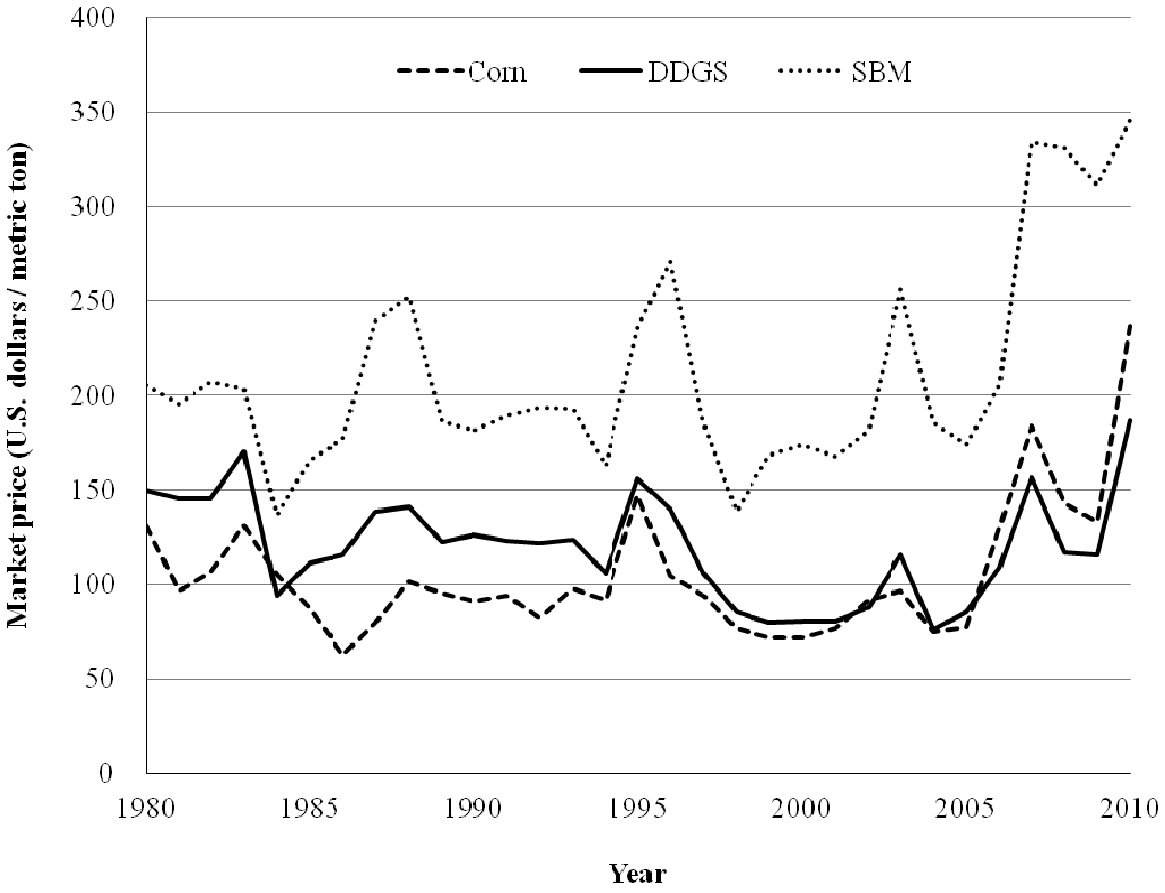


Figure 2.2. Changes in average cash market prices of corn, soybean meal (SBM), and distillers dried grains with solubles (DDGS) in the U.S. from 1980 to 2010.

(Source: USDA, Agricultural Marketing Service, Grain and Feed Market News, 2012)

As an alternative feed ingredient, DDGS usage is sensitive to corn and SBM market prices. Changes in the cash market prices of corn, SBM, and DDGS in the U.S. are shown in **Figure 2.2**. Cash market prices have been fluctuating for the past 30 years, but have ended with a recent steep incline. Between 2005 and 2010, the cash market prices tripled for corn and doubled for SBM. Such price increase forced DDGS market price to increase as well. Although DDGS prices doubled during this period, the unit price relationship versus corn was reversed, as cash corn market prices have been higher than DDGS since 2006 (USDA, 2012).

Table 2.2. Maximum price that can be paid for distillers dried grains with solubles (DDGS) at different costs of corn and soybean meal (SBM) without increasing cost of complete swine diet[1,2]

| | Corn | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SBM | 93 | 111 | 129 | 147 | 165 | 183 | 201 | 219 | 237 |
| 175 | 109 | 119 | 129 | 139 | 149 | 159 | 169 | 179 | 189 |
| 200 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 |
| 225 | 131 | 141 | 151 | 161 | 171 | 181 | 191 | 201 | 211 |
| 250 | 142 | 152 | 162 | 172 | 182 | 192 | 202 | 212 | 222 |
| 275 | 153 | 163 | 173 | 183 | 193 | 203 | 213 | 223 | 233 |
| 300 | 164 | 174 | 184 | 194 | 204 | 214 | 224 | 234 | 244 |
| 325 | 175 | 185 | 195 | 205 | 215 | 225 | 235 | 245 | 255 |
| 350 | 186 | 196 | 206 | 216 | 226 | 236 | 246 | 256 | 266 |

[1] For each combination of costs for corn and SBM, the price indicated for DDGS will result in identical diet costs for a corn-SBM and a corn-SBM-DDGS diet.
[2] All units are U.S. dollars/metric ton.

(adapted from Stein, 2007)

**Table 2.2** shows the maximum price that can be paid for DDGS at different costs of corn and SBM without increasing the cost of complete swine diet (Stein, 2007). By combining values in **Figure 2.2** and **Table 2.2** to determine the market price advantage of DDGS as partial substituent for corn and SBM, it can be seen that DDGS market prices have been competitive since 1995. Moreover, since 1995, the gap between actual versus breakpoint DDGS prices showed a widening trend as the difference was $11.70/metric ton in 1995, but increased to

$79.05/metric ton in 2010. While DDGS is economically favorable as a feed ingredient, concerns regarding its high degree of nutrient variability among sources (Cromwell et al., 1993) have limited its use in the swine and poultry industries.

Nutritional and physical variations among DDGS products are largely due to factors such as the source of grain, type of fermentation, and drying conditions (Carpenter, 1970; Olentine, 1986). As DDGS is the co-product of alcohol production, where most starch is removed from the source grain, the concentration of the remaining nutrients increases approximately 3-fold (Spiehs et al., 2002). This characterizes DDGS as a good source of protein with 27.8% of crude protein (CP), higher than that of the corn at 7.2% (Pedersen et al., 2007).

The AA concentrations in DDGS, standardized ileal AA digestibility (SID) coefficients, and their derived standardized digestible AA concentration of DDGS for poultry and swine are presented in **Table 2.3**. Most indispensable AA in DDGS have a medium digestibility, and except for Lys, the variability among different samples is within the normal range of variation found in other feed ingredients (Stein, 2007). The reason for the greater variation in Lys digestibility compared with the digestibility of other AA is believed to be that some sources of DDGS have been heat damaged during the drying process (Cromwell et al., 1993; Stein et al., 2006).

Meanwhile, DDGS is also high in total non-starch polysaccharide (NSP) content at 22.7% (Ward et al., 2008), which is higher than corn and SBM at 6.1% and 12.6%, respectively (Cowieson and Adeola, 2005). Because non-ruminants lack production of carbohydrases to digest NSP and limited fermentation occurs prior to the small intestine, the metabolizable energy (ME) content of DDGS for monogastrics is generally lower than corn. While Spiehs et al. (2002) and Pedersen et al. (2007) reported no differences in digestible energy (DE) and ME

Table 2.3. Amino acid (AA) concentration (Conc.), standardized ileal AA digestibility (SID), and standardized digestible AA concentration ($_{SD}$Conc.) of distillers dried grain with solubles for poultry and swine

| | Poultry[1] | | | Swine[2] | | |
|---|---|---|---|---|---|---|
| | Conc. (%) | SID (%) | $_{SD}$Conc. (%) | Conc. (%) | SID (%) | $_{SD}$Conc. (%) |
| Indispensable | | | | | | |
| Arginine | 1.16 | 88.5 | 1.03 | 1.14 | 81.1 | 0.92 |
| Histidine | 0.70 | 86.7 | 0.61 | 0.71 | 77.1 | 0.55 |
| Isoleucine | 0.99 | 83.5 | 0.83 | 1.00 | 75.3 | 0.75 |
| Leucine | 3.11 | 91.0 | 2.83 | 3.11 | 83.5 | 2.60 |
| Lysine | 0.77 | 61.4 | 0.47 | 0.76 | 60.6 | 0.46 |
| Methionine | 0.51 | 86.9 | 0.44 | 0.54 | 81.8 | 0.44 |
| Phenylalanine | 1.31 | 87.8 | 1.15 | 1.32 | 80.8 | 1.07 |
| Threonine | 0.99 | 77.6 | 0.77 | 1.04 | 70.4 | 0.73 |
| Tryptophan | 0.17 | - | - | 0.21 | 69.6 | 0.15 |
| Valine | 1.35 | 84.4 | 1.14 | 1.34 | 74.4 | 1.00 |
| Dispensable | | | | | | |
| Alanine | 1.85 | 85.7 | 1.59 | 1.89 | 78.1 | 1.48 |
| Aspartate | 1.66 | 77.1 | 1.28 | 1.80 | 68.1 | 1.23 |
| Cystine | 0.49 | 83.3 | 0.41 | 0.52 | 72.5 | 0.38 |
| Glutamate | 3.68 | 87.5 | 3.22 | 4.24 | 80.4 | 3.41 |
| Glycine | 1.04 | - | - | 1.02 | 63.2 | 0.64 |
| Proline | 1.59 | 72.0 | 1.14 | 2.06 | 74.1 | 1.53 |
| Serine | 1.17 | 84.5 | 0.99 | 1.14 | 75.9 | 0.87 |
| Tyrosine | 1.02 | 88.9 | 0.91 | 1.00 | 80.9 | 0.81 |

[1] Data from Pahm et al. (2009), based on 7 DDGS sources.
[2] Data from Stein and Shurson (2009), based on 39 DDGS sources.

between DDGS and corn in growing pigs, the National Research Council, along with others have previously reported lower ME for poultry (Adeola and Zhai, 2012; Batal and Dale, 2006; Lumpkins et al., 2004; NRC, 1994) and swine (Fu et al., 2004; NRC, 1998). These inconsistencies are presumably due to actual energy differences among DDGS sources used and oil content. Distillers dried grains with solubles produced in "new generation" ethanol plants have higher ME (Spiehs, 1999) than DDGS produced in traditional ethanol plants. Although these DDGS contain greater amounts of crude fiber, they are also high in crude fat content which results in DDGS with higher ME, similar to that of corn (Shurson et al., 2004).

At present, the recommended DDGS inclusion levels for poultry and swine are: 10-20% for broilers, 15-20% for chicken layers, 20-30% for nursery to finishing pigs, 20-30% for lactating sows, and 40-50% for gestating sows (Shurson and Noll, 2005; Stein, 2007). Research is needed to determine practical ways to enhance DM and energy digestibility in DDGS because there is a great opportunity for improvement of the feeding value of DDGS. Therefore, strategies to improve the digestibility of the NSP fraction should be developed.

### 2.2.2 Carbohydrase

A general description of current carbohydrase usage in non-ruminant animal nutrition was recently reviewed by Adeola and Cowieson (2011). Broadly, carbohydrases refer to a class of enzymes that function as a catalyst in reduction of non-digestible polymeric carbohydrates. Because polymeric carbohydrates exist in various shapes and sizes, the number of carbohydrases can be somewhat overwhelming, leading to considerable confusion in its terminology. For example, xylanases are often mistakenly referred to as pentosanases, NSPases, hemicellulases, and vice versa. One way to minimize confusion is to follow the enzyme nomenclature scheme

of the Enzyme Commission (EC), where enzymes are classified based on the chemical reactions they catalyze. All exogenous carbohydrases that are relevant to non-ruminant animal nutrition are under the EC family of hydrolases (EC 3), specifically the subfamily of glycosidases (EC 3.2) that hydrolyze O- and S-glycosyl compounds (EC 3.2.1).

Two types of carbohydrases dominate the global carbohydrase market, namely xylanase and glucanase. Xylanases refer to endo-1,4-β-xylanase (EC 3.2.1.8) which cleave the linear polysaccharide β-1,4-xylan into xylose. Glucanases are identified as endo-1,3(4)-β-glucanases (EC 3.2.1.6) and are often partially mistaken for cellulases or glycosidases. Cellulases have their own assigned EC identifier (EC 3.2.1.4), but technically refer to enzymes with a series of glycosidase activities that depolymerize cellulose into glucose (e.g., endo-1,4-β-glucanase, cellobiohydrolase, and β-glucosidase). Other popular commercial carbohydrases are; α-amylase, β-mannanase, α-galactosidase, and pectinase. Sometimes, a collective term is used to refer to a group of carbohydrases. Hemicellulase is a collective term for enzymes that hydrolyze hemicelluloses including xylan, arabinoxylan, xyloglucan and glucomannan. Xylanases (EC 3.2.1.8) and galactanases (EC 3.2.1.89) are representative enzymes that belong to the hemicellulase group.

Poultry and swine lack the abilities to produce endogenous enzymes or host pre-gastric microbial fermentation that hydrolyze some complex carbohydrates. Because monogastric livestock are generally fed plant-based diets, significant amount of NSP are introduced into the gastrointestinal tract (GIT) that are indigestible by the host. These are largely the structural carbohydrates, abundant in plant cell walls. Other than the fact that these complex carbohydrates are indigestible, the physical structure of the endosperm cell wall further impairs the digestibility of other nutrients, as they obstruct access to digestive enzymes, or encapsulate

digestible substrates causing them to leave the GIT undigested (Campbell and Bedford, 1992). This is more evident when diets include viscous grain sources, which are generally high in NSP content, as it induces increased intestinal viscosity, which slows down the rate of digestion (Jeroch et al., 1995). Also, high NSP contents are considered anti-nutritional as it leads to reductions in voluntary feed intake and nutrient digestibility (Kyriazakis and Emmans, 1995; Zijlstra et al., 1999).

These constraints can be overcome effectively by using carbohydrases to increase the diffusivity of digestive enzymes and digesta, allowing a more rapid and complete digestion and absorption processes. Previous studies have shown that xylanase attacks the arabinoxylan backbone, causing a decrease in the degree of polymerization and release of oligomers (Courtin and Delcour, 2002; Hu et al., 2008). Reduction in digesta viscosity has been shown to directly enhance animal performance in several studies (Adeola and Bedford, 2004; Zhang et al., 2000). Thus, along with its ability to release carbohydrate from otherwise insoluble fiber substrate (Wagner, 2007), carbohydrase supplementation can theoretically improve nutrient digestibility of poultry and swine by reducing digesta viscosity when fed diets high in NSP. Furthermore, reduction of the fibrous matrix of a feedstuff can result in higher digestibility of protein, starch, and fat due to a greater access of digestible enzymes to their respective substrates.

Many poultry studies have reported improvements in nutrient utilization when diets high in NSP content are supplemented with carbohydrases. Although not a nutrient *per se*, energy utilization was improved when diets high in NSP content were supplemented with carbohydrases (Adeola et al., 2007; MacLeod et al., 2008; Mathlouthi et al., 2003). Because energy is also derived from fat and AA, the improvement in energy utilization should be interpreted as a collective effect of overall increased nutrient digestibility, not only the enzymatic hydrolysis of

NSP to oligosaccharides. Improvements in DM (Leslie et al., 2007; Yang et al., 2010), fat (Boguhn and Rodehutscord, 2010; Mathlouthi et al., 2002), mineral (Olukosi et al., 2008), and AA (Cowieson and Ravindran, 2008; Rutherfurd et al., 2007) digestibility have been reported in response to carbohydrase supplementation.

In contrast to poultry, the use of carbohydrase enzymes has shown inconsistent results in swine studies. Inclusion of carbohydrases to high NSP diets have shown improvements in energy utilization (Diebold et al., 2004; Yin et al., 2000), DM (Li et al., 1996; Nortey et al., 2007), mineral (He et al., 2010; Olukosi et al., 2007b) or AA digestibility (Barrera et al., 2004; Diebold et al., 2004; Emiola et al., 2009; Nitrayová et al., 2009; Vahjen et al., 2007), whereas others have not observed responses in energy (Olukosi et al., 2007a,b), DM (Woyengo et al., 2008), mineral (Nortey et al., 2008), nor AA digestibility (Yáñez et al., 2011). In general, poultry species respond better to carbohydrase supplementation than swine, presumably due to differences in the anatomy and physiology of GIT as well as water content of digesta. Again, as the primary mode of action of carbohydrase in a high NSP environment is to reduce viscosity of digesta, the lower moisture content of poultry digesta (i.e., higher viscosity) should theoretically be more sensitive to changes in viscosity compared to the higher water content of pig digesta (Bedford and Schulze, 1998).

Exogenous carbohydrases are used to reduce inherent inefficiency of nutrient utilization associated with diets that contain a high level of NSP. In order to obtain optimum enzyme efficiency, strategic reductions in dietary energy and nutrient content, as well as careful choice of feed ingredients are required, to maximize the economic benefits of the enzyme supplementation. The efficacy of dietary exogenous enzymes will vary depending on their adequacy to breakdown the fibrous matrix of feedstuffs or a mixed diet at a proper timing to allow for a higher activity of

endogenous digestive enzymes on their substrates. It is important to continue the effort to understand the use and limitations of matrix values of enzymes, which, if inappropriately applied, will result in depressed performance because of nutritional inadequacy of diets, which will lead to wastage of resources.

### 2.3 Enteric *Salmonella* infection and amino acid digestibility

Enteric diseases occur subsequent to infection by various pathogens, responsible for a detrimental performance in livestock production. In swine, *Salmonella* infections are a major public health concern as pork is associated with many cases of human illness (Foley et al., 2008). Moreover, *Salmonella* enterocolitis is recognized as a serious problem for the swine industry (Veldhuizen et al., 2008), as it causes significant mortality in nursery pigs and retards growth performance (Nielsen et al., 1997) resulting in considerable economic losses. *Salmonella enterica* serological variant (serovar) Typhimurium (*S*. Typhimurium) is one of the most frequently isolated serovars in pigs (Chiu et al., 2004; Schwartz, 1999), which is likely to cause clinical disease with a mild transient diarrhea. Previous studies with *S*. Typhimurium have reported fever, growth suppression, anorexia, and acute phase immune response in pigs (Balaji et al., 2000; Loughmiller et al., 2007; Fraser et al., 2007). Stimulation of the immune system by enteric pathogens results in physiological changes of the host including its digestive system. Catabolism of AA during an immunological stress emphasizes the importance of AA digestion and absorption for optimal immune responses and sound recovery (Johnson et al., 2001).

2.3.1 *Salmonella enterica* serovar Typhimurium

The genus, *Salmonella*, refers to a group of Gram-negative, facultative, rod-shaped, motile bacteria. There are two species of this genus, *Salmonella enterica* and *Salmonella bongori*. Most attention is given to *Salmonella enterica* subspecies *enterica*, which includes the serovars Typhi and Typhimurium. *Salmonella* Typhi (*S*. Typhi) is a human specific pathogen that causes the systemic infection of typhoid fever. The bacteria multiply in the spleen and liver, and large numbers of bacteria enter the blood stream, eventually leading to septic shock. Except for humans, no other animals have been reported to be affected by *S*. Typhi in such a manner. *Salmonella* Typhimurium, as hinted from its name, causes typhoid-like disease in murine species. Mice models are often used to study the pathogenesis of *S*. Typhimurium in an effort to understand the pathogenesis of *S*. Typhi in humans. In other species such as pigs, *S*. Typhimurium causes a mild-diarrheal and self-contained enterocolitis.

Because animals do not appear ill, afflicted animals are often not treated and leads to *Salmonella* contamination of animal-derived products. This poses as a major public health threat to humans. Salmonella ranks as the number one food-borne pathogen in annual disease burden (Batz et al., 2011). Furthermore, *Salmonella* induced enterocolitis is the single most common cause of death from food-borne illnesses associated with viruses, parasites, or bacteria in the U.S., responsible for approximately 378 annual deaths (Scallon et al., 2011). The serotype associated most frequently with this diarrheal disease syndrome in the U.S. is *S*. Typhimurium, accounting for 26% of all *Salmonella* reported isolates to the Centers for Diseases Control in 1998 (CDC, 1999). Moreover, in 1984, a multi-antibiotic resistant strain of *S*. Typhimurium (DT104) was discovered in the U.K. as the responsible agent for many disease outbreaks in Europe and the U.S. (USDA, 1997).

2.3.2 *Salmonella* Typhimurium pathogenesis

The pathogenesis of *S*. Typhimurium was summarized by Salyers (2002) and reviewed by Foley and Lynne (2008). As with all pathogenic bacterial infections, the pathogenesis of *S*. Typhimurium obeys the steps of: introduction, adhesion, invasion, and host inflammation.

*Salmonella* Typhimurium is a natural occurring microorganism that can be easily found in livestock production sites and processing plants. Pigs frequently harbor subclinical levels of *Salmonella*, allowing the organism to be transmitted among animals in a herd. The primary route of *Salmonella* transmission is the fecal-oral route. During events where stress levels are high (e.g., transportation and lairage), the fecal shedding and transmission rate of *Salmonella* from infected pigs to naïve pigs is increased (Hurd et al., 2002; McKean et al., 2001). The infectious dose of *Salmonella* is rather broad and variable, ranging from approximately 30 to more than $10^9$ colony forming units (Morgan et al., 1994; Vought and Tatini, 1998).

Once *Salmonella* is introduced orally, the bacteria journey through the GIT. *Salmonella* that survive the low pH environment of the stomach are able to attach and adhere to multiple sites including the small intestine, cecum, and colon. Intestinal adhesion is mediated by fimbriae or pili present on the bacterial cell surface. There are many types of fimbriae associated with *Salmonella* that may play a role in colonization including type 1 fimbria (Fim), long polar fimbria (Lpf), plasmid-encoded fimbria (Pef), and thin aggregative or curli fimbria (Darwin and Miller, 1999). The Fim specifically targets α-D-mannose receptors on the surface of multiple epithelial cells in the small intestine. Other types of fimbriae also appear to have host tissue specificity, as Lpf binds to the surface of the Peyer's patches and M cells, Pef to the villous intestine, and curli fimbria likely to the small intestine (Althouse et al., 2003; Baumler et al., 1996; Sukupolvi et al., 1997).

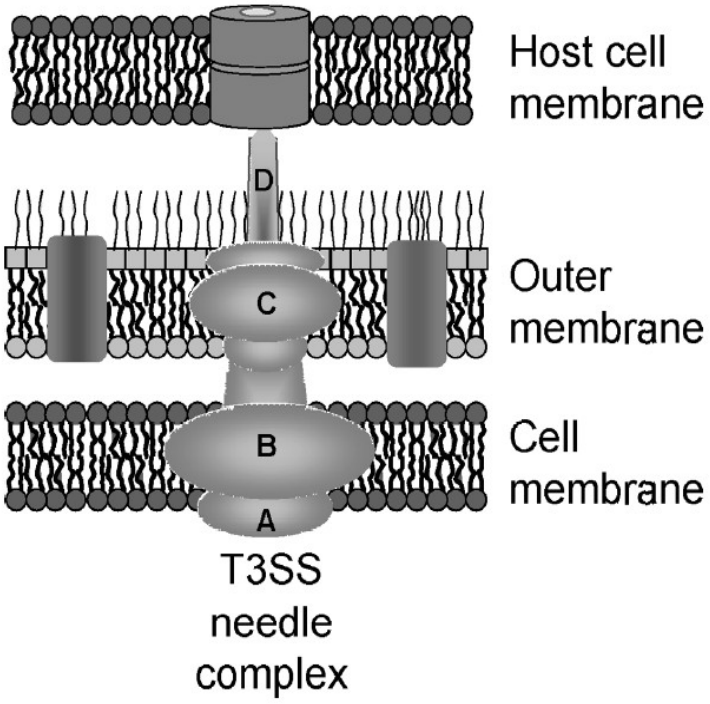


Figure 2.3. Illustration of the *Salmonella* pathogenicity island 1 associated type 3 secretion system (T3SS). The base structure of the T3SS is composed of the inner ring structure (A) and the export machinery (B). The outer ring structure (C) is connected to the inner ring structure. The completed base structure allows for the assembly of the needle (D) and inner rod structures.

(redrawn from Foley and Lynne, 2008)

Once successfully attached, *Salmonella* invades the intestinal epithelium by two pathways. First, if taken up by M cells (surveillance cells on surface of villi passing possible antigens to underlying immune cells), *Salmonella* directly passes the epithelium. But more often, *Salmonella* injects proteins into the epithelial cell using the type 3 secretion system (T3SS). The T3SS is a complex of proteins that allows for the injection of virulence factors directly into the host cells and it is associated with at least 20 structural and regulatory proteins involved in cellular invasion (Marlovits et al., 2004; Galán and Wolf-Watz, 2006). The base structure of the T3SS complex expands the cell membrane and the cell wall of *Salmonella,* and a needle structure assembles from the base that links with host cells. Within the base and needle structure is an inner rod that forms the passage between the bacterial cytoplasm and the host cell membrane (**Figure 2.3**). The genes that encode T3SS are associated with *Salmonella* pathogenicity island-

1 (SPI-1). Pathogenicity islands are genetic elements that carry genes encoding virulence factors, such as adhesion, invasion, and toxin genes (Hacker et al., 1997). The injected proteins rearrange the actin of the enterocyte apical surface, causing a membrane ruffling which leads to macropinocytosis of the bacteria.

Once *Salmonella* is internalized, it resides in *Salmonella* containing vacuoles (SCV). As the SCV matures, it migrates to the basal membrane of the enterocyte where the *Salmonella* enter macrophages (Pegues et al., 2005). Formations of SCV protect *Salmonella* as it disrupts the normal phago-lysosomal processing pathways (Holden, 2002) by avoiding fusion with lysosomal compartments (Unsworth and Holden, 2000). This mechanism of intracellular survival is associated with genes encoded on SPI-2, which are expressed when internalized.

Although the innate immune system is partially deceived, macrophages are immediately activated by *Salmonella* invasion and neutrophils release granules of powerful cytotoxic enzymes to fight the pathogens. Neutrophils also release prostaglandins which increase the cellular cyclic adenosine monophosphate (cAMP) of enterocytes leading to decreased $Na^+$ intake and $Cl^-$ secretion. This leads to loss of water by the enterocytes and consequently, diarrhea. The cytotoxic enzymes released by neutrophils damage host tissue as well, leading to the loss of intestinal barrier function, and hence, also diarrhea. The diarrheal disease induced by *S.* Typhimurium is discussed further in the following section. The acquired immune system takes over soon after, and with time, the pathogen is removed. However, *Salmonella* can survive inside inactivated macrophages or other cells, resulting in a prolonged disease state for several months.

2.3.3 *Salmonella* Typhimurium-induced diarrhea

The mechanisms of porcine diarrheal disease induced by *S*. Typhimurium were reviewed by Zhang et al. (2003) and Moeser and Blikslager (2007). Fluid secretion in the intestine is a natural physiological process that flushes mucus into the lumen from the crypts and provides an optimal aqueous environment required for digestive process. Secretion is also an important defense mechanism by flushing out enteric pathogens. However, uncontrolled secretion can induce fluid loss sufficient to be potentially fatal.

Diarrheal diseases in pigs have been characterized into 4 major types: hypersecretion, malabsorption, hypermotility, and increased permeability (Moon, 1978). Generally, diarrheal disease in pigs involves multiple factors including alterations in secretory and absorptive functions. In addition, increased intestinal permeability, due to a disruption of the intestinal barrier function, is a common pathophysiologic event occurring in hypersecretory and malabsorptive diarrheal diseases, which can result in activation of inflammatory cascades that may further increase secretion and induce diarrhea. A relative increase in luminal ions as a result of secretion and reduced absorption may also aggravate diarrhea as a result of the increased osmotic load within the lumen. Enterocolitis induced by *S*. Typhimurium is accompanied by diarrheal disease with combined characteristics of hypersecretion and increased intestinal permeability.

The principal secreted ion that results in fluid movement into the lumen is $Cl^-$. Cyclic AMP stimulates a number of kinases, including protein kinase A (PKA), which regulates the cystic fibrosis transmembrane regulator (CFTR), the principal $Cl^-$ channel. Thus, an increase of intracellular cAMP concentration by any cellular or pathologic process results in opening of the CFTR, enhancing $Cl^-$ secretion. During *Salmonella* infection, a variety of effector proteins are

injected into the host cell and trigger inflammatory signaling cascades. Neutrophils are attracted to the basolateral surface of the epithelium by interleukin-8 (IL-8). Upon arrival at the epithelium, neutrophils produce prostaglandin $E_2$ via cyclooxygenase-2 within subepithelial fibroblasts. The prostaglandins increase the cellular cAMP of enterocytes leading to $Cl^-$ secretion. This leads to loss of water by the enterocytes and consequently, diarrhea. Giannela et al. (1975) speculated that an increase in cAMP concentration in the mucosa of rabbit ligated ileal loops infected with *S*. Typhimurium was a mechanism for fluid secretion.

Unlike $Cl^-$ secretion, an inflammatory mechanism of fluid secretion would be expected to result in the release of serum proteins into the intestinal lumen and increase endogenous nitrogen losses (ENL), due to the loss of the intestinal permeability barrier. Indeed, a comprehensive evaluation of the hematology and blood chemistry profile of orally infected calves has shown that *S*. Typhimurium-induced diarrhea results in a non-specific effusion of serum proteins (Santos et al., 2002a). If *S*. Typhimurium causes diarrhea by an inflammatory mechanism, then the intestinal fluid accumulation that occurs during enteric inflammation should coincide with the loss of the intestinal permeability barrier. This hypothesis has been tested using a bovine ligated ileal loop model, in which fluid accumulation and the development of lesions were examined at various time points post-infection (Santos et al., 2001, 2002b). Bacterial invasion of the epithelial cells was detected at 15 min post-infection, and by 1 h, *Salmonella* was observed in the lamina propria. With early inflammatory changes at 1 h post-infection, fluid accumulation began at 3 h post-infection. Injury to the intestinal epithelium (i.e., detachment of epithelial cells from the tips of absorptive villi) was first detectable at 3 h post-infection in *S*. Typhimurium-infected loops, thus coinciding with the onset of fluid accumulation. The injury progressed to a severe neutrophilic inflammation associated with necrosis of the mucosa at 8 h,

and this was accompanied by an increase in the volume of fluid in the lumen of *S*. Typhimurium-infected loops.

In summary, previous studies have suggested an inflammatory mechanism for the loss of fluid and protein during *S*. Typhimurium-induced enterocolitis. However, contribution of $Cl^-$ secretion to the severe fluid loss should not be ruled out. Neutrophils have been shown to play a crucial role in mediating diarrhea by an inflammatory mechanism, since this cell type is known to release substances that lead to tissue injury. It has been shown in rats that the alterations of the intestinal mucosal permeability can be prevented by neutrophil depletion (Sir et al., 2000). These findings suggest that the study of virulence mechanisms responsible for eliciting a neutrophils-based inflammatory infiltrate is of prime importance for understanding the pathogenesis of *S*. Typhimurium enterocolitis.

### 2.3.4 *Salmonella* Typhimurium studies in swine

Because of its impact on the physiology of the host, including its digestive system, immune system stimulation has been a topic of interest to many animal scientists. Most of the experimental evidence documenting the physiological responses has been gathered from model systems of infection and inflammation, typically treatments with bacterial lipopolysaccharide (LPS) (Johnson et al., 1994; Mazzocchi et al., 1995). In pigs, LPS induces a rapid febrile response (Parrot and Vellucci, 1998), complete anorexia (Webel et al., 1997), reduced circulating insulin-like growth factor-1 (IGF-1) (Hevener et al., 1997), and elevated plasma inflammatory cytokines (Johnson et al., 2005). Nonetheless, these acute responses are of short duration, typically 6 to 8 h after LPS administration, and therefore are not suitable to study prolonged changes in digestive processes. For example, a recent study reported no differences in apparent

ileal amino acid digestibility (AID) in pigs using the LPS model (Rakhshandeh et al., 2010).

Without question, the LPS model has provided valuable information on the effect of immune system stimulation on the physiology of the host. The LPS model is favorable over an actual bacterial model, as the experimental burden (e.g., time, biosafety, labor, required resources) are significantly reduced. However, the LPS model has certain limitations, as it does not fully simulate an enteric bacterial pathogenesis. For instance, LPS challenge produces more of a systemic cytokine response (Johnson et al., 2005) as opposed to a localized production of proinflamatory cytokines in cases such as Salmonellosis (Balaji et al., 2000; Burkey et al., 2004).

Previous studies with *S*. Typhimurium have shown fever, growth suppression, feed intake reduction and acute phase immune response in pigs (Balaji et al., 2000; Loughmiller et al., 2007; Fraser et al., 2007). The systemic concentrations of plasma inflammatory cytokines tumor necrosis factor α (TNFα) or IL-6 were not elevated in *S*. Typhimurium challenged pigs (Balaji et al., 2000; Burkey et al., 2004), supporting the self-limiting nature of *S*. Typhimurium enterocolitis. Although the importance of AA metabolism under immune system stimulation is emphasized (Johnson et al., 2001), and various insights have been previously provided regarding the AA requirement of diseased animals (Webel et al., 1998; Wu, 1998; Yoo et al., 1997), the digestibility of specific AA have not been reported using an actual bacterial challenge model in laboratory or agricultural animals.

## 2.4 Conclusion

The unique chemical properties of AA grant the power to play a key role in all aspects of life. Thus, the study of AA metabolism is important to all fields in life sciences. Determining

the AA digestibility in a specific environment is one of the fundamentals, as it serves as a starting point to study general AA metabolism. This is also required for extended studies such as in AA requirement determination. Of many practical situations, two specific topics were selected to be examined in depth.

Increasing use of corn-derived DDGS in the poultry and swine industries has led to the development of various strategies to improve nutrient digestibility. Supplementation of dietary exogenous enzymes has proven to be an efficient solution to the matter. However, enzymes should be carefully selected to maximize the economic benefits of the enzyme supplementation.

*Salmonella* infection and presence are major concern for both the general public and the livestock industry. The catabolism of AA during an immunological stress emphasizes the importance of AA digestion for optimal immune responses and a sound recovery. Although the importance of AA metabolism under immune system stimulation has been emphasized and studied, the digestibility of individual AA has not been reported using an actual bacterial challenge model in animals.

The cited literatures in current review were selected to construct scientific basis for the following chapters. The studies conducted in this dissertation were designed to test two of the exogenous factors that were hypothesized to affect AA digestibility in monogastric animals. In chapters 3 and 4, a novel mixture of carbohydrases were tested for its effect on energy and AA digestibility when supplemented to diets containing high level of DDGS in poultry and swine, respectively. Finally, chapters 5 and 6 presents the results of AA digestibility and endogenous AA losses from pigs orally challenged with *S*. Typhimurium using the comparative slaughter method or simple ileal T-cannula.

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# CHAPTER 3

## Increased amino acid digestibility, apparent metabolizable energy, and growth rate in broiler chicks fed an energy-restricted corn DDGS diet supplemented with a novel mixture of carbohydrases

### 3.1 Abstract

High-fiber content of distillers dried grains with solubles (DDGS) is partially responsible for its low ME, which may be increased with dietary inclusion of a mixture of carbohydrases. Broiler chicks (7-d-old, 8 chicks/pen, 6-7 pens/diet) were randomly allotted to 4 dietary treatments with ad libitum access to feed and water. Corn and DDGS (20%) diets were supplemented or not with a novel mixture of carbohydrases. DDGS diets were formulated to be deficient only in metabolizable energy (ME) (90% of corn diets). In wk 2, average weekly weight gain (AWG), gain to feed ratio (G:F), and apparent ME (aME) of chicks receiving corn diets were higher ($P < 0.0001$) than DDGS diets regardless of enzyme supplementation. Although enzyme inclusion increased ($P < 0.05$) aME by 9.4%, it had no effect on AWG. In wk 3, AWG and G:F were higher ($P < 0.0001$) in chicks receiving corn diets compared to DDGS diets regardless of enzyme supplementation. Inclusion of enzyme improved ($P < 0.05$) AWG in both corn and DDGS diets. Supplementation of enzyme increased ($P < 0.01$) aME by 11.7%, which made it comparable in ME to both corn diets. However, AWG of chicks fed DDGS+Enzymes was lower than corn groups with or without enzyme supplementation ($P < 0.01$). While overall standardized ileal digestibility (SID) for all amino acids (AA) were not

affected by inclusion of DDGS except for Leu, Met, and Phe at wk 3, enzyme supplementation increased ($P < 0.05$) the SID of all AA except for Asp+Asn at wk 2 and Met at wk 3. Regardless of diet, SID increased with age ($P < 0.05$) except for Ser, His, Thr, Val, Met, and Ala. These results indicate the ability of a novel mixture of carbohydrases to increase aME of ME-deficient diets containing 20% DDGS. Further, a lag in AWG response at equal aME suggests a potential metabolic adaptation to the use of reducing sugars freed by carbohydrases for growth.

## 3.2 Introduction

The production of distillers dried grains with solubles (DDGS) has continued to increase and is estimated that by 2011-2012, approximately 1.16 million metric tons of corn DDGS will be used by the U.S. poultry industry (Wisner, 2008). While DDGS is economically favorable as feed ingredient for broiler diets, concerns regarding high degree of variability in the physical, chemical, and nutritional properties have limited its use (Cromwell et al., 1993).

The three most expensive components provided in animal feeds are energy, amino acid (AA), and phosphorus. For poultry, 1 unit of corn DDGS can replace 0.59 unit of corn, 0.45 unit of soybean meal (SBM), and 0.02 unit of inorganic phosphate, with small additions of synthetic AA, fat and calcium supplement (Shurson, 2009). However, higher levels of corn DDGS use may result in high supplemental fat levels due to its low metabolizable energy (ME) value.

The total non-starch polysaccharide (NSP) content of corn DDGS is 22.7% (Ward et al., 2008), which is higher than corn and SBM at 6.1% and 12.6%, respectively (Cowieson and

Adeola, 2005). The high NSP content of corn DDGS is partially responsible for its low ME. Inclusion of carbohydrases have been shown to increase apparent ME (aME) in broiler chickens fed corn-SBM diets (Meng and Slominski, 2005; Saleh et al., 2005; Rutherfurd et al., 2007). Moreover, carbohydrases are also known to improve ileal AA digestibility in monogastric animals (Cowieson and Bedford, 2009). The objective of this study was to elucidate the effect of a novel mixture of carbohydrases on growth performance, aME, and ileal AA digestibility in broiler chicks fed an energy-restricted diet containing 20% corn DDGS.

## 3.3 Materials and methods

### 3.3.1 General

All animal procedures were approved by the Institutional Animal Care and Use Committee of Virginia Tech. A total of 216 one-day-old Cobb 500 broilers of mixed sex (Cobb Vantress Inc., Wadesboro, NC) were housed in a thermostatically controlled stainless steel brooder battery (Alternative Design, Siloam Springs, AR) divided into pens, each equipped with raised wire floors, height-adjustable nipple waterers, and feeder. The brooder battery was located in a discretely ventilated room with 100% clean air (i.e., no recirculation), under constant negative pressure, temperature controlled by an automated system, and under continuous fluorescent lighting. Broilers had ad libitum access to water and feed unless otherwise indicated. During the 7-d pre-experimental period a standard starter corn-soy-based diet was offered. On d 8 of age, broilers were food-deprived for 4 h, individually weighed, and assigned to 1 of 8 body weight (BW) groups. One broiler from each BW group was randomly assigned

to each pen (8 broilers/pen). Dietary treatments were randomly assigned to pens in each side of the brooder battery. A total of 6-7 pens were allocated to each dietary treatment. On d 8, 14 (wk 2), and 21 (wk 3) of age broilers were group-weighed and feeders were individually weighed to determine feed disappearance (i.e., referred as feed intake henceforth). Average weekly weight gain (AWG), average weekly feed intake (AWFI), and gain to feed ratio (G:F) were calculated to determine growth performance.

### 3.3.2 Diets

Diets were formulated to meet or exceed NRC (1994) nutrient recommendations except for ME. A control diet (CORN) was formulated to contain 3,000 kcal ME/kg whereas the 20% corn DDGS diet (DDGS) was formulated to contain 90% of the ME of CORN (i.e., 2,700 kcal ME/kg). The inclusion of 0.2% carbohydrase mixture to CORN (CORN+Enzymes) and DDGS (DDGS+Enzymes) was made at the expense of ground corn (**Table 3.1**). Chromic oxide was included at 0.4% as an indigestible marker. All diets were offered in mash form. Calculated carbohydrase (all from Bio-Cat Inc., Troy, VA) activity (unit/kg of diet) in the final mixed diet were: 20,360 for cellulase, 3,284 for hemicellulase, 99 for ß-glucanase, and 35,466 for xylanase.

### 3.3.3 Sample collection and chemical analyses

Total excreta were collected daily at 0800 on d 13, 14, and 15 for wk 2 and d 20, 21, and 22 for wk 3 and stored at -20ºC to determine aME. At the end of the study, excreta from each 3-d collection period were thawed combined and thoroughly mixed to obtain a representative sample, which was dried at 68ºC in a dry-heat oven for 72 h and ground in a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ) fitted with a 1 mm screen. Dried and ground excreta

samples were stored at 4ºC until analyzed for gross energy (GE).

On d 15 and 22, 4 birds from each pen were euthanized with $CO_2$ asphyxiation followed by cervical dislocation. The contents of the ileum, defined from Meckel's diverticulum to 1 cm above the ileocecal junction, were manually removed by gentle squeezing, immediately frozen in liquid nitrogen, freeze-dried (Genesis 25EL, VirTis, Gardiner, NY), pre-ground with a mortar and pestle, and ground with a Cyclotec 1093 Sample Mill (FOSS North America, Eden Prairie, MN) fitted with a 1 mm screen to determine apparent ileal AA digestibility. Feed samples were collected after mixing and at the end of wk 2 and 3. At the end of the trial, feed samples for each diet were combined, thoroughly mixed, and ground with a Cyclotec 1093 Sample Mill fitted with a 1 mm screen.

Chromic oxide in diets and digesta was analyzed as previously described (Fenton and Fenton, 1979). Briefly, samples were dry-ashed overnight at 450ºC and digested with a mixture containing 20 g/L of sodium molybdate dehydrate in a 3:3:4 (v/v/v) solution of concentrated sulfuric acid:water:70% perchloric acid on a hot plate. Digested samples were quantitatively diluted and an aliquot was transferred to 16×100 test tubes, which were centrifuged (5810R, Eppendorf, Hamburg, Germany) at 1,000×$g$ for 5 min at room temperature. Absorbency of supernatants was determined at 440 nm using PowerWave XS (Bio-Tek, Winooski, VT) microplate spectrophotometers.

Amino acid content in diets and digesta was determined using the Pico·Tag method as previously described (Albin et al., 2000; Cohen et al., 1989). Briefly, samples were hydrolyzed in 6 M HCl in screw-capped $N_2$-purged tubes at 110ºC in a dry oven (Isotemp Oven, Thermo Fisher Scientific Inc., Pittsburgh, PA) for 24 h. Resulting hydrolysates were syringe-filtered using 0.45 μm PTFE membrane (Acrodisc CR 13 mm, Pall Corp., Port Washington, NY) and

stored at -20ºC. Equal volumes of filtered hydrolysate and norleucine (2 mM in 0.1 M HCl) were combined, vacuum-dried (Acid-Resistant CentriVap Vacuum Concentrator, Labconco, Kansas City, MS) at 50ºC for 1 h, mixed with 10 μl of 2:2:1 (v/v/v) 1 M Na acetate:methanol:triethylamine, vacuum-dried, derivatized with 20 μl of 7:1:1:1 (v/v/v/v) methanol:water:triethylamine:phenyl isothiocyanate, incubated at room temperature for 20 min, vacuum-dried, and reconstituted in 100 μl of sample diluent [188 mg/L of $CaNa_2$·EDTA, 0.47 ml/L triethylamine, 17.86 g/L Na acetate trihydrate, and 6% (v/v) acetonitrile]. Derivatized AA were separated using an Alliance 2695 Separations Module equipped with a 3.9×300 mm Pico-Tag column, 2487 Dual λ Absorbance Detector (all from Waters Corp., Milford, MA) using previously described chromatographic solutions and conditions (Cohen et al., 1989). Chromatograms were collected, recorded, and analyzed with Empower 2 software (Waters Corp.). Amino acid concentrations are expressed in g/100 g of digesta or diet sample.

A Parr 1271 Automatic Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL) was used to determine the gross energy content of diets and excreta; benzoic acid was used as a standard according to manufacturer's specifications.

3.3.4 Calculations and statistical analyses

Apparent ME values were calculated using the following formula (Ravindran et al., 2001):

$$aME\ (kcal/kg) = [\ (\ Intake_{Feed} \times GE_{Feed}\ ) - (\ E \times GE_{Excreta}\ )\ ] / Intake_{Feed}$$

where $Intake_{Feed}$ is feed intake (g), $GE_{Feed}$ is the gross energy (GE) of the diet (kcal/kg), E is the excreta (g), and $GE_{Excreta}$ is the gross energy of excreta (kcal/kg).

Values for apparent energy metabolizability (AEM) and apparent ileal digestibility of amino acids (AID) were calculated based on the following equation (Dänicke et al., 2000; Stein

et al., 2007):

$$AID\ (\%) = \{ 1 - [ ( AA_{Digesta} / AAGE_{Feed} ) \times ( M_{Feed} / M_{Digesta} ) ] \} \times 100$$

$$AEM\ (\%) = \{ 1 - [ ( GE_{Excreta} / GE_{Feed} ) \times ( M_{Feed} / M_{Excreta} ) ] \} \times 100$$

where $AA_{Digesta}$ and $GE_{Excreta}$ is the AA [g/kg dry matter (DM)] or GE (kcal/kg DM) content in the ileal digesta or excreta, $AA_{Feed}$ $GE_{Feed}$ is the AA (g/kg DM) or GE (kcal/kg DM) content in the feed, $M_{Feed}$ is the chromic oxide concentration (g/kg DM) in the feed, and $M_{Digesta\ or\ excreta}$ is the chromic oxide concentration (g/kg DM) in the ileal digesta or excreta.

Standardized ileal amino acid digestibility (SID) was calculated using the following formula (Lemme et al., 2004):

$$SID\ (\%) = AID + [ (AA_{End} ) / ( AA_{Feed} ) \times 100 ]$$

where $AA_{End}$ is the basal endogenous AA losses (g/kg DM), and the $AA_{Feed}$ is the AA content of the feed (g/kg DM). Values for basal endogenous AA losses were adapted from a previous study obtained by the regression method using enzymatically hydrolyzed casein (Golian et al., 2008).

The experimental design was a completely randomized design blocked by side of the brooder battery in a 2 × 2 factorial arrangement of treatments (Kaps and Lamberson, 2004). Data were checked for outliers using the PROC UNIVARIATE procedure of SAS (SAS Institute, Cary, NC) and removed. To test main effects of DDGS inclusion or multicarbohydrase supplementation, all data were subjected to ANOVA using the PROC GLM procedure of SAS. A post-ANOVA was performed for independent treatments using the PROC MIXED procedure of SAS. Differences between least square means were tested using Tukey's multiple comparison procedure of SAS. Significance was declared at $P < 0.05$, and tendencies at $P < 0.10$. Pen was considered as the experimental unit.

## 3.4 Results

### 3.4.1 Growth performance

Growth performance results for wk 2, wk 3, and the combined 14-d trial are presented on **Table 3.2**. In wk 2, the main effect of enzyme supplementation was an increase ($P < 0.05$) in AWG (3.2%) and AWFI (2.8%) without affecting G:F regardless of diet. Inclusion of carbohydrases to CORN, however, tended to increase ($P = 0.06$) AWFI during wk 2. AWG and AWFI of broilers receiving CORN or CORN+Enzymes were higher ($P < 0.007$) than those consuming DDGS-containing diets regardless of enzyme inclusion. In wk 3, AWG and G:F of broilers receiving CORN or CORN+Enzymes were higher ($P < 0.009$) than those consuming DDGS-containing diets regardless of enzyme inclusion. Supplementation of basal diets with carbohydrases increased ($P < 0.006$) AWG (8.1%) and AWFI (3.9%), and tended to increase ($P = 0.06$) G:F by 3.2%. Most importantly, enzyme supplementation increased ($P = 0.05$) AWG by 8.0% of broilers consuming the energy-deficient DDGS-containing diet. Overall growth performance of broilers consuming CORN and CORN+Enzymes, both adequate in ME, was better ($P < 0.0004$) than those consuming the DDGS-containing diets. Nevertheless, inclusion of carbohydrase increased ($P < 0.0004$) AWG and AWFI and tended to increase ($P = 0.10$) G:F during the entire 14-d trial.

### 3.4.2 Dietary energy content and utilization

Measured aME of CORN was close to the calculated value (**Tables 3.1** and **3.3**) and higher ($P < 0.0001$) than DDGS. Energy content of DDGS diet, however, was about 83% of the CORN perhaps due to an overestimation of the ME value of corn DDGS. Inclusion of

carbohydrase mixture increased ($P < 0.01$) aME by 9.5% during wk 2 and by 11.9% during wk 3 without affecting the energy content of CORN diets. The aME of DDGS+Enzymes was lower ($P = 0.004$) compared to CORN during wk 2 but this difference disappeared ($P = 0.31$) by the end of wk 3. Despite the differences in dietary aME content, AEM was not different ($P = 0.44$ to 0.99) among CORN, CORN+Enzymes, and DDGS+Enzymes and lower ($P < 0.0001$) for DDGS during both wks of the study. Finally, both aME and AEM increased ($P < 0.02$) from wk 2 to wk 3 of the study regardless of dietary treatment.

3.4.3 Amino acid digestibility

Standardized ileal AA digestibility of broiler chicks fed CORN or DDGS diets supplemented with or without a mixture of a novel mixture of carbohydrases at wk 2 and wk 3 are shown on **Tables 3.6** and **3.7**, respectively. Multicarbohydrase inclusion increased ($P < 0.05$) overall SID of all AA with the exception of Asp+Asn at wk 2 and Met at wk 3. Inclusion of enzymes did not affect the SID of chicks fed CORN-based diets for all AA in both wk 2 and 3. However, inclusion of carbohydrases improved the SID for all AA present in DDGS diets ($P < 0.05$) except for Met, Asp+Asn, and Glu+Gln in wk 2. In wk 3, the significant differences in SID of chicks fed corn DDGS diets observed during wk 2 were diminished except for His ($P = 0.02$) and Pro ($P = 0.02$). Overall, SID of chicks were not affected by inclusion of corn DDGS for all AA throughout the experiment except Leu ($P = 0.004$), Met ($P = 0.001$), and Phe ($P = 0.05$) at wk 3. Regardless of diet, SID increased with age ($P < 0.05$) except for Ser, His, Thr, Val, Met, and Ala.

### 3.5 Discussion

It has been well-studied as to how poultry productivity can benefit from inclusion of carbohydrases when given diets that contain fibrous ingredients that increase the viscosity of digesta (Campbell and Bedford, 1992; Jeroch et al., 1995; Simon, 1998). Ingredients high in NSP content generally increase the viscosity of digesta, which slows down the rate of digestion. The physical structure of the endosperm cell walls of these ingredients is also known to obstruct access to digestive enzymes. These constraints can be overcome effectively by using carbohydrases to degrade NSP and thus reduce viscosity and consequently increase the diffusivity of digesta, allowing a more rapid and complete digestion and absorption process. Thus, along with its ability to directly release oligosaccharides from otherwise insoluble fiber substrates, carbohydrases can increase the AA digestibility of diets that reduce digesta viscosity in broilers (Rutherfurd et al., 2007).

When given a typical corn-SBM diet that is sufficient in both ME and protein levels, the effects of carbohydrases are known to be less dramatic. Zanella et al. (1999) reported only a numerical increase in BW gain and AA digestibility in broilers given a corn-SBM diet supplemented with a mixture of xylanase, protease, and amylase. While current results showed a numerical increase in the AWG and AWFI of broilers fed CORN+Enzymes for wk 2, significant increases were observed in wk 3 and for overall combined period. This improvement in performance without an increment in aME or SID is therefore attributed to increased feed intake determined for CORN+Enzymes. Although we have not determined changes in digesta viscosity in the current study, it has been demonstrated that digesta viscosity is reduced with inclusion of carbohydrases in broilers given a typical corn-soy diet sufficient in

both ME and AA (Adibmoradi and Mehri, 2007). The effect of carbohydrases on corn-SBM diet seems to be inconsistent regarding feed intake (Kocher et al., 2002; Jackson et al., 2004; Tahir et al., 2008), possibly due to the high variability of corn (Leeson et al., 1993; Collins et al., 1998). Nevertheless, a decrease in digesta viscosity should facilitate gastric emptying (Spiller, 1994), leading to higher feed intake, hence, resulting in an increase in weight gain.

In the current study, 20% corn DDGS depressed AWG and G:F of broiler regardless of carbohydrase supplementation in wk 2 and 3. This is in agreement to study by Lumpkins et al. (2004) where an inclusion level of 15% corn DDGS was concluded to be excessive for broiler starters. However, in contrast to their study, diets formulated for the present experiment were supplemented with exogenous Lys to prevent marginal Lys deficiency. Wang et al. (2008) reported a decline in growth performance in broiler chicks when energy-deficient diets with corn DDGS content of 15% or more were given. On the other hand, Parsons and Baker (1983) have shown that even more than 25% corn DDGS could be used in broiler starter diets if Lys and dietary energy levels were sufficient. Since our experimental DDGS diets were deficient only in ME, and not AA, the measured growth depression in broilers given DDGS diets should be first attributed to insufficient energy content of the diet and then to any potential detrimental effects of high NSP content on nutrient digestibility.

While energy-restricted 20% corn DDGS diets had detrimental effects on the growth performance of broilers regardless of enzyme inclusion, DDGS+Enzymes did increase the AWG of broilers by 8.3% compared to DDGS alone at wk 3. This result is in agreement to Moran and Lehman (2008) where a mixture of xylanase, amylase, protease and phytase improved BW gain of broilers given a energy-restricted 10% DDGS diet by 12.3% at wk 8. Our results share a similar context as inclusion of carbohydrases to a 20% corn DDGS diet improved AWG by 8.0%

and aME by 11.7%, which made this diet comparable in ME content to CORN or CORN+Enzymes at wk 3.

The corn DDGS used in current study had similar (Pahm et al., 2009) or lower (Lumpkins et al., 2004; Wang et al., 2008) AA concentration compared to values reported in previous studies. Moreover, the SID of the broilers given corn DDGS was lower than values reported by Pahm et al. (2009) except for Lys.

As described in a recent review (Cowieson, 2010), SID of a specific AA is positively correlated ($R^2 = 0.65$; $P < 0.001$) with its response to exogenous carbohydrase supplementation. Again, carbohydrases can be expected to have greater impact on the SID of AA with lower digestibility coefficients. Indeed, our results showed that AA with lower SID respond more to carbohydrase supplementation. Namely, while Thr (lowest in overall SID) showed an overall 24.4% increase in SID when enzyme was supplemented, Met (highest in overall SID) increased by only 3.8%. Additionally, while the SID of CORN (relatively higher SID) displayed only numerical increases in response to enzyme, significant increases were observed in DDGS (relatively lower SID). Similar improvements in AA digestibility with carbohydrase supplementation have been reported in chicks when given diets based on viscous/fibrous grains like barley (Perttilä et al., 2001) and wheat (Hew et al., 1998; Ravindran et al., 1999), wheat DDGS-barley diets in pigs (Emiola et al., 2009), and in wheat-rye-barley in turkeys (Boguhn and Rodehutscord, 2010).

The increase in AWG of broilers given DDGS+Enzymes was not proportional to the significant improvement aME and SID. This finding suggests three main points. First, extra energy can be extracted from fibrous dietary feed ingredients and used for metabolic processes. Second, carbohydrases do have limitations to increase nutrient digestibility and bioavailability.

And third, a lag in AWG response to improved aME suggests a metabolic adaptation of broiler chicks to the "new" source of energy for growth.

Exogenous enzymes are known to be more effective for younger birds, before their microflora is fully established (Olukosi et al., 2007) which is known to be at approximately wk 3 of age (Kirjavainen and Gibson, 1999). As studied in pigs (Adeola and King, 2006) and domestic fowl (Gonzalez and Vinardell, 1996), digestive and absorptive capacities of the intestine for young animals are under-developed. We speculate that the premature absorptive capacity of the intestine may have resulted in a lag of AWG response in the current study.

### 3.6 Conclusion

In conclusion, the addition of a novel carbohydrase mixture improved the growth rate of broilers that were fed an energy-restricted diet with 20% corn DDGS inclusion. The improvement in AWG is likely accounted for an increase in aME induced by the novel carbohydrase mixture. While significant improvements in SID were observed, it is likely that the extra digested AA contributed more as a source of energy rather than protein building blocks. The current study also confirms that the magnitude of response of SID to carbohydrase supplementation is greater when ingredients with low SID are present in the diet. Finally, we demonstrate a lag of AWG response to carbohydrase-induced increase in aME increment, suggesting an adaptation period of the intestine to the extra contributors of energy supply for growth.

### 3.7 Tables

Table 3.1. Diet formulation and calculated nutrient composition of experimental diets, as fed basis

| | CORN | CORN + Enzyme | DDGS | DDGS + Enzyme |
|---|---|---|---|---|
| Ingredients, % | | | | |
| Corn | 58.14 | 57.94 | 44.59 | 44.39 |
| Soybean meal (47.5%) | 34.80 | 34.80 | 25.00 | 25.00 |
| Corn DDGS | - | - | 20.00 | 20.00 |
| Sand | - | - | 5.44 | 5.44 |
| Soybean oil | 2.03 | 2.03 | - | - |
| Monocalcium phosphate | 1.56 | 1.56 | 1.34 | 1.34 |
| Limestone | 1.73 | 1.73 | 1.82 | 1.82 |
| DL-Methionine | 0.50 | 0.50 | 0.47 | 0.47 |
| L-Lysine-HCl | 0.13 | 0.13 | 0.39 | 0.39 |
| L-Threonine | 0.02 | 0.02 | 0.08 | 0.08 |
| Vitamin premix[1] | 0.11 | 0.11 | 0.10 | 0.10 |
| Mineral premix[2] | 0.12 | 0.12 | 0.12 | 0.12 |
| Salt | 0.46 | 0.46 | 0.25 | 0.25 |
| Enzyme[3] | - | 0.20 | - | 0.20 |
| Chromic oxide | 0.40 | 0.40 | 0.40 | 0.40 |
| | | | | |
| Calculated nutrients | | | | |
| ME, kcal/kg | 3000.00 | 3000.00 | 2700.00 | 2700.00 |
| CP, % | 22.28 | 22.28 | 22.10 | 22.10 |
| Ca, % | 1.00 | 1.00 | 1.00 | 1.00 |
| Non-phytate P, % | 0.45 | 0.45 | 0.45 | 0.45 |
| Lysine, % | 1.32 | 1.32 | 1.34 | 1.34 |
| $_{TD}$Lysine, %[4] | 1.19 | 1.19 | 1.18 | 1.18 |
| Methionine, % | 0.58 | 0.58 | 0.58 | 0.58 |
| $_{TD}$Methionine, %[4] | 0.55 | 0.55 | 0.54 | 0.54 |
| Threonine, % | 0.87 | 0.87 | 0.90 | 0.90 |
| $_{TD}$Threonine, %[4] | 0.77 | 0.77 | 0.76 | 0.76 |
| Tryptophan, % | 0.27 | 0.27 | 0.23 | 0.23 |
| $_{TD}$Tryptophan, %[4] | 0.23 | 0.23 | 0.20 | 0.20 |

[1] Provided the following per kg of diet: Vitamin A, 3,227 IU; Vitamin D3, 445 IU; Vitamin E, 19 IU; Vitamin K, 3 mg; biotin, 0.14 mg; choline, 242 mg; folic acid, 0.61 mg, niacin, 14 mg; D-pantothenic acid, 9 mg; riboflavin, 3 mg; and B12, 0.01 mg (from 77320014, ADM, Decatur, IL)

[2] Provided the following as mg/kg of diet: Zn, 144 mg from ZnSO4; Fe, 144 mg from $FeSO_4$; Mn, 44 mg from $MnSO_4$; Cu, 9 mg $CuSO_4$; and Se, 0.2 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[3] Carbohydrase mixture provided per kg of diet: cellulase, 20,360 unit (from *Trichoderma longibrachiatum*); hemicellulase, 3,284 unit (from *Aspergillus niger*); ß-glucanase, 99 unit (from *Trichoderma longibrachiatum*); and xylanase, 35,466 unit (from *Trichoderma longibrachiatum*); all carbohydrase from Bio-Cat Inc., Troy, VA

[4] True digestibility values

Table 3.2. Growth performance of broiler fed diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase at week 2 and week 3[1]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Week 2 | | | | | | | | |
| AWG[3] | 248.1[a] | 253.7[a] | 214.5[b] | 223.7[b] | 3.5 | < 0.0001 | 0.0469 | 0.6126 |
| AWFI[4] | 304.3[ab] | 316.4[a]* | 298.7[b] | 303.7[b] | 3.1 | 0.0075 | 0.0118 | 0.2770 |
| G:F[5] | 816[a] | 802[a] | 719[b] | 734[b] | 7 | < 0.0001 | 0.9059 | 0.0442 |
| Week 3 | | | | | | | | |
| AWG[3] | 312.4[b] | 338.4[a] | 262.1[d] | 283.0[c] | 5.3 | < 0.0001 | 0.0003 | 0.6382 |
| AWFI[4] | 488.2[b] | 515.1[a] | 477.8[b] | 489.1[ab] | 7.6 | 0.0086 | 0.0061 | 0.2292 |
| G:F[5] | 634[a] | 653[a] | 554[b] | 573[b] | 9 | < 0.0001 | 0.0593 | 0.9801 |
| Overall | | | | | | | | |
| AWG[3] | 280.3[b] | 296.0[a] | 238.3[d] | 253.4[c] | 3.2 | < 0.0001 | < 0.0001 | 0.6121 |
| AWFI[4] | 398.0[b] | 417.3[a] | 389.9[b] | 398.2[b] | 3.7 | 0.0004 | 0.0004 | 0.1281 |
| G:F[5] | 725[a] | 728[a] | 636[b] | 654[b] | 6 | < 0.0001 | 0.1015 | 0.2191 |

[a-d] Least square means within a row with no common superscript differ at $P < 0.05$; * differ from CORN at $P = 0.06$.
[1] Least square means represent 6-7 pens of 8 chicks per treatment for week 2 and 4 chicks per treatment for week 3.
[2] Pooled standard error of the mean
[3] Average weekly weight gain, g
[4] Average weekly feed intake, g
[5] Gain to feed ratio, g/kg

Table 3.3. Apparent metabolizable energy (aME) and apparent energy metabolizability (AEM) diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase fed to of broiler chicks at week 2 and week 3[1]

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | SEM[2] | DDGS | Enzymes | DDGS × Enzymes |
| Week 2 | | | | | | | | |
| aME[3] | 3,089[a] | 3,039[a] | 2,505[c] | 2,742[b] | 60 | < 0.0001 | 0.0796 | 0.0102 |
| AEM[4] | 78.2[a] | 78.1[a] | 68.5[b] | 74.8[a] | 1.6 | < 0.0001 | 0.0278 | 0.0263 |
| Week 3 | | | | | | | | |
| aME | 3,073[a] | 3,075[a] | 2,610[b] | 2,920[a] | 75 | < 0.0001 | 0.0183 | 0.0201 |
| AEM | 77.8[a] | 79.1[a] | 71.5[b] | 79.7[a] | 2.0 | 0.1017 | 0.0089 | 0.0485 |

[a-c] Least square means within a row with no common superscript differ at $P < 0.05$.
[1] Least square means represent 6-7 pens of 8 chicks per treatment for week 2 and 4 chicks per treatment for week 3.
[2] Pooled standard error of the mean
[3] Apparent metabolizable energy, kcal/kg of diet
[4] Apparent energy metabolizability, %

Table 3.4. Apparent ileal amino acid digestibility (%) of diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase fed to broiler chicks at week 2[1]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable | | | | | | | | |
| Arg | 79.0$^{ab}$ | 83.3$^{a}$ | 76.5$^{b}$ | 83.9$^{a}$ | 1.48 | 0.4783 | 0.0004 | 0.2566 |
| His | 71.0$^{bc}$ | 76.7$^{ab}$ | 67.0$^{c}$ | 80.4$^{a}$ | 2.50 | 0.9891 | 0.0004 | 0.0920 |
| Ile | 62.9$^{b}$ | 69.1$^{ab}$ | 61.4$^{b}$ | 73.0$^{a}$ | 2.98 | 0.6619 | 0.0032 | 0.3219 |
| Leu | 66.8$^{b}$ | 72.2$^{ab}$ | 69.1$^{b}$ | 79.5$^{a}$ | 2.59 | 0.0525 | 0.0029 | 0.2987 |
| Lys | 73.6$^{ab}$ | 79.3$^{a}$ | 71.0$^{b}$ | 79.0$^{a}$ | 1.90 | 0.4291 | 0.0009 | 0.5070 |
| Met | 87.9$^{a}$ | 91.7$^{a}$ | 80.9$^{b}$ | 86.5$^{ab}$ | 1.50 | 0.0007 | 0.0056 | 0.5553 |
| Phe | 68.5$^{b}$ | 73.5$^{ab}$ | 69.0$^{b}$ | 78.5$^{a}$ | 2.57 | 0.2447 | 0.0052 | 0.3465 |
| Thr | 53.6$^{bc}$ | 65.0$^{ab}$ | 51.8$^{c}$ | 68.9$^{a}$ | 3.13 | 0.7316 | 0.0002 | 0.3735 |
| Val | 59.7$^{b}$ | 66.4$^{ab}$ | 58.6$^{b}$ | 71.5$^{a}$ | 3.19 | 0.4984 | 0.0027 | 0.2895 |
| Dispensable | | | | | | | | |
| Ala | 63.8$^{b}$ | 70.3$^{ab}$ | 65.0$^{b}$ | 77.2$^{a}$ | 2.81 | 0.1254 | 0.0014 | 0.2869 |
| Asp+Asn | 76.4 | 79.9 | 73.9 | 78.1 | 2.13 | 0.2817 | 0.0559 | 0.8464 |
| Glu+Gln | 77.9$^{ab}$ | 82.1$^{ab}$ | 75.8$^{b}$ | 82.4$^{a}$ | 1.85 | 0.6057 | 0.0038 | 0.4987 |
| Gly | 54.7$^{b}$ | 63.3$^{ab}$ | 51.4$^{b}$ | 67.2$^{a}$ | 3.54 | 0.9131 | 0.0010 | 0.2746 |
| Pro | 62.7$^{b}$ | 69.5$^{ab}$ | 62.8$^{b}$ | 76.7$^{a}$ | 2.77 | 0.1605 | 0.0005 | 0.1768 |
| Ser | 60.4$^{bc}$ | 70.5$^{ab}$ | 59.2$^{c}$ | 74.1$^{a}$ | 2.87 | 0.6557 | < 0.0001 | 0.3706 |

[a-c] Least square means within a row with no common superscript differ at $P < 0.05$.
[1] Least square means represent 6-7 cages of 4 chicks per treatment.
[2] Pooled standard error of the mean

Table 3.5. Apparent ileal amino acid digestibility (%) of diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase fed to broiler chicks at week 3[1]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable | | | | | | | | |
| Arg | 82.7 | 85.0 | 81.0 | 86.7 | 1.58 | 0.9861 | 0.0152 | 0.2728 |
| His | 72.2$^{b}$ | 77.5$^{a}$ | 71.2$^{b}$ | 81.6$^{a}$ | 2.11 | 0.4539 | 0.0011 | 0.2304 |
| Ile | 66.7$^{b}$ | 69.9$^{ab}$ | 68.5$^{ab}$ | 77.7$^{a}$ | 2.51 | 0.0626 | 0.0190 | 0.2234 |
| Leu | 70.8$^{b}$ | 73.5$^{b}$ | 75.3$^{ab}$ | 83.7$^{a}$ | 2.20 | 0.0025 | 0.0172 | 0.1967 |
| Lys | 77.5 | 81.1 | 76.6 | 81.4 | 1.83 | 0.8700 | 0.0270 | 0.7395 |
| Met | 89.1$^{ab}$ | 91.0$^{a}$ | 84.5$^{b}$ | 84.9$^{b}$ | 1.45 | 0.0007 | 0.4020 | 0.5806 |
| Phe | 72.2$^{b}$ | 74.9$^{b}$ | 74.3$^{b}$ | 82.9$^{a}$ | 2.12 | 0.0227 | 0.0127 | 0.1618 |
| Thr | 54.6$^{b}$ | 62.2$^{ab}$ | 55.2$^{b}$ | 68.4$^{a}$ | 3.33 | 0.3045 | 0.0045 | 0.3897 |
| Val | 62.3$^{b}$ | 66.7$^{ab}$ | 64.4$^{ab}$ | 74.3$^{a}$ | 2.79 | 0.0887 | 0.0156 | 0.3103 |
| Dispensable | | | | | | | | |
| Ala | 68.4$^{b}$ | 71.0$^{ab}$ | 70.1$^{b}$ | 79.4$^{a}$ | 2.59 | 0.0416 | 0.0188 | 0.1651 |
| Asp+Asn | 75.8 | 82.2 | 79.3 | 83.6 | 2.18 | 0.2646 | 0.0224 | 0.6539 |
| Glu+Gln | 79.9 | 83.3 | 80.8 | 86.5 | 1.74 | 0.2374 | 0.0139 | 0.4889 |
| Gly | 60.2 | 64.6 | 59.8 | 71.2 | 3.08 | 0.3085 | 0.0155 | 0.2441 |
| Pro | 68.1$^{b}$ | 71.9$^{ab}$ | 69.3$^{b}$ | 80.0$^{a}$ | 2.38 | 0.0556 | 0.0051 | 0.1505 |
| Ser | 61.1$^{b}$ | 68.1$^{ab}$ | 62.1$^{b}$ | 73.8$^{a}$ | 2.97 | 0.2552 | 0.0041 | 0.4134 |

[a-c] Least square means within a row with no common superscript differ at $P < 0.05$.
[1] Least square means represent 6-7 cages of 4 chicks per treatment.
[2] Pooled standard error of the mean

Table 3.6. Standardized ileal amino acid digestibility (%) of diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase fed to broiler chicks at week 2[1,2]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable | | | | | | | | |
| Arg | 80.5[ab] | 84.7[a] | 77.9[b] | 85.1[a] | 1.48 | 0.4238 | 0.0004 | 0.2757 |
| His | 72.2[bc] | 77.7[ab] | 68.1[c] | 81.3[a] | 2.50 | 0.8928 | 0.0005 | 0.1015 |
| Ile | 66.6[ab] | 72.8[ab] | 64.8[b] | 76.0[a] | 2.99 | 0.7916 | 0.0040 | 0.3603 |
| Leu | 68.8[b] | 74.2[ab] | 70.8[b] | 80.9[a] | 2.59 | 0.0783 | 0.0033 | 0.3339 |
| Lys | 75.4[ab] | 81.0[a] | 72.6[b] | 80.7[a] | 1.90 | 0.3827 | 0.0009 | 0.5034 |
| Met | 89.1[a] | 92.8[a] | 82.4[b] | 88.1[ab] | 1.64 | 0.0011 | 0.0055 | 0.5076 |
| Phe | 72.9[b] | 77.9[ab] | 73.0[b] | 81.9[a] | 2.57 | 0.3815 | 0.0068 | 0.4051 |
| Thr | 60.8[bc] | 72.1[ab] | 57.8[c] | 74.4[a] | 3.43 | 0.9179 | 0.0002 | 0.4017 |
| Val | 63.4[b] | 70.1[ab] | 61.9[b] | 74.4[a] | 3.19 | 0.6347 | 0.0033 | 0.3226 |
| Dispensable | | | | | | | | |
| Ala | 66.6[b] | 73.2[ab] | 67.4[b] | 79.2[a] | 2.81 | 0.1966 | 0.0016 | 0.3145 |
| Asp+Asn | 78.8 | 82.2 | 76.2 | 80.2 | 2.14 | 0.2414 | 0.0633 | 0.8800 |
| Glu+Gln | 79.8 | 84.1 | 77.6 | 83.9 | 1.85 | 0.4866 | 0.0047 | 0.5380 |
| Gly | 58.7[b] | 67.2[ab] | 55.0[b] | 70.5[a] | 3.54 | 0.9533 | 0.0012 | 0.2842 |
| Pro | 65.5[b] | 72.4[ab] | 65.1[b] | 78.6[a] | 2.77 | 0.2600 | 0.0006 | 0.1960 |
| Ser | 66.8[bc] | 76.8[ab] | 64.7[c] | 78.9[a] | 2.86 | 0.9892 | 0.0001 | 0.4313 |

[a-c] Least square means within a row with no common superscript differ at $P < 0.05$.

[1] Least square means represent 6-7 cages of 4 chicks per treatment.

[2] Standardized ileal digestibility values were calculated using basal ileal endogenous AA losses estimates (g/kg of DM intake) for enzymatically hydrolyzed casein adapted from Golian et al., 2008.

[3] Pooled standard error of the mean

Table 3.7. Standardized ileal amino acid digestibility (%) of diets containing corn or 20% corn DDGS supplemented with or without a mixture of carbohydrase fed to broiler chicks at week 3[1,2]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable | | | | | | | | |
| Arg | 84.1 | 86.5 | 82.3 | 88.0 | 1.58 | 0.9401 | 0.0164 | 0.2845 |
| His | 73.4[b] | 78.8[ab] | 72.3[b] | 82.5[a] | 2.11 | 0.5242 | 0.0012 | 0.2514 |
| Ile | 70.4[b] | 73.6[ab] | 71.9[ab] | 80.7[a] | 2.50 | 0.0924 | 0.0231 | 0.2515 |
| Leu | 72.8[b] | 75.5[b] | 77.0[ab] | 85.1[a] | 2.20 | 0.0043 | 0.0208 | 0.2167 |
| Lys | 79.3 | 82.9 | 78.2 | 83.0 | 1.82 | 0.7852 | 0.0256 | 0.7351 |
| Met | 90.4[ab] | 92.1[a] | 85.9[b] | 86.4[b] | 1.46 | 0.0012 | 0.4053 | 0.6528 |
| Phe | 76.7[b] | 79.3[ab] | 78.4[ab] | 86.4[a] | 2.13 | 0.0463 | 0.0177 | 0.2019 |
| Thr | 61.9 | 69.2 | 61.2 | 73.8 | 3.33 | 0.5520 | 0.0057 | 0.4163 |
| Val | 66.0[b] | 70.4[ab] | 67.6[ab] | 77.2[a] | 2.78 | 0.1313 | 0.0176 | 0.3401 |
| Dispensable | | | | | | | | |
| Ala | 71.2[b] | 73.9[ab] | 72.4[ab] | 81.4[a] | 2.59 | 0.0756 | 0.0222 | 0.1875 |
| Asp+Asn | 78.2 | 84.6 | 81.6 | 85.8 | 2.18 | 0.3039 | 0.0247 | 0.6213 |
| Glu+Gln | 81.8 | 85.2 | 82.5 | 88.0 | 1.75 | 0.3169 | 0.0160 | 0.5262 |
| Gly | 64.1 | 68.4 | 63.3 | 74.4 | 3.07 | 0.3971 | 0.0174 | 0.2613 |
| Pro | 70.9[b] | 74.8[ab] | 71.6[b] | 81.9[a] | 2.38 | 0.1036 | 0.0060 | 0.1764 |
| Ser | 67.5 | 74.3 | 67.7 | 78.6 | 2.97 | 0.4427 | 0.0059 | 0.4757 |

[a-b] Least square means within a row with no common superscript differ at $P < 0.05$.

[1] Least square means represent 6-7 cages of 4 chicks per treatment.

[2] Standardized ileal digestibility values were calculated using basal ileal endogenous AA losses estimates (g/kg of DM intake) for enzymatically hydrolyzed casein adapted from Golian et al., 2008.

[3] Pooled standard error of the mean

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# CHAPTER 4

## Amino acid digestibility and energy utilization of a high corn DDGS diet supplemented with a novel mixture of carbohydrases in ileal cannulated pigs

### 4.1. Abstract

Increasing availability of corn distillers dried grains with solubles (DDGS) to the swine industry has resulted in development of strategies to overcome nutritional limitations of its high non-starch polysaccharide content. A novel mixture of carbohydrases was supplemented to pigs fed a high corn DDGS diet. Ileal cannulated, growing pigs (n = 8, BW = 64.3 ± 0.5 kg) were allotted to 4 dietary treatments in a replicated 4 × 4 Latin Square design. Control (CORN) and DDGS (40%) diets were supplemented or not with a mixture of carbohydrases. Higher metabolizable energy (ME) was measured for DDGS diets ($P < 0.0001$). However, increased fecal and urinary energy output ($P < 0.0001$) of pigs fed DDGS diets resulted in lower apparent energy metabolizability (AEM) compared to corn diets. Apparent ileal amino acid (AA) digestibility (AID) coefficients were lower for Arg, His, Ile, Lys, Phe, Asx, Glx, Gly, and Ser ($P < 0.04$), or tended to be lower for Thr and Val ($P < 0.09$) in pigs offered DDGS diets. Standardized ileal AA digestibility (SID) coefficients in DDGS diets were also lower for all measured AA ($P < 0.04$), except Leu, Met, Ala, Glx, and Tyr. Supplementation of carbohydrases did not affect dietary ME or AEM of pigs ($P = 0.85$ and 0.48, respectively). There was a trend for an interaction indicating that carbohydrase supplementation reduced the

urinary energy output of pigs when fed DDGS diet ($P < 0.09$). Carbohydrase mixture supplementation improved the AID of Arg, Met, Ser, Glx, and Tyr ($P \leq 0.05$), or tended to increase the AID of Ile, Phe, and Thr ($P < 0.09$). Improvements in SID were measured for Arg, Ile, Met, Phe, Thr, Ala, Glx, Ser, and Tyr ($P \leq 0.04$), while Leu, Lys, Val, and Gly showed improvement tendencies in response to carbohydrases ($P < 0.10$). Although not significant, numeric increases for SID of AA, along with a decreased tendency of urinary energy output suggested a possibility for improved nutrient utilization in pigs when carbohydrases were supplemented to 40% corn DDGS diet. Finally, carbohydrase seems less effective for swine application due to greater water content and consequently a lower viscosity of pig digesta.

## 4.2 Introduction

The increased production of corn distillers dried grains with solubles (DDGS) has lead to increased use in the livestock feed industry. Though the majority of corn DDGS is used for ruminant feed, it is projected that by 2012-2013, approximately 2.21 million metric tons of corn DDGS will be used by the U.S. swine feed industry (Wisner, 2008). The total non-starch polysaccharide (NSP) content of corn DDGS (22.7%, Ward et al., 2008) is higher than corn or soybean meal (SBM, 6.1% and 12.6%, respectively; Cowieson and Adeola, 2005). Monogastrics lack endogenous enzymes to digest NSP, which at high levels can be considered anti-nutritional factors because it reduces voluntary feed intake and nutrient digestibility (Kyriazakis and Emmans, 1995; Zijlstra et al., 1999). Supplementation of exogenous dietary enzymes has been widely practiced to overcome such matters, especially in poultry.

The use of carbohydrases has shown inconsistent effects in swine diets with high NSP content. Previous studies have reported improvements in growth performance (Barrera et al., 2004; Kiarie et al., 2007), energy utilization (Diebold et al., 2004; Yin et al., 2000), or amino acid (AA) digestibility (Emiola et al., 2009; Nitrayová et al., 2009; Vahjen et al., 2007), whereas others have shown no responses in body weight (BW) gain (Mavromichalis et al., 2000; Woyengo et al., 2008), energy (Olukosi et al., 2007), or AA digestibility (Yáñez et al., 2011).

Although previous studies have been conducted using a carbohydrase in swine diets with corn DDGS, there seems to be an opportunity of improvement as the effect of enzyme supplementation should increase with higher levels of corn DDGS inclusion. The objective of this study was to elucidate the effect of a novel mixture of carbohydrases on apparent metabolizable energy (aME) and ileal AA digestibility in growing pigs fed a diet containing 40% corn DDGS.

## 4.3 Materials and methods

### 4.3.1 General

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. A total of 8 pigs were obtained from the Swine Center at Virginia Tech (Blacksburg, VA) at approximately 93 d of age (BW = 47.0 ± 1.5 kg). Pigs were fitted with a simple T-cannula at the distal ileum, approximately 5 cm cranial to the ileo-cecal junction. A detailed description of cannula preparation, surgical procedure and postsurgical care was previously given by Wubben et al. (2001). One ml of ceftiofur HCl (Excenel RTU, 50 mg

ceftiofur/ml, Pfizer Animal Health, Fort Dodge, IA) was administered per 10 kg BW i.m. for 3 d following surgery. Neomycin sulfate (NeoMed 325, 0.5 g neomycin/g, Bimeda, Inc., Oakbrook terrace, IL) and carprofen (Rimadyl, 75 mg carprofen/tbl, Pfizer Animal Health, Fort Dodge, IA) were ground and mixed into feed for 6 d post surgery at 2 g and 300 mg per day, respectively. Pigs were allowed to recover for 3 wk in individual pens (1.5 × 1.5 m) equipped with a feeder and nipple waterer. Feed allotments were increased in 200 g increments over 7 d until a daily intake of 1.4 kg was attained. Skin sutures were removed 13 d after surgery. After a 3-wk recovery period, the average BW of the pigs increased to 51.0 ± 1.4 kg. Following the recovery period, feed allotments were calculated as 3 times the estimated energy requirement for maintenance (i.e., 106 kcal ME/kg $BW^{0.75}$; NRC, 1998), divided into 2 equal meals given at 0800 and 1600. The facility was independently ventilated with 100% clean air (i.e., no recirculation) under negative pressure. Temperature (24°C) and lighting (18-h light:6-h dark with lights on at 0600) were controlled by automated systems.

4.3.2 Sample collection and experimental design

Basal endogenous AA losses (EAAL) were obtained from two observations with a N-free diet (NFD) at 135 d of age (n = 8, BW = 53.3 ± 1.7 kg) and at 309 d of age (n = 4, 76.0 ± 1.4 kg). Following 5-d adaptation period, digesta were collected for 2 d from 0800 to 1800 into plastic bags attached to the cannula with a rubber band. The plastic bags were changed when full or at least every 30 min to minimize bacterial fermentation. Digesta were immediately poured into glass jars stored in -20°C. Digesta were later thawed at 4°C, homogenized (Waring Commercial CB-5, Waring Products Inc., Stamford, CT), sub-sampled, freeze dried (Lyph-Lock

12, Labconco Corp., Kansas City, MI), ground (BCG100WH, KitchenAid, St. Joseph, MI), and stored at -20°C until analyses.

On d 191 of age, pigs (BW = 64.3 ± 0.5 kg) were moved into metabolic cages (0.6 × 1.4 m) and allotted to 4 dietary treatments in 4 × 4 Latin Square design involving 4 periods with 2 replicates. Each period totaled 16 d, consisting of a 7-d washout period (fed a typical corn-SBM diet without chromic oxide), a 5-d acclimation period to the experimental diet, a 2-d digesta collection period, and a 2-d feces/urine collection period. Metabolic cages were adjusted to immobilize pigs at 1800 on the day before feces/urine collection. Urine was collected for 2 d into urine collectors containing 100 ml of 6 N HCl placed under the collection drain of each cage. Urine was collected daily, measured, diluted with water to a constant volume, thoroughly mixed, and a representative aliquot was collected and stored at -20°C until subsequent analyses. Feces were also collected daily and stored at -20°C. At the end of the study, feces from each 2-d collection period were thawed, combined and thoroughly mixed to obtain a representative sample, which was dried at 68ºC in a dry-heat oven (Memmert oven 800, Wisconsin Ovens Corp., East Troy, WI) for 72 h, ground (BCG100WH, KitchenAid, St. Joseph, MI), and stored at -20°C until analyses.

4.3.3 Dietary treatments

The ingredient and the calculated nutrient composition of all diets used in current study are shown in **Table 4.1** (NFD and washout diet) and **Table 4.2** (experimental diets). The N-free diet was formulated based on cornstarch and sucrose. Solka-Floc was used as source of fiber and vitamins $B_1$ and $B_6$ (Vitamin World Inc., Ronkonkoma, NY) were supplemented to meet NRC recommendations. All other diets were formulated to meet or exceed NRC (1998)

nutrients recommendations except for metabolizable energy (ME). The control diet (CORN) was formulated to contain approximately 3,370 kcal ME/kg whereas the 40% corn DDGS diet (DDGS) was formulated to contain 92% of the ME of CORN (i.e., approximately 3,100 kcal ME/kg). The inclusion of 0.2% carbohydrase mixture to CORN (CORN+Enzymes) and DDGS (DDGS+Enzymes) was made at the expense of ground corn. Chromic oxide was included at 0.4% as an indigestible marker. Calculated carbohydrase (all from Bio-Cat Inc., Troy, VA) activities (unit/kg of diet) in the final mixed diet were: 25,085 for cellulase, 4,046 for hemicellulase, 121 for β-glucanase, and 43,694 for xylanase.

4.3.4 Chemical analyses

Chromic oxide was analyzed using the procedures described by Fenton and Fenton (1979). Amino acid contents were determined using the Pico·Tag method as previously described (Albin et al., 2000; Cohen et al., 1989). Methionine and Cys were analyzed as Met sulfone and cysteic acid after overnight performic acid oxidation before hydrolysis (method 982.30; AOAC, 2005). Gross energy (GE) content of diets, feces, and urine were measured with a bomb calorimeter (Parr 1271 Automatic Oxygen Bomb Calorimeter, Parr Instrument Co., Moline, IL); benzoic acid was used as a standard according to manufacturer's specifications. Gross energy of urine was determined as described by Kim et al. (2009) where cotton balls were used as absorbent.

4.3.5 Calculations and statistical analyses

Gross energy consumed (kcal/d) was calculated by multiplying the GE value of the diet fed by feed intake over the 2-d collection period on a dry matter (DM) basis. Apparent ME (kcal/kg) of the experimental diets were calculated by subtracting fecal energy and urinary

energy from intake energy. The percentage of apparent energy metabolizability (AEM) was calculated by dividing the ME by the GE of DM intake (Adeola, 2001).

Apparent ileal AA digestibility (AID) was calculated based on the following equation (Stein et al., 2007):

$$AID\ (\%) = \{ 1 - [ ( AA_{Digesta} / AA_{Feed} ) \times ( M_{Feed} / M_{Digesta} ) ] \} \times 100$$

where $AA_{Digesta}$ and $AA_{Feed}$ are the AA content in the ileal digesta and the feed (g/kg DM), respectively, and $M_{Feed}$ and $M_{Digesta}$ are the chromic oxide concentration in the feed and the ileal digesta (g/kg DM), respectively.

Standardized ileal AA digestibility (SID) was calculated using the following formula (Lemme et al., 2004):

$$SID\ (\%) = AID + [ (AA_{End} ) / ( AA_{Feed} ) \times 100 ]$$

where $AA_{End}$ is the basal EAAL (g/kg DM), determined based on values obtained by feeding NFD.

Potential outliers were removed using the PROC UNIVARIATE procedure of SAS (SAS Institute, Cary, NC). Energy utilization (aME and AEM) and AA digestibility (AID and SID) data were subjected to one-way ANOVA using the PROC GLM procedure of SAS to test the main effect of multicarbohydrase supplementation or DDGS inclusion. A post-ANOVA was performed for independent treatments using the PROC MIXED procedure of SAS. Pig was set as a random variable. Differences between least square means were tested using Tukey's multiple comparison procedure of SAS. Significance was declared at $P \leq 0.05$ and tendency at $P < 0.10$.

## 4.4 Results

### 4.4.1 Energy utilization

Apparent metabolizable energy of experimental diets and its AEM were calculated from daily energy intake, fecal output, and urinary output (**Table 4.4**). Carbohydrases did not affect energy utilization in pigs ($P$ = 0.85 and 0.48). A tendency for DDGS × Enzyme interaction indicated that carbohydrase supplementation reduced urinary energy output of pigs when fed a high corn DDGS diet ($P < 0.09$). Inclusion of 40% corn DDGS significantly increased ($P < 0.0001$) the fecal and urinary energy output of pigs. Although diets with DDGS had increased ME ($P < 0.0001$), the AEM was reduced ($P < 0.0001$) when compared to CORN-based diets.

### 4.4.2 Amino acid digestibility

Supplementation of a carbohydrase mixture improved AID (**Table 4.5**) of Arg, Met, Ser, Glx, and Tyr ($P \leq 0.05$), and tended to increase Ile, Phe, and Thr ($P < 0.09$). Absence of DDGS × Enzyme interaction indicated that carbohydrases did not affect the AID when specifically supplemented to either CORN or DDGS diets. Apparent ileal AA digestibility of pigs given CORN-based diets was higher than DDGS-based diets ($P < 0.04$) for Arg, His, Ile, Lys, Phe, Asx, Glx, Gly, and Ser; a tendency ($P < 0.09$) was measured for Thr and Val.

Endogenous AA losses (**Table 4.6**) were measured and used to estimate SID (**Table 4.7**). Carbohydrase supplementation improved the SID of Arg, Ile, Met, Phe, Thr, Ala, Glx, Ser, and Tyr ($P \leq 0.04$), while Leu, Lys, Val, and Gly ($P < 0.10$) showed tendencies to be increased. Pigs given DDGS-based diets had lower SID for all measured AA ($P < 0.04$), except Leu, Met, Ala, Glx, and Tyr.

## 4.5 Discussion

Washout periods (fed a typical corn-SBM diet without chromic oxide) in the current study were applied to minimize the carryover effects of dietary treatments on the microbial population of the GIT (Moya et al., 2009). As another part of the study observed changes of the microbial population in response to the dietary treatments (data not shown), minimum washout periods of 7 d were required.

While CORN diets showed similar aME between measured (**Table 4.4**) and tabulated (**Table 4.2**) values (2.7% differential: average of 3,281 vs. 3,372 kcal/kg), the measured aME of DDGS diets were approximately 11.7% higher than the anticipated tabulated values (average of 3,476 vs. 3,106 kcal/kg). The original objective of this study was to observe the ability of carbohydrases to increase ME of a diet containing high level of NSP. In this effort, diets containing 40% corn DDGS were formulated to be lower in ME (92%) compared to the CORN diets. The measured ME of CORN diets were within an acceptable range in comparison to calculated values, indicating that the unexpected high ME measurements for DDGS diets were likely due to an underestimated ME of the corn DDGS source used in the study.

The ME value for the corn DDGS used in the nutrient matrix to formulate experimental diets was 2,805 kcal/kg as fed basis (Feed Ingredient Catalog, ADM, Decatur, IL). However, a back-calculation showed that the actual ME of the DDGS source was approximately 3,722 kcal/kg as fed basis, agreeing with previously reported values (Pedersen et al., 2007; Stein and Shurson, 2009). Furthermore, pigs given DDGS-based diets also had higher GE intake by approximately 11.1% than when offered CORN-based diets. Thus, the ME of DDGS-based diets were rather higher ($P < 0.0001$) by approximately 5.7% than CORN-based diets (average of

3,476 vs 3,281 kcal/kg), failing to create an energy-restricted environment in DDGS-based diets.

Increased GE of daily fecal and urinary output has been demonstrated previously in pigs given high DDGS inclusion diets (Pedersen et al., 2007). Amino acid concentration of corn DDGS is generally 2-3 times higher when compared to corn, but 2-4 times lower than SBM (NRC, 1998). Especially, Lys content of DDGS is approximately 5-9 times lower than SBM, ranking Lys as the first limiting AA in diets with DDGS inclusion (Pedersen et al., 2007). Analyzed AA composition of the experimental diets showed that except for Lys and Arg, most AA were higher in DDGS diets than C ORN diets (**Table 4.3**). Corn-SBM-DDGS diets had increased CP to meet the Lys requirement likely causing AA imbalances and excesses. Moreover, because corn DDGS has a higher level of CP compared to corn (NRC, 1998) and the majority of DDGS inclusion was at the expense of corn, DDGS diets had approximately 30% more CP than CORN diets (average of 20.61 vs. 15.84%). Thus, it was expected that more N would be excreted in urine due to AA imbalances, resulting in higher urinary energy output for DDGS diets.

Endogenous AA losses measured in current study were in agreement with previously reported values evaluated by Jansman et al. (2002). The EAAL of Pro and Gly are known to be overestimated when measured using a NFD method (de Lange et al., 1989). Indeed, high endogenous losses for Pro and Gly were measured, consequently leading to high values of its relative percentages to experimental diets, as well as its SID.

Both AID and SID coefficients were lower in DDGS diets compared to CORN diets for most AA. Although nutrient digestibility, including AA digestibility, has been improved with the introduction of “new generation” DDGS (Shurson et al., 2004), its SID are lower than that of corn (Stein et al., 2006) as a result of the anti-nutritional property of high NSP content in DDGS

(Ward et al., 2008).

Supplementation of a novel carbohydrase mixture did not improve feed aME or AEM in present study for either CORN or DDGS diets. When fed a typical corn-SBM diet, the effect of exogenous carbohydrases on energy utilization of pigs is generally accepted to be insignificant (Adeola and Cowieson, 2011). Meanwhile, the results are controversial for diets with more viscous/fibrous ingredients (e.g., wheat), which have higher NSP content. While Yin et al. (2000) and Diebold et al. (2004) reported improvements in energy utilization in pigs when offered wheat by-product or wheat-based diets supplemented with xylanase, Olukosi et al. (2007) showed that xylanase supplementation had no effect on the apparent digestible energy (aDE) of wheat-based diets. Yáñez et al. (2011) showed that supplementation of xylanase did not improve aDE in pigs fed corn DDGS co-fermented with wheat.

In the present study, carbohydrase supplementation tended ($P < 0.09$) to reduce urinary energy output of pigs when DDGS diet was given. However, as urinary energy output generally accounts for only a small portion of the ME (Farrell, 1978), the observed reducing tendency was not converted to improvements in aME or AEM.

The main effect of carbohydrase supplementation was significant for both AID and SID of most AA, regardless of diet composition. Previous studies have reported improvements in AA digestibility in pigs when carbohydrases were supplemented to diets with rye (Nitrayová et al., 2009), and wheat DDGS (Emiola et al., 2009; Vahjen et al., 2007), whereas Yáñez et al. (2011) reported no improvements when xylanase was supplemented to a corn DDGS diet.

The primary mode of action of carbohydrase in a high NSP environment is to reduce viscosity of digesta. Although it has been demonstrated that supplementation of carbohydrase reduced the viscosity of digesta in pigs (Vahjen et al., 2007), carbohydrase seems less effective

for swine application. This is due to the fact that the viscosity of digesta in pigs is considerably less than in poultry in respect to greater water content in pig digesta (Bedford and Schulze, 1998).

## 4.6 Conclusion

Inclusion of corn DDGS in swine diets at a high level significantly reduced energy utilization and AA digestibility in ileal cannulated growing pigs. Although DDGS diets were higher in aME, increased fecal and urinary energy output resulted in lower energy utilization. Amino acid digestibility coefficients were also reduced in high DDGS diets. Supplementation of a novel carbohydrase mixture to DDGS diet did not improve energy utilization but increased AA digestibility and tended to reduce urinary nitrogen in pigs. Collectively, results suggested a possibility for improved nutrient utilization in pigs, when carbohydrases are supplemented to a diet with high corn DDGS content.

### 4.7 Tables

Table 4.1. Diet formulation and calculated nutrient composition of N-free and washout diets, as fed basis

| | N-free[1] | Washout |
|---|---|---|
| Ingredients, % | | |
| Corn | - | 76.86 |
| Soybean meal (47.5%) | - | 20.00 |
| Cornstarch | 78.80 | - |
| Sucrose | 10.00 | - |
| Solca-Floc | 4.00 | - |
| Soy oil | 3.00 | 1.00 |
| Limestone | 1.15 | 1.00 |
| Dicalcium phosphate | 1.10 | 0.80 |
| Salt | 0.35 | 0.22 |
| Vitamin premix | 0.15[2] | 0.08[3] |
| Mineral premix | 0.15[4] | 0.04[5] |
| Potassium chloride | 0.60 | - |
| Magnesium oxide | 0.25 | - |
| Sodium carbonate | 0.05 | - |
| Chromic oxide | 0.40 | - |
| Calculated nutrients | | |
| ME, kcal/kg | 3756 | 3389 |
| CP, % | - | 15.88 |
| Ca, % | 0.61 | 0.59 |
| P, %[6] | 0.23 | 0.23 |
| Lysine, %[7] | - | 0.80 |
| Methionine, %[7] | - | 0.26 |
| Threonine, %[7] | - | 0.59 |
| Arginine, %[7] | - | 0.98 |

[1] Additional vitamins supplemented per kg of diet: Vitamins B1, 47 mg; Vitamin B6, 20 mg (from Vitamin World Inc., Ronkonkoma, NY)

[2] Provided the following per kg of diet: Vitamin A, 4,400 IU; Vitamin D3, 606 IU; Vitamin E, 25 IU; Vitamin K, 4 mg; biotin, 0.19 mg; choline, 330 mg; folic acid, 0.83 mg, niacin, 19 mg; D-pantothenic acid, 13 mg; riboflavin, 4 mg; and B12, 0.02 mg (from 77320014, ADM, Decatur, IL)

[3] Provided the following per kg of diet: Vitamin A, 2,347 IU; Vitamin D3, 323 IU; Vitamin E, 13 IU; Vitamin K, 2 mg; biotin, 0.10 mg; choline, 176 mg; folic acid, 0.44 mg, niacin, 10 mg; D-pantothenic acid, 7 mg; riboflavin, 2 mg; and B12, 0.01 mg (from 77320014, ADM, Decatur, IL)

[4] Provided the following as mg/kg of diet: Zn, 180 mg from ZnSO4; Fe, 180 mg from $FeSO_4$; Mn, 55 mg from $MnSO_4$; Cu, 11 mg $CuSO_4$; and Se, 0.3 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[5] Provided the following as mg/kg of diet: Zn, 48 mg from ZnSO4; Fe, 48 mg from $FeSO_4$; Mn,15 mg from $MnSO_4$; Cu, 3 mg $CuSO_4$; and Se, 0.1 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[6] Available phosphorus

[7] Total values

Table 4.2. Diet formulation and nutrient composition of the experimental diets, as fed basis

| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes |
|---|---|---|---|---|
| Ingredients, % | | | | |
| Corn | 76.46 | 76.26 | 45.08 | 44.88 |
| Soybean meal (47.5%) | 20.00 | 20.00 | 13.20 | 13.20 |
| Corn DDGS | - | - | 40.00 | 40.00 |
| Limestone | 1.00 | 1.00 | 1.20 | 1.20 |
| Soy oil | 1.00 | 1.00 | - | - |
| Dicalcium phosphate | 0.80 | 0.80 | - | - |
| Salt | 0.22 | 0.22 | - | - |
| Vitamin premix[1] | 0.08 | 0.08 | 0.08 | 0.08 |
| Mineral premix[2] | 0.04 | 0.04 | 0.04 | 0.04 |
| Chromic oxide | 0.40 | 0.40 | 0.40 | 0.40 |
| Enzyme[3] | - | 0.20 | - | 0.20 |
| Calculated nutrients | | | | |
| ME, kcal/kg | 3375 | 3368 | 3110 | 3103 |
| CP, % | 15.85 | 15.83 | 20.61 | 20.60 |
| Ca, % | 0.59 | 0.59 | 0.57 | 0.57 |
| P, %[4] | 0.23 | 0.23 | 0.25 | 0.25 |
| Lysine, %[5] | 0.80 | 0.80 | 0.66 | 0.66 |
| Methionine, %[5] | 0.26 | 0.26 | 0.32 | 0.31 |
| Threonine, %[5] | 0.59 | 0.59 | 0.62 | 0.62 |
| Arginine, %[5] | 0.98 | 0.98 | 0.97 | 0.97 |

[1] Provided the following per kg of diet: Vitamin A, 2,347 IU; Vitamin D3, 323 IU; Vitamin E, 13 IU; Vitamin K, 2 mg; biotin, 0.10 mg; choline, 176 mg; folic acid, 0.44 mg, niacin, 10 mg; D-pantothenic acid, 7 mg; riboflavin, 2 mg; and B12, 0.01 mg (from 77320014, ADM, Decatur, IL)

[2] Provided the following as mg/kg of diet: Zn, 48 mg from ZnSO4; Fe, 48 mg from $FeSO_4$; Mn, 15 mg from $MnSO_4$; Cu, 3 mg $CuSO_4$; and Se, 0.1 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[3] Carbohydrase mixture provided per kg of diet: cellulase, 25,085 unit (from *Trichoderma longibrachiatum*); hemicellulase, 4,046 unit (from *Aspergillus niger*); ß-glucanase, 121 unit (from *Trichoderma longibrachiatum*); and xylanase, 43,694 unit (from *Trichoderma longibrachiatum*); all carbohydrase from Bio-Cat Inc., Troy, VA

[4] Available phosphorus

[5] True digestibility values

Table 4.3. Analyzed amino acid composition (%) of the experimental diets, as fed basis

| | N-Free[1] | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes |
|---|---|---|---|---|---|
| Indispensable | | | | | |
| Arg | 0.05 | 1.01 | 0.93 | 0.80 | 0.75 |
| His | 0.02 | 0.46 | 0.39 | 0.44 | 0.41 |
| Ile | 0.04 | 0.56 | 0.52 | 0.52 | 0.49 |
| Leu | 0.03 | 1.21 | 1.20 | 1.47 | 1.43 |
| Lys | 0.05 | 0.82 | 0.78 | 0.59 | 0.57 |
| Met | 0.01 | 0.17 | 0.18 | 0.23 | 0.19 |
| Phe | 0.03 | 0.68 | 0.66 | 0.70 | 0.65 |
| Thr | 0.02 | 0.53 | 0.47 | 0.56 | 0.50 |
| Val | 0.03 | 0.62 | 0.59 | 0.64 | 0.61 |
| Dispensable | | | | | |
| Ala | 0.02 | 0.69 | 0.66 | 0.88 | 0.83 |
| Asp+Asn | 0.05 | 1.41 | 1.18 | 0.97 | 0.98 |
| Glu+Gln | 0.03 | 2.62 | 2.34 | 2.49 | 2.32 |
| Gly | 0.02 | 0.53 | 0.49 | 0.52 | 0.48 |
| Pro | 0.03 | 0.90 | 0.87 | 1.02 | 1.01 |
| Ser | 0.04 | 0.74 | 0.69 | 0.73 | 0.69 |
| Tyr | 0.04 | 0.43 | 0.46 | 0.54 | 0.48 |

[1] Nitrogen free diet

Table 4.4. Energy measurements, apparent metabolizable energy (aME), and energy metabolizability of ileal cannulated pigs of diets containing corn or 40% corn DDGS supplemented with or without a mixture of carbohydrase fed to ileal cannulated pigs, as fed basis[1]

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | SEM[2] | DDGS | Enzymes | DDGS × Enzymes |
| GE intake, kcal/d | 5278 | 5262 | 5883 | 5832 | - | - | - | - |
| Fecal energy, kcal/d | 290[ab] | 282[b] | 501[a] | 482[a] | 74 | <0.0001 | 0.9034 | 0.9987 |
| Urine energy, kcal/d | 61[b] | 63[b] | 155[a] | 125[a] | 18 | <0.0001 | 0.1178 | 0.0874 |
| Feed aME, kcal/kg | 3284[b] | 3278[b] | 3484[a] | 3468[a] | 37 | <0.0001 | 0.8503 | 0.7395 |
| Energy metabolizability, % | 93.3[a] | 93.4[a] | 88.8[b] | 89.2[b] | 1.0 | <0.0001 | 0.4781 | 0.9355 |

[a-b] Least square means within a row with no common superscript differ at $P < 0.05$.
[1] Least square means
[2] Pooled standard error of the mean

Table 4.5. Apparent ileal amino acid digestibility (%) of diets containing corn or 40% corn DDGS supplemented with or without a mixture of carbohydrase fed to ileal cannulated pigs[1]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable | | | | | | | | |
| Arg | 87.8[a] | 88.8[a] | 80.0[b] | 85.2[ab]* | 2.68 | <0.0001 | 0.0318 | 0.2040 |
| His | 86.2 | 84.9 | 81.2 | 84.1 | 2.63 | 0.0057 | 0.5937 | 0.2958 |
| Ile | 85.1[ab] | 87.7[a] | 80.9[b] | 83.5[ab] | 2.87 | 0.0015 | 0.0622 | 0.8466 |
| Leu | 88.2 | 89.5 | 88.8 | 90.0 | 1.92 | 0.9942 | 0.1681 | 0.5188 |
| Lys | 85.2[a] | 86.5[a] | 76.5[b] | 78.9[b] | 2.90 | <0.0001 | 0.1866 | 0.8468 |
| Met | 86.0 | 88.3 | 85.2 | 89.0 | 2.45 | 0.5430 | 0.0136 | 0.9317 |
| Phe | 87.5 | 88.9 | 85.3 | 87.8 | 2.07 | 0.0330 | 0.0699 | 0.9939 |
| Thr | 77.8 | 79.0 | 73.2 | 78.8 | 4.18 | 0.0845 | 0.0871 | 0.5116 |
| Val | 82.2 | 83.9 | 80.0 | 82.6 | 3.08 | 0.0820 | 0.1397 | 0.8634 |
| Dispensable | | | | | | | | |
| Ala | 83.6 | 85.3 | 84.0 | 86.5 | 2.54 | 0.7845 | 0.0963 | 0.9118 |
| Asp+Asn | 88.1[a] | 87.9[a] | 81.0[b] | 82.2[b] | 1.79 | <0.0001 | 0.4143 | 0.6015 |
| Glu+Gln | 91.0[ab] | 92.4[a] | 88.6[b] | 89.9[ab] | 1.35 | 0.0008 | 0.0524 | 0.9204 |
| Gly | 77.7 | 78.6 | 71.5 | 75.8 | 4.31 | 0.0158 | 0.1873 | 0.4200 |
| Pro | 86.1 | 89.6 | 85.9 | 86.0 | 2.44 | 0.1275 | 0.1621 | 0.2267 |
| Ser | 81.6[ab] | 84.6[a] | 77.0[b] | 80.9[ab] | 3.56 | 0.0090 | 0.0515 | 0.9129 |
| Tyr | 82.1 | 85.6 | 82.2 | 85.7 | 3.14 | 0.6758 | 0.0285 | 0.6231 |

[a-b] Least square means within a row with no common superscript differ at $P < 0.05$; * differ from DDGS at $P < 0.08$.

[1] Least square means

[2] Pooled standard error of the mean

Table 4.6. Endogenous amino acid losses (EAAL) in ileal cannulated pigs and the relative percentage to diets containing corn or 40% corn DDGS supplemented with or without a mixture of carbohydrase

| | EAAL[1] | Relative percentage[2] | | | |
|---|---|---|---|---|---|
| | | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes |
| Indispensable | | | | | |
| Arg | 0.44 | 3.86 | 4.16 | 4.97 | 5.30 |
| His | 0.20 | 3.88 | 4.56 | 4.15 | 4.45 |
| Ile | 0.28 | 4.42 | 4.70 | 4.81 | 5.04 |
| Leu | 0.41 | 3.01 | 3.02 | 2.54 | 2.58 |
| Lys | 0.41 | 4.50 | 4.73 | 6.37 | 6.57 |
| Met | 0.09 | 4.84 | 4.53 | 3.63 | 4.39 |
| Phe | 0.26 | 3.40 | 3.49 | 3.37 | 3.61 |
| Thr | 0.47 | 7.91 | 8.79 | 7.65 | 8.37 |
| Val | 0.38 | 5.54 | 5.77 | 5.41 | 5.62 |
| Dispensable | | | | | |
| Ala | 0.41 | 5.35 | 5.53 | 4.27 | 4.50 |
| Asp+Asn | 0.75 | 4.71 | 5.63 | 6.96 | 6.84 |
| Glu+Gln | 0.97 | 3.29 | 3.68 | 3.53 | 3.75 |
| Gly | 0.78 | 13.25 | 14.32 | 13.60 | 14.70 |
| Pro | 1.13 | 11.21 | 11.55 | 10.10 | 10.04 |
| Ser | 0.57 | 6.83 | 7.39 | 7.06 | 7.44 |
| Tyr | 0.25 | 5.10 | 4.79 | 4.13 | 4.56 |

[1] Endogenous AA loss, g/kg of dry matter (DM) intake
[2] Calculated as: [( EAAL ) / (Feed AA, g/kg DM) ] × 100

Table 4.7. Standardized ileal amino acid digestibility (%) of diets containing corn or 40% corn DDGS supplemented with or without a mixture of carbohydrase fed to ileal cannulated pigs[1]

| | Treatment | | | | SEM[2] | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CORN | CORN + Enzymes | DDGS | DDGS + Enzymes | | DDGS | Enzymes | DDGS × Enzymes |
| Indispensable AA | | | | | | | | |
| Arg | 91.7[a] | 93.8[a] | 85.0[b] | 90.4[ab]* | 2.67 | 0.0003 | 0.0105 | 0.2682 |
| His | 90.1 | 91.0 | 85.4 | 88.3 | 2.95 | 0.0035 | 0.2160 | 0.6054 |
| Ile | 89.5[ab] | 92.4[a] | 85.7[b] | 88.6[ab] | 2.87 | 0.0031 | 0.0407 | 0.8320 |
| Leu | 91.2 | 93.6 | 91.3 | 92.4 | 2.11 | 0.3991 | 0.0926 | 0.3542 |
| Lys | 89.7[ab] | 92.7[a] | 82.9[c] | 85.1[bc] | 3.15 | <0.0001 | 0.0875 | 0.6460 |
| Met | 90.8 | 94.3 | 88.8 | 93.1 | 2.75 | 0.1455 | 0.0046 | 0.8487 |
| Phe | 90.9[ab] | 93.4[a] | 88.6[b] | 91.2[ab] | 2.25 | 0.0203 | 0.0279 | 0.8198 |
| Thr | 85.7[ab] | 89.9[a] | 80.8[b] | 86.8[ab] | 4.51 | 0.0333 | 0.0204 | 0.7530 |
| Val | 87.8 | 91.2 | 85.4 | 87.9 | 3.32 | 0.0378 | 0.0609 | 0.6332 |
| Dispensable AA | | | | | | | | |
| Ala | 88.9 | 92.1 | 88.3 | 90.7 | 2.74 | 0.3503 | 0.0378 | 0.6650 |
| Asp+Asn | 90.4 | 92.0 | 87.8 | 88.7 | 2.27 | 0.0080 | 0.2122 | 0.9364 |
| Glu+Gln | 93.0 | 94.7 | 92.2 | 93.6 | 1.35 | 0.1262 | 0.0249 | 0.9049 |
| Gly | 90.9 | 93.0 | 85.1 | 90.5 | 4.31 | 0.0251 | 0.0603 | 0.4142 |
| Pro | 97.3 | 99.8 | 96.3 | 96.1 | 2.73 | 0.0294 | 0.2594 | 0.3205 |
| Ser | 88.4[ab] | 91.9[a] | 84.1[b] | 88.3[ab] | 3.56 | 0.0112 | 0.0269 | 0.9586 |
| Tyr | 87.2 | 91.8 | 86.3 | 90.0 | 3.29 | 0.2841 | 0.0143 | 0.6101 |

[a-c] Least square means within a row with no common superscript differ at $P < 0.05$; * differ from DDGS at $P < 0.07$.

[1] Least square means

[2] Pooled standard error of the mean

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# CHAPTER 5

## Increased endogenous amino acid losses and altered amino acid digestibility in nursery pigs challenged orally with *Salmonella enterica* serovar Typhimurium

### 5.1 Abstract

Pigs are exposed to various pathogens throughout production phases. Many studies have determined amino acid (AA) digestibility in pigs as affected by various feed ingredients, feed additives, or physiological states, but little is known about it under actual bacterial pathogenesis. Pigs (n = 48, BW = 17.9 ± 0.5 kg) were randomly assigned to a 2 × 2 factorial arrangement consisting of diet (control or N-free) and inoculation (sterile broth or *Salmonella*). Animals were orally inoculated with $9.8 \times 10^9$ colony forming units of an antibiotic resistant *Salmonella* strain (*S. enterica* serovar Typhimurium DT104 $Nal^R Nov^R$) or sterile tryptic soy broth. Inoculation of *Salmonella* was associated with reduced average daily gain (ADG) and gain to feed ratio (G:F) ($P < 0.10$). Compared to healthy uninfected controls, *Salmonella* inoculated pigs showed a significant decrease in apparent ileal AA digestibility (AID) for Ile, Thr, Gly and Tyr ($P < 0.05$), and a tendency of the same for Arg, Met, Phe, Val, Pro and Ser ($P < 0.10$) 24 h after inoculation. This was due to an increase in endogenous AA losses (EAAL) for all AA ($P \leq 0.05$). *Salmonella* challenge had no effect on standardized ileal AA digestibility (SID) except for His and Gly, where an increase was measured ($P < 0.05$). At 72 h post-inoculation, decreases in AID were determined for Lys, Phe, Thr, Val and Ser ($P < 0.05$), while Arg, Ile, Leu, Ala, Asx, Glx, Gly and Tyr tended to be reduced ($P < 0.10$) with *Salmonella* inoculation. Measured EAAL were not different

between the control and *Salmonella* treatments ($P > 0.05$). The SID for Lys was decreased ($P < 0.05$) and Arg, Ile, Phe, Thr and Glx tended to be lower ($P < 0.10$) in *Salmonella* inoculated pigs. These results indicate that AA digestibility of pigs is impaired through the initial phase of *Salmonella* infection and gradually restored, but not fully by 72 h. The marked increases in EAAL during initial *Salmonella* infection resulted in an overestimation of SID. Thus, AID appears to be a better indicator of digestive capacity during initial phase of an infection than SID. In conclusion, diarrheal response induced by epithelial invasion during a *Salmonella* infection leads to a reduction in growth performance and AA digestibility.

## 5.2 Introduction

Enteric diseases occur subsequent to host infection following adherent of various pathogens, and are responsible for detrimental growth in livestock. In swine, *Salmonella* infections are a major public health concern as pork is an important cause of Salmonellosis (Foley et al., 2008). Moreover, *Salmonella* enterocolitis is recognized as a serious problem for the swine industry (Veldhuizen et al., 2008), as it cause significant mortality in nursery pigs, resulting in considerable economic loss. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is one of the most frequently isolated serovars in pigs (Chiu et al., 2004; Schwartz, 1999) which is likely to cause clinical disease with a mild diarrhea. In pigs, *S.* Typhimurium infection is associated with fever, growth suppression, anorexia and an acute phase immune response in pigs (Balaji et al., 2000; Loughmiller et al., 2007).

Because young animals are more susceptible to Salmonellosis (Timoney et al., 1988), pigs are more susceptible to *Salmonella* infection during the nursery phase. It has been demonstrated that pigs that are exposed to *Salmonella* infection have retarded growth

performance resulting in 10 to 15-d lag to reach market weight (Nielsen et al., 1997). The lean growth of swine at their early production stage is highly dependent on protein metabolism. While previous studies have reported N balance of the *S*. Typhimurium challenge model in pigs (Loughmiller et al., 2007), the digestibility of individual amino acids (AA) have not been reported using an actual bacterial challenge model in pigs.

This study was conducted to determine the impact of oral *S*. Typhimurium challenge on apparent ileal AA digestibility (AID), endogenous AA losses (EAAL), and its derived standardized ileal AA digestibility (SID) in late-stage nursery pigs.

## 5.3 Materials and methods

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee.

### 5.3.1 Bacterial strain and inoculant preparation

*Salmonella* Typhimurium used in this experiment was cultured and inoculant was prepared as previously described (Price et al., 2010). Briefly, *Salmonella enterica* subspecies *enterica* serovar Typhimurium DT104 (ATCC, BAA-185, Manassas, VA) was resuscitated in 10 ml of tryptic soy broth (TSB) at 37°C for 24 h and plated onto tryptic soy agar (TSA). By sequentially transferring single colonies onto TSA plates that were finalized to contain 20 μg /ml of nalidixic acid (Acros Organics, Morris Plains, NJ) and 25 μg/ml of novobiocin (BD Bioscience), an antibiotic resistant strain was prepared (*Salmonella* Typhimurium $Nal^{R}Nov^{R}$, henceforth $ST^{RR}$). An antibiotic resistant strain was chosen to ensure any *Salmonella* detected were of inoculant origin. *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ was cultured overnight at 37°C in TSB medium on an orbital shaker (New

Brunswick Scientific, Edison, NJ) at 150 rpm and bacterial populations were estimated by spectrophotometry at 600 nm. For inoculant preparation, $ST^{RR}$ were harvested at 7,500 × *g* for 10 min at 4°C, and resuspended in sterile TSB.

5.3.2 General

A total of 48 pigs were obtained from the Swine Center at Virginia Tech (Blacksburg, VA) and transported to an Animal Biosafety Level (ABSL)-2 facility at 54 d of age (BW = 17.9 ± 0.5 kg). Animals were housed in individual pens equipped with a self feeder and a nipple waterer. The facility was independently ventilated with 100% clean air (i.e., no recirculation) under constant negative pressure. Temperature (24°C) and lighting (18 h light:6 h dark with lights on at 0600) were controlled by an automated system.

Diet formulation and calculated nutrient composition are shown in **Table 5.1**. A typical corn-soybean meal (SBM) diet was formulated to meet or exceed NRC recommendations for nutrients (NRC, 1998). The N-free diet (NFD) was formulated based on cornstarch and sucrose. Solka-Floc was used as source of fiber and vitamins $B_1$ and $B_6$ (Vitamin World Inc., Ronkonkoma, NY) were supplemented to meet NRC recommendations. All diets contained 0.3% chromic oxide as an indigestible marker and contained no antibiotics. Prior to feeding, random composite feed samples were collected from both diets and were screened for the presence of *Salmonella* by enriching in Gram-negative Hanja broth at 37°C for 24 h and plating onto Brilliant Green Agar (BGA, BD Bioscience) plates. Both diets were negative for *Salmonella* and pigs were given ad libitum access to feed and water throughout the experiment.

After a 5-d pre-adjustment period with the control diet, pigs were randomly assigned to treatments. Treatments were arranged in a 2 × 2 factorial arrangement consisting of diet (control or NFD) and inoculation (sterile broth or $ST^{RR}$) with 12 pigs per treatment.

Animals were segregated to 2 identical rooms according to their inoculants to minimize the potential of cross-contamination. Pigs were screened for the presence of *Salmonella* on d 5 by directly plating rectal swab onto BGA and were all negative. An additional 3 d were provided allowing the animals to adjust to their respective experimental diets. On d 9, pigs were given a 10 ml oral dose of TSB containing $9.8 \times 10^9$ colony forming units (CFU) of $ST^{RR}$ or sterile TSB. Animals were screened daily for *Salmonella* shedding on d 9 to d 12.

Body weight (BW) and feed intake of control pigs (n = 24) were measured on d 6, 9, 10 and 12 with each cage as the experimental unit. Average daily weight gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were calculated to determine the growth performance at pre-inoculation (d 6-9) and post-inoculation (d 9-12).

5.3.3 Sample collection and chemical analyses

On d 10 (24 h post-inoculation) and 12 (72 h post-inoculation), half of the pigs were randomly selected and euthanized with a lethal dose of 120 mg/kg BW of sodium pentobarbital i.v. (Beuthanasia-D, Shering-Plough, Union, NJ). The ileal regions of the digestive tract were removed and its contents were collected by gentle squeezing, immediately frozen, freeze-dried (Genesis 25EL, VirTis, Gardiner, NY), and ground to determine AID and EAAL.

Chromic oxide was analyzed using the procedure described by Fenton and Fenton (1979) and AA contents were determined using the Pico·Tag method as previously described (Albin et al., 2000; Cohen et al., 1989).

5.3.4 Calculations and statistical analyses

Apparent ileal AA digestibility was calculated using the following equation (Stein et al., 2007):

$$AID\ (\%) = \{ 1 - [ ( AA_{Digesta} / AA_{Feed} ) \times ( M_{Feed} / M_{Digesta} ) ] \} \times 100$$

where $AA_{Digesta}$ is the AA concentration in the ileal digesta [g/kg dry matter (DM)], $AA_{Feed}$ is the AA concentration in the feed (g/kg DM), $M_{Feed}$ is the marker concentration in the feed (g/kg DM), and $M_{Digesta}$ is the marker concentration in the ileal digesta (g/kg DM).

The obtained AID was transformed to standardized values by correcting for the EAAL by the equation as previously reported by Lemme et al. (2004), with the SID derived from the following formula:

$$SID\ (\%) = AID\ (\%) + [ ( EAAL ) / ( AA_{Feed} ) \times 100 ]$$

The PROC UNIVARIATE procedure of SAS (SAS Institute, Cary, NC) was used to eliminate outliers. Pre- and post-inoculation growth performance (ADG, ADFI, G:F) of control treatments (n = 24) were subjected to one-way ANOVA using the PROC GLM procedure of SAS. Amino acid digestibility (AID, SID) of the control diet and EAAL for 24 and 72 h post-inoculation were subjected to one-way ANOVA using the PROC GLM procedure of SAS. Differences between least square means were tested using Tukey's multiple comparison procedure of SAS. Significance was declared at $P \leq 0.05$ and tendency at $P < 0.10$.

## 5.4 Results

### 5.4.1 Growth performance

Calculated values for ADG, ADFI and G:F, as well as BW measurements for pre- and post-inoculation are shown in **Table 5.2**. Pre-inoculation, no differences were detected in ADG, G:F and BW. Although difference in ADFI ($P < 0.05$) was detected, it was not converted to an increase in ADG nor G:F ($P > 0.10$). Following inoculation, ADG and G:F tended to be lower ($P < 0.08$) for pigs inoculated with $ST^{RR}$. No differences were measured

in ADFI ($P$ = 0.51) between the two treatments. Pigs challenged with $ST^{RR}$ showed numeric decreases of ADG and G:F by 61% and 64%, respectively, post-inoculation.

5.4.2 Amino acid digestibility and endogenous amino acid losses

At 24 h post-inoculation, the AID of animals subjected to $ST^{RR}$ challenge showed numeric decreases for all measured AA compared to the controls (**Table 5.3**). The AID for Ile, Thr, Gly and Tyr were reduced ($P < 0.05$), and Arg, Met, Phe, Val, Pro and Ser showed decreased tendency ($P < 0.10$) in pigs that were inoculated with $ST^{RR}$. At 72 h post-inoculation, pigs challenged with $ST^{RR}$ still showed decreased the AID (**Table 5.4**). Decreases were detected for Lys, Phe, Thr, Val and Ser ($P < 0.05$) while Arg, Ile, Leu, Ala, Asx, Glx, Gly and Tyr tended to be lower ($P < 0.10$).

Oral challenge of *Salmonella* increased ($P \leq 0.05$) EAAL of all AA at 24 h post-inoculation (**Table 5.5**). However, at 72 h post-inoculation, these drastic elevations were diminished (**Table 5.6**). Except for Asx, where EAAL for $ST^{RR}$ inoculated pigs tended to be lower ($P$ = 0.09), all measured values did not differ between the two groups ($P$ = 0.11 to 0.92).

At 24 h post-inoculation, pigs subjected to $ST^{RR}$ challenge showed numerical increases in SID for all AA (**Table 5.7**). Especially, marked increases were observed for His ($P$ = 0.01) and Gly ($P$ = 0.001). However, at 72 h post-inoculation, SID for Lys was decreased ($P < 0.05$) and tended to be lower for Arg and Phe ($P < 0.10$) in $ST^{RR}$ inoculated pigs (**Table 5.8**). While all other calculated SID values showed a numerical decrease with $ST^{RR}$ inoculation, the value for Pro tended to be higher ($P < 0.10$).

## 5.5 Discussion

In the present study, *Salmonella* inoculation did not affect the ADFI of animals. Enteric pathogen challenge models (e.g., *S*. Typhimurium), as well as non-infectious endotoxin challenge models (i.e., lipopolysaccharide; LPS), are known to reduce feed intake of pigs, due to increased concentrations of proinflammatory cytokines (Dritz et al., 1996; Klasing et al., 1987; van Heugten et al., 1996). Although Fraser et al. (2007) and Davis et al. (2010) have shown that oral inoculation of *S*. Typhimurium at $10^8$ CFU did not cause a reduction in feed intake, it is widely accepted that the stimulation of the immune system reduces ADFI. While considerable amount of caution was taken to measure leftover feed as accurately as possible, many pigs in current study developed significant diarrhea which led to contaminated feeders. Thus, error was inevitable, most likely resulting in biased ADFI calculations.

Pigs inoculated with $ST^{RR}$ tended to have lower ADG compared to their non-challenged counterparts. This is in agreement with previous studies where significant decreases or tendencies in ADG were observed in immune stimulated pigs (Williams et al., 1997), specifically in models using *S*. Typhimurium (Balaji et al., 2000; Price et al., 2010). A decreased BW gain during an acute phase immune response have been suggested to be associated with reductions in circulating insulin-like growth factor 1 (IGF-1). Of factors that are known to regulate plasma IGF-1 concentrations (i.e., feed intake, growth hormone, insulin thyroid hormones $T_3$ and $T_4$), decreases in feed intake as a consequence of increased proinflamatory cytokines during an acute phase immune response have been proposed as the main reason (Loughmiller et al., 2007). In fact, in previous pig studies where ADG had not been affected by oral *S*. Typhimurium challenges, the ADFI and plasma IGF-1 concentrations were not affected as well (Davis et al., 2010; Fraser et al., 2007). Loughmiller et al. (2007)

further demonstrated that non-infected pigs have similar ADG when pair-fed to pigs challenged with *S*. Typhimurium.

Activation of the acute phase immune response numerically reduced the AID of all AA in pigs orally challenged with $ST^{RR}$ compared to the non-challenged controls at 24 h post-inoculation. These reductions were significant for Ile, Thr, Gly, and Tyr, while tendencies were shown for Arg, Met, Phe, Val, Pro, and Ser. To the author's knowledge, the only available data on the AID of immune system stimulated pigs have reported no differences for all AA using the LPS model (Rakhshandeh et al., 2010). However, the short-lived LPS model does not fully simulate an enteric bacterial pathogenesis. For instance, LPS challenge produces more of a systemic cytokine response (Johnson et al., 2005) opposed to a localized production of proinflamatory cytokines as in cases such as Salmonellosis (Balaji et al., 2000; Burkey et al., 2004).

The pathogenesis of *S*. Typhimurium involve injuries to the intestinal epithelium that progress to severe neutrophilic inflammations associated with necrosis of the mucosa in calves (Santos et al., 2002b). Moreover, Salmonellosis accompanies a mild diarrheal response based on an inflammatory mechanism of fluid secretion where serum proteins are released into the intestinal lumen, which is attributable to the loss of the intestinal permeability barrier. Thus, a reduction in AID is expected in pigs infected with $ST^{RR}$, not only due to the impaired absorptive capacity of the physically damaged ileum, but also because of the amplified EAAL.

Pigs showed an average of a 5-fold increase of EAAL at 24 h post-inoculation with $ST^{RR}$. The significant increases in EAAL were not limited to a specific category of AA but were observed in all measured AA. A comprehensive evaluation of the hematology and blood chemistry profile of orally infected calves has been performed to test the prediction that *S*. Typhimurium-induced diarrhea results in a non-specific effusion of serum proteins (Santos

et al., 2000a). Furthermore, the concentration of total plasma protein decreased significantly and continuously after infection, indicating that the loss of protein was non-selective.

Under normal physiological conditions with low stress levels, the SID is a better representation of the "true" value than the AID. The AID is an underestimated value, as the EAAL is not reflected. By adjusting the AID for EAAL, the SID serves as a relatively accurate, practical, and yet convenient method to determine AA digestibility. However, in the current study, EAAL were increased between 4 and 9 fold at 24 h post-inoculation, resulting in higher SID values for pigs challenged with $ST^{RR}$ compared to the healthy controls. Thus, results suggest that the concept of SID may not be as applicable during initial enteric disease conditions, where EAAL is drastically elevated, as opposed to normal "healthy" experimental conditions (i.e., comparison of SID between healthy and sick animals).

The significant increases of EAAL at 24 h post-inoculation were not sustained at 72 h post-inoculation. This implies that the intestinal permeability barrier of pigs challenged with $ST^{RR}$ was adequately restored by 72 h post-inoculation. As a key component of the innate immune system, neutrophils have been shown to play a crucial role in mediating diarrhea by an inflammatory mechanism, since this cell type is known to release substances that lead to tissue injury. It has been shown in rats that alterations of intestinal mucosal permeability can be prevented by neutrophil depletion (Sir et al., 2000). The non-specific innate immune system subsides after the initial *Salmonella* invasion of the epithelium, and the adaptive immune system comes to full effect, allowing the intestinal epithelium to replenish and regain its normal digestive function. The decreased AID and SID of pigs challenged with $ST^{RR}$ at 72 h post-inoculation without differences in the EAAL, suggests that the AA digestibility of pigs was gradually restored, but not fully by 72 h.

Of the entire AA examined in current study, Gly was most affected by $ST^{RR}$ challenge. During the acute immune response (i.e., at 24 h post-inoculation), the AID of Gly had a

negative value (-15.4%), while the SID value was calculated to be of over 100% (148%). This was due to the very large EAAL value for Gly, as it was significantly increased by 9-fold, accounting for about 37% the total EAAL. The EAAL of Gly, along with Pro, is known to be overestimated when measured using the NFD method (de Lange et al., 1989; Moughan et al., 1992). The abundance of Gly in EAAL when fed a NFD is known to be a collective result of the disruptions in absorption of bile acids (BA, de Lange et al., 1989) and reflux back into the lumen as free AA following intracellular digestion (Taverner et al., 1981). However, EAAL of Gly has not been reported to exceed that of Pro under any circumstances.

An inflammatory diarrhea should not only result in a reduction of nutrient absorption, but also disrupt the enterohepatic circulation of BA. Under normal physiological conditions, BA are efficiently recycled through the enterohepatic circulation which readily absorbs approximately 95% of conjugated BA at the ileum. Bile acids are mainly conjugated with Gly or Tau, hence having enhanced water solubility (Martínez-Augustin and de Medina, 2008). Pigs are principally Gly conjugators, and in cases when BA turnover is accelerated by disease, Gly conjugation becomes even more dominant (Shonsey et al., 2005). Thus, the marked increase of endogenous Gly loss induced by *Salmonella* challenge could be in part, explained by an altered enterohepatic recycling of BA.

## 5.6 Conclusion

An inflammatory diarrhea induced by oral inoculation of $ST^{RR}$ reduced the ADG and AID of pigs at 24 h post-inoculation. The EAAL for all AA were significantly increased during the acute phase immune response. However, the current concept of SID does not seem to be applicable for situations where the gut health between treatments is expected to be

different, as the drastic elevations in EAAL resulted in an increased SID of $ST^{RR}$ challenged pigs over their healthy controls. By 72 h post-inoculation, the EAAL of *Salmonella* challenged pigs returned to normal levels, but the AID and SID were lower, suggesting a gradual recovery of the AA digestibility. Also, we report a marked increase of endogenous Gly loss in pigs challenged with oral inoculation of $ST^{RR}$, possibly due to a disruption in the enterohepatic recycle of BA.

## 5.7 Tables

Table 5.1. Diet formulation and calculated nutrient composition of N-free and control diets, as fed basis

| | N-free[1] | Control |
|---|---|---|
| Ingredients, % | | |
| Corn | - | 67.15 |
| Soybean meal (47.5%) | - | 26.65 |
| Fish meal | - | 3.00 |
| Cornstarch | 78.80 | - |
| Sucrose | 10.00 | - |
| Solca-Floc | 4.00 | - |
| Soy oil | 3.00 | 1.00 |
| Dicalcium phosphate | 1.55 | 0.70 |
| Limestone | 0.95 | 0.70 |
| Salt | 0.30 | 0.30 |
| Vitamin premix | 0.18[2] | 0.15[3] |
| Mineral premix | 0.14[4] | 0.05[5] |
| Potassium chloride | 0.55 | - |
| Magnesium sulfate | 0.10 | - |
| Sodium carbonate | 0.05 | - |
| Chromic oxide | 0.30 | 0.30 |
| Calculated nutrients | | |
| ME, kcal/kg | 3759 | 3382 |
| CP, % | - | 20.12 |
| Ca, % | 0.70 | 0.68 |
| P, %[6] | 0.29 | 0.28 |
| Lysine, %[7] | - | 1.00 |
| Methionine, %[7] | - | 0.32 |
| Threonine, %[7] | - | 0.66 |
| Arginine, %[7] | - | 1.20 |

[1] Additional vitamins supplemented per kg of diet: Vitamins B1, 47 mg; Vitamin B6, 20 mg (from Vitamin World Inc., Ronkonkoma, NY)

[2] Provided the following per kg of diet: Vitamin A, 5,281 IU; Vitamin D3, 727 IU; Vitamin E, 30 IU; Vitamin K, 5 mg; biotin, 0.22 mg; choline, 396 mg; folic acid, 0.99 mg, niacin, 22 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; and B12, 0.02 mg (from 77320014, ADM, Decatur, IL)

[3] Provided the following per kg of diet: Vitamin A, 4,400 IU; Vitamin D3, 606 IU; Vitamin E, 25 IU; Vitamin K, 4 mg; biotin, 0.19 mg; choline, 330 mg; folic acid, 0.83 mg, niacin, 19 mg; D-pantothenic acid, 13 mg; riboflavin, 4 mg; and B12, 0.02 mg (from 77320014, ADM, Decatur, IL)

[4] Provided the following as mg/kg of diet: Zn, 144 mg from ZnSO4; Fe, 144 mg from $FeSO_4$; Mn, 44 mg from $MnSO_4$; Cu, 9 mg $CuSO_4$; and Se, 0.2 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[5] Provided the following as mg/kg of diet: Zn, 60 mg from ZnSO4; Fe, 60 mg from $FeSO_4$; Mn, 18 mg from $MnSO_4$; Cu, 4 mg $CuSO_4$; and Se, 0.1 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[6] Available phosphorus

[7] True digestibility values

Table 5.2. Growth performance of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) during pre- and post-inoculation periods[1]

| | Inoculant | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Pre-inoculation | | | | |
| ADG[3], kg | 0.550 | 0.591 | 0.048 | 0.5523 |
| ADFI[4], kg | 1.141 | 1.264 | 0.034 | 0.0036 |
| G:F[5] | 0.48 | 0.45 | 0.038 | 0.6189 |
| BW[6], kg | 21.54 | 21.32 | 1.225 | 0.9016 |
| Post-inoculation | | | | |
| ADG[3], kg | 0.517 | 0.232 | 0.104 | 0.0668 |
| ADFI[4], kg | 1.489 | 1.445 | 0.046 | 0.5060 |
| G:F[5] | 0.34 | 0.16 | 0.070 | 0.0784 |
| BW[6], kg | 22.38 | 21.72 | 1.234 | 0.7040 |

[1] Least square means. Pigs (n=24, control diet) were inoculated on d 9 of the trial. Pre-inoculation: d 6 to d 9; Post-inoculation: d 9 to d 12
[2] Pooled standard error of the mean
[3] Average daily gain
[4] Average daily feed intake
[5] Gain to feed ratio
[6] Body weight of pigs

Table 5.3. Apparent ileal amino acid digestibility (%) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 24 h post-inoculation[1]

| | Inoculant | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 77.9 | 63.7 | 5.45 | 0.0710 |
| His | 63.6 | 62.5 | 7.06 | 0.9038 |
| Ile | 75.5 | 56.8 | 6.50 | 0.0509 |
| Leu | 74.6 | 57.1 | 8.03 | 0.1187 |
| Lys | 77.4 | 66.8 | 4.90 | 0.1211 |
| Met | 78.5 | 59.9 | 7.24 | 0.0751 |
| Phe | 77.3 | 61.5 | 5.77 | 0.0607 |
| Thr | 62.5 | 33.0 | 10.26 | 0.0512 |
| Val | 70.4 | 45.6 | 9.26 | 0.0648 |
| Dispensable | | | | |
| Ala | 69.6 | 48.3 | 9.91 | 0.1231 |
| Asp+Asn | 73.7 | 70.6 | 8.07 | 0.7638 |
| Glu+Gln | 77.7 | 70.9 | 5.39 | 0.3345 |
| Gly | 50.3 | -15.4 | 12.64 | 0.0038 |
| Pro | 68.9 | 46.1 | 9.95 | 0.1034 |
| Ser | 65.0 | 48.6 | 6.67 | 0.0859 |
| Tyr | 71.4 | 49.0 | 7.74 | 0.0504 |

[1] Least square means represent n = 6 and 4 for TSB and $ST^{RR}$ treatments, respectively.
[2] Pooled standard error of the mean

Table 5.4. Apparent ileal amino acid digestibility (%) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 72 h post-inoculation[1]

| | Inoculant | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 81.9 | 73.6 | 2.93 | 0.0626 |
| His | 85.4 | 80.1 | 4.57 | 0.4123 |
| Ile | 76.8 | 65.8 | 3.86 | 0.0604 |
| Leu | 76.8 | 68.6 | 3.17 | 0.0827 |
| Lys | 85.6 | 75.9 | 2.54 | 0.0171 |
| Met | 77.9 | 68.6 | 4.02 | 0.1208 |
| Phe | 79.8 | 70.8 | 2.88 | 0.0427 |
| Thr | 73.7 | 58.3 | 4.89 | 0.0411 |
| Val | 72.7 | 59.6 | 4.37 | 0.0505 |
| Dispensable | | | | |
| Ala | 71.5 | 59.7 | 4.26 | 0.0667 |
| Asp+Asn | 96.0 | 87.7 | 3.20 | 0.0766 |
| Glu+Gln | 92.8 | 83.4 | 3.37 | 0.0589 |
| Gly | 48.9 | 34.8 | 5.84 | 0.1041 |
| Pro | 65.0 | 62.1 | 2.59 | 0.4275 |
| Ser | 81.6 | 70.7 | 3.60 | 0.0480 |
| Tyr | 76.7 | 67.2 | 3.60 | 0.0795 |

[1] Least square means represent n = 6 and 8 for TSB and $ST^{RR}$ treatments, respectively.
[2] Pooled standard error of the mean

Table 5.5. Endogenous amino acid losses (g/kg of dry matter intake) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 24 h post-inoculation[1]

| | Inoculant | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 0.63 | 3.84 | 0.088 | <0.0001 |
| His | 0.26 | 1.34 | 0.037 | <0.0001 |
| Ile | 0.41 | 1.77 | 0.080 | 0.0003 |
| Leu | 0.66 | 3.41 | 0.134 | 0.0001 |
| Lys | 0.49 | 2.56 | 0.137 | 0.0004 |
| Met | 0.16 | 0.72 | 0.029 | 0.0002 |
| Phe | 0.38 | 1.99 | 0.073 | <0.0001 |
| Thr | 0.55 | 2.58 | 0.067 | <0.0001 |
| Val | 0.54 | 2.59 | 0.091 | <0.0001 |
| Dispensable | | | | |
| Ala | 0.60 | 2.52 | 0.095 | 0.0001 |
| Asp+Asn | 0.63 | 3.56 | 0.158 | 0.0002 |
| Glu+Gln | 1.08 | 5.15 | 0.307 | 0.0007 |
| Gly | 1.27 | 11.04 | 2.530 | 0.0524 |
| Pro | 0.75 | 3.25 | 0.509 | 0.0255 |
| Ser | 0.66 | 2.90 | 0.013 | 0.0005 |
| Tyr | 0.34 | 1.67 | 0.054 | <0.0001 |

[1] Least square means represent n = 6 and 8 for TSB and $ST^{RR}$ treatments, respectively.
[2] Pooled standard error of the mean

Table 5.6. Endogenous amino acid losses (g/kg of dry matter intake) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 72 h post-inoculation[1]

| | Inoculant | | SEM[2] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 0.76 | 0.80 | 0.213 | 0.8729 |
| His | 0.08 | 0.12 | 0.034 | 0.3492 |
| Ile | 0.46 | 0.58 | 0.163 | 0.5563 |
| Leu | 0.72 | 0.96 | 0.284 | 0.5039 |
| Lys | 0.32 | 0.50 | 0.123 | 0.2556 |
| Met | 0.17 | 0.20 | 0.052 | 0.6330 |
| Phe | 0.42 | 0.56 | 0.160 | 0.5080 |
| Thr | 0.49 | 0.69 | 0.165 | 0.3592 |
| Val | 0.60 | 0.83 | 0.221 | 0.4335 |
| Dispensable | | | | |
| Ala | 0.56 | 0.85 | 0.208 | 0.2966 |
| Asp+Asn | 0.10 | 0.26 | 0.067 | 0.0956 |
| Glu+Gln | 0.28 | 0.71 | 0.188 | 0.1057 |
| Gly | 1.83 | 1.89 | 0.490 | 0.9253 |
| Pro | 2.04 | 2.96 | 0.986 | 0.4709 |
| Ser | 0.39 | 0.60 | 0.136 | 0.2551 |
| Tyr | 0.34 | 0.52 | 0.136 | 0.3299 |

[1] Least square means represent n = 6 and 4 for TSB and $ST^{RR}$ treatments, respectively.
[2] Pooled standard error of the mean

Table 5.7. Standardized ileal amino acid digestibility (%) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 24 h post-inoculation

| | Inoculant | | SEM[1] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 83.2 | 95.8 | 5.45 | 0.1004 |
| His | 70.9 | 99.9 | 7.06 | 0.0121 |
| Ile | 81.1 | 81.1 | 6.50 | 0.9944 |
| Leu | 79.0 | 79.8 | 8.03 | 0.9405 |
| Lys | 82.5 | 93.3 | 4.90 | 0.1127 |
| Met | 84.9 | 87.8 | 7.24 | 0.7566 |
| Phe | 81.7 | 84.7 | 5.77 | 0.6839 |
| Thr | 72.4 | 79.3 | 10.26 | 0.6026 |
| Val | 77.1 | 77.9 | 9.26 | 0.9481 |
| Dispensable | | | | |
| Ala | 76.5 | 77.5 | 9.91 | 0.9363 |
| Asp+Asn | 77.8 | 93.8 | 8.07 | 0.1499 |
| Glu+Gln | 81.3 | 87.9 | 5.39 | 0.3507 |
| Gly | 69.1 | 148.0 | 12.64 | 0.0014 |
| Pro | 76.5 | 79.1 | 9.95 | 0.8387 |
| Ser | 73.9 | 87.8 | 6.67 | 0.1335 |
| Tyr | 77.5 | 79.5 | 7.74 | 0.8395 |

[1] Pooled standard error of the mean

Table 5.8. Standardized ileal amino acid digestibility (%) of pigs inoculated with sterile tryptic soy broth (TSB) or *Salmonella* Typhimurium $Nal^{R}Nov^{R}$ ($ST^{RR}$) at 72 h post-inoculation

| | Inoculant | | SEM[1] | *P*-value |
|---|---|---|---|---|
| | TSB | $ST^{RR}$ | | |
| Indispensable | | | | |
| Arg | 88.3 | 80.4 | 2.93 | 0.0734 |
| His | 87.6 | 83.6 | 4.57 | 0.5303 |
| Ile | 83.1 | 73.8 | 3.86 | 0.1036 |
| Leu | 81.6 | 75.0 | 3.17 | 0.1539 |
| Lys | 88.9 | 81.1 | 2.54 | 0.0459 |
| Met | 84.4 | 76.6 | 4.02 | 0.1834 |
| Phe | 84.7 | 77.3 | 2.88 | 0.0865 |
| Thr | 82.5 | 70.7 | 4.89 | 0.1032 |
| Val | 80.2 | 69.9 | 4.37 | 0.1129 |
| Dispensable | | | | |
| Ala | 78.0 | 69.5 | 4.26 | 0.1723 |
| Asp+Asn | 96.7 | 89.4 | 3.20 | 0.1162 |
| Glu+Gln | 93.7 | 85.7 | 2.85 | 0.1002 |
| Gly | 76.1 | 62.8 | 5.84 | 0.1237 |
| Pro | 85.7 | 92.2 | 5.59 | 0.0996 |
| Ser | 86.9 | 78.8 | 3.60 | 0.1270 |
| Tyr | 83.0 | 76.7 | 3.60 | 0.2286 |

[1] Pooled standard error of the mean

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# CHAPTER 6

## Dynamic alterations of the endogenous amino acid losses and subsequent amino acid digestibility in ileal cannulated pigs challenged orally with *Salmonella enterica* serovar Typhimurium

### 6.1 Abstract

Dynamic fluctuations of endogenous amino acid (AA) losses (EAAL) and subsequent AA digestibility in response to *Salmonella* Typhimurium challenge were determined in pigs. Ileal cannulated pigs (n = 8, 76.0 ± 1.4 kg) were randomly assigned to either a control or N-free diet (NFD). Rectal temperatures (RT) were monitored 3 times daily and ileal digesta were collected for 8-16 h daily. Pigs were orally inoculated with $1.3 \times 10^{10}$ colony forming units of an antibiotic resistant *Salmonella* strain (*S. enterica* serovar Typhimurium DT104 $Nal^{R}Nov^{R}$) at 0 h. Daily RT displayed patterns of circadian body temperatures with post-inoculation nocturnal fever. The daily peak RT showed increases at 16 h post-inoculation ($P < 0.0001$). The EAAL peaked during 8-16 h post-inoculation, showing 2-4 fold increments compared to pre-inoculation levels. Apparent ileal AA digestibility (AID) and standardized ileal AA digestibility (SID) showed similar changes, as the lowest AID were observed 8-16 h post-inoculation, with decreases in all AA ($P < 0.09$). Final measurements at 72-80 h post-inoculation seemed to be decreased with 6 and 8% reductions in AID and SID, respectively. *Salmonella* Typhimurium induced enterocolitis reduced AA digestibility in ileal cannulated pigs having greatest impact at 8-16 h post-inoculation with highest EAAL. Alterations in AID, EAAL, and its derived SID were gradually recovered to near pre-

inoculation values by 56-64 h post-inoculation, but showed impaired digestibility at 72-80 h post-inoculation.

## 6.2 Introduction

Stimulation of the immune system by enteric pathogens results in physiological changes of the host including its digestive system. The catabolism of amino acids (AA) during an immunological stress emphasizes the importance of AA digestion for immune responses and sound recovery (Johnson et al., 2001).

Only a few studies have shown the effect of immune system stimulation on AA digestibility. A recent study reported no differences in apparent ileal AA digestibility (AID) in pigs using the lipopolysaccharide (LPS) model (Rakhshandeh et al., 2010). However, the LPS model does not fully simulate an enteric bacterial pathogenesis, as a LPS challenge produces more of a systemic cytokine response (Johnson et al., 2005) opposed to a localized production of proinflamatory cytokines in cases such as Salmonellosis. In poultry, reductions in AID were reported during acute infection phase of *Eimeria acervulina* (Persia et al., 2006).

While we have previously shown alterations of AA digestibility at 24 h and 72 h post *Salmonella* infection using the comparative slaughter technique (**Chapter 5**); however, measuring dynamic fluctuations of endogenous AA losses (EAAL) required further examination. Thus, the objective of current study was to determine the impact of *Salmonella* on dynamic alterations of AA digestibility with multiple time-point observations using ileal-cannulated pigs.

## 6.3 Materials and methods

### 6.3.1 Animals, maintenance, and diets

All experimental procedures were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee. A total of 8 pigs were obtained from the Swine Center at Virginia Tech (Blacksburg, VA) and transported to an Animal Biosafety Level (ABSL)-2 facility at approximately 93 d of age (47.0 ± 1.5 kg). A simple T-cannula was surgically implanted on the animals according to Wubben et al. (2001). Following a 3-wk post-surgical recovery period, pigs were fed 3 times the estimated energy requirement for maintenance (i.e., 106 kcal ME/kg $BW^{0.75}$; NRC, 1998), divided into 2 equal meals given at 0800 and 1600 throughout the entire study. Pigs were housed in individual pens (0.6 × 1.4 m) equipped with a feeder and a nipple waterer. The facility was independently ventilated with 100% clean air (i.e., no recirculation) under negative pressure. Temperature (24°C) and lighting (18-h light:6-h dark with lights on at 0600) were controlled by automated system.

Pigs were kept on an antibiotic-free control diet for 54 d prior to the current experiment. At approximately 309 d of age, pigs (76.0 ± 1.4 kg) were randomly assigned to either a control diet or N-free diet (NFD) (i.e., n = 4 for each diet). The ingredient composition and nutrient composition of the experimental diets are shown in **Table 6.1**. A typical corn-soybean meal (SBM) diet was formulated to meet or exceed NRC recommendations for nutrients (NRC, 1998). A NFD was formulated based on cornstarch and sucrose. Solka-Floc was used as source of fiber and vitamins $B_1$ and $B_6$ (Vitamin World Inc., Ronkonkoma, NY) were supplemented to meet NRC recommendations. All diets contained 0.3% chromic oxide as an indigestible marker and contained no antibiotics. Prior to experiment, both diets and all of the pigs were screened for the presence of *Salmonella*. Random composite feed samples were enriched in Gram-negative Hanja broth

at 37°C for 24 h, diluted and plated onto Brilliant Green agar (BGA) plates. Pigs were screened by directly plating rectal swabs onto BGA plates. All screening results were negative for *Salmonella* prior to inoculation.

6.3.2 Inoculant preparation

*Salmonella* Typhimurium used in this experiment was prepared and cultured as previously described (Price et al., 2010) with slight modifications. Briefly, *S. enterica* subspecies *enterica* serovar Typhimurium DT104 (ATCC, BAA-185, Manassas, VA) was resuscitated in 50 ml of tryptic soy broth (TSB) at 37°C for 8 h, then incubated in fresh TSB overnight. *Salmonella* was identified using XLT4 agar plate. By sequentially transferring single colonies onto BGA plates that were finalized to contain 20 μg/ml of nalidixic acid (Acros Organics, Morris Plains, NJ) and 25 μg/ml of novobiocin (BD Bioscience, Sparks, MD), the antibiotic resistant strain was prepared (*Salmonella* Typimurium $Nal^{R}Nov^{R}$, $ST^{RR}$ henceforth). The choice of an antibiotic strain was to ensure any *Salmonella* detected were only of inoculant origin. *Salmonella* Typimurium $Nal^{R}Nov^{R}$ was cultured overnight at 37°C in TSB on an orbital shaker (New Brunswick Scientific, Edison, NJ) at 200 rpm and bacterial populations were estimated by spectrophotometry at 600 nm. To increase its virulence, L-norephinephrine was added at 2 mM to the TSB as described by Toscano et al. (2006). For inoculant preparation, $ST^{RR}$ was harvested at 3,000 × *g* for 10 min at 4°C, and resuspended in sterile TSB. The inoculant was immediately placed on ice and used within 2 h. Inoculant dose was assayed for bacterial content using serial dilution with peptone water and plating, and it was determined to contain $1.3 \times 10^{9}$ CFU/ml.

6.3.3 Experiment procedures

Animals were given a 4-d acclimation period to experiment diets. From d 5, rectal temperatures (RT) were monitored 3 times daily (0800, 1600, and 2400) throughout the rest of the trial. On d 5, ileal digesta were collected for 8 h (0800 to 1600) into plastic bags attached to the cannula with a rubber band. The plastic bags were changed when full or at least every 30 min to minimize bacterial fermentation. Digesta from each 8-h collection period was immediately poured into glass jars stored in ice bath. Jars were moved to a -20°C freezer at the end of each 8-h period. On d 6, 10 ml of $ST^{RR}$ inoculant was carefully soaked and mixed into the 0800 meal. Water valves were turned off until all pigs were confirmed to have consumed all of the given diet. Ileal digesta was collected for 16 h (two 8-h periods; 0800 to 1600 and 1600 to 2400) each day for 3 d following inoculation (i.e., d 6 to 8). On d 9, final digesta were collected for 8 h (0800 to 1600) and pigs were euthanized with a lethal dose of 120 mg/kg body weight (BW) of sodium pentobarbital i.v. (Beuthanasia-D, Shering-Plough, Union, NJ). Feces, ileocecal lymph node, and cecum contents were collected for detection of $ST^{RR}$. Collected samples were diluted with peptone water, and homogenized in a stomacher (BagMixer 400W, Interscience, Rockland, MA). A 100 µl aliquot of each homogenate was plated onto BGA and XLT4 agar plates containing 20 µg/ml of nalidixic acid and 25 µg/ml of novobiocin. Presence of $ST^{RR}$, by colony appearance was confirmed for all samples from $ST^{RR}$ inoculated pigs. Digesta were later thawed at 4°C, homogenized (Waring Commercial CB-5, Waring Products Inc., Stamford, CT), sub-sampled, freeze dried (Lyph-Lock 12, Labconco Corp., Kansas City, MI), ground (BCG100WH, KitchenAid, St. Joseph, MI), and stored in -20°C until analyses.

6.3.4 Calculations, chemical and statistical analyses

Chromic oxide was analyzed using the procedures described by Fenton and Fenton (1979). Ileal digesta were measured for dry matter (DM, method 930.15; AOAC, 2005).

Amino acid contents were determined using the Pico·Tag method as previously described (Albin et al., 2000; Cohen et al., 1989). Methionine and Cys were analyzed as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30; AOAC, 2005).

Apparent ileal AA digestibility was calculated based on the following equation:

$$AID\ (\%) = \{ 1 - [ ( AA_{Digesta} / AA_{Feed} ) \times ( M_{Feed} / M_{Digesta} ) ] \} \times 100$$

where $AA_{Digesta}$ and $AA_{Feed}$ are the AA content in the ileal digesta and the feed (g/kg DM), respectively, and $M_{Feed}$ and $M_{Digesta}$ are the chromic oxide concentration in the feed and the ileal digesta (g/kg DM), respectively.

Standardized ileal AA digestibility (SID) was calculated using the following formula (Lemme et al., 2004):

$$SID\ (\%) = AID + [ ( EAAL ) / ( AA_{Feed} ) \times 100 ]$$

All values were subjected to PROC UNIVARIATE procedure of SAS (SAS Institute, Cary, NC) to discard outliers. One-way ANOVA using the PROC MIXED procedure of SAS was used to compare each time-point measurements to pre-inoculation values. Pig was set as a random variable. Differences between least square means were tested using Tukey's multiple comparison procedure of SAS. Significance was declared at $P < 0.10$.

## 6.4 Results

### 6.4.1 Rectal temperatures and ileal digesta dry matter

Changes in RT of pigs and their comparison to average pre-inoculation value (37.9°C) are shown in **Figure 6.1**. Daily patterns of RT were observed post-inoculation with nocturnal (12 AM) fever. The RT of pigs were increased and peaked at 16 h post-inoculation (39.5°C, $P < 0.0001$). Peak RT of the second (38.6°C) and third day (38.3°C)

post-inoculation showed signs of febrile response. Effect of $ST^{RR}$ infection on the DM of pig ileal digesta are shown in **Figure 6.2**. The DM of ileal digesta was significantly reduced at 8-16 h post-inoculation ($P = 0.002$) and fluctuated until returning to pre-inoculation value at 72-80 h post-inoculation. Thus, the febrile response, significant decreases in DM of ileal digesta, as well as presence of $ST^{RR}$ in feces, ileocecal lymph node, and cecum contents from all pigs at the end of trial, collectively showed that inoculation was successful with clinical symptoms of Salmonellosis.

6.4.2 Amino acid digestibility

Pre-inoculation means of AID, EAAL, and its derived SID are shown in **Table 6.2**. Based on pre-inoculation values, effects of $ST^{RR}$ infection for individual AA are illustrated on **Figures 6.3-18**. High variation was detected for many measurements, especially during the initial 56 h post-inoculation observations when pigs appear to become sick then start recovering from infection. Thus, most descriptions were based on numerical evaluations unless *P*-values were mentioned.

Similar patterns were shown for AID of all measured AA, where the lowest points were observed during 8-16 h post-inoculation, with decreases in Arg ($P = 0.04$), His ($P = 0.02$), Ile ($P = 0.03$), Leu ($P = 0.06$), Lys ($P = 0.08$), Met ($P = 0.08$), Phe ($P = 0.03$), Thr ($P = 0.008$), Val ($P = 0.01$), Ala ($P = 0.05$), Asx ($P = 0.09$), Glx ($P = 0.06$), Gly ($P = 0.0007$), Pro ($P = 0.03$), Ser ($P = 0.02$), and Tyr ($P = 0.01$) compared to pre-inoculation values. The AID gradually recovered to near pre-inoculation values by 56-64 h post-inoculation, fluctuating at 48-56 h post-inoculation. Final AID measurements at 72-80 h post-inoculation showed an average of 6% reduction compared to pre-inoculation values.

Except for Gly (**Figure 6.15**) and Pro (**Figure 6.16**), EAAL of all measured AA showed similar patterns. The EAAL were unaffected during 0-8 h post-inoculation except

for Gly and Pro where peak increases were measured. Most AA showed the highest endogenous losses during 8-16 h post-inoculation. The peak values were 2-4 fold increments of pre-inoculation levels. The EAAL gradually decreased to near pre-inoculation values by 56-64 h post-inoculation, fluctuating at 48-56 h post-inoculation. Final measurements at 72-80 h post-inoculation showed an average of 32% reduction compared to pre-inoculation values.

Changes in SID showed similar patterns as AID in all measured AA except for Pro. The SID were unaffected during 0-8 h post-inoculation, whereas Pro increased by 12%. The lowest SID were measured during 8-16 h or 24-32 h post-inoculation, with an average reduction of 16% from pre-inoculation values, except for Gly (53% reduction). Especially, Gly showed decreased SID at 8-16 and 24-32 h post-inoculation (both at $P = 0.01$), while Pro measured decrements at 24-32 h post inoculation ($P = 0.03$). The SID gradually recovered to near pre-inoculation values by 56-64 h post-inoculation, fluctuating at 48-56 h post-inoculation. Final SID measurements at 72-80 h post-inoculation showed an average of 8% reduction compared to pre-inoculation values.

## 6.5 Discussion

### 6.5.1 Rectal temperatures

Daily RT displayed patterns of circadian body temperatures with post-inoculation nocturnal fever. The circadian body temperature rhythm begins low in the mornings, increasing throughout the day, ending with a high plateau at night. Although a rhythmical change of daily body temperature has been known to be related to activity, feeding and sleep patterns in confined pigs (Ingram and Legge, 1970), existence of such rhythm suggest that body temperature results could be affected simply by the time of the day of its measurement.

In fact, a daily RT measurement in the morning (0800) would have failed to detect the febrile response in present study.

It is important to mention that the febrile response was relatively small considering the high infectious dosage level ($1.3 \times 10^{10}$ CFU) and presumed increased virulence of $ST^{RR}$ (L-norephinephrine treatment) used in present study. Previous swine studies using *S.* Typhimurium models have shown prolonged RT elevations for days at lower infectious doses of approximately $10^9$ CFU (Balaji et al., 2000; Price et al., 2010). Nonetheless, the occurrence of diarrhea and presence of $ST^{RR}$ in tissues confirm the presence of active enteric bacterial infection in the experimental pigs.

An acute immune response to *S.* Typhimurium induced enterocolitis accompanies a diarrheal disease through an inflammatory mechanism (Zhang et al., 2003). Initial stage of *Salmonella* pathogenesis is crucial as severity of the disease should be determined by number of *Salmonella* that successfully penetrate the outer epithelium. The diarrheal response by the host is an effective method to physically flush pathogens out into the lumen. In this study, pigs were fitted with a simple-T cannula at the distal ileum for digesta collection, and copious amounts of digesta were collected during *Salmonella* infection compared to pre-inoculation periods. Especially, the amount of digesta collected during the 8-16 h post-inoculation period was increased 2-3 fold compared to pre-inoculation collection periods, which would have significantly reduced the pathogenic load within GIT. We speculate that the effective infectious dose was near or lower than $10^8$ CFU, where a previous study showed no RT increases when measured in mornings (Davis et al., 2010).

### 6.5.2 Amino acid digestibility

Pre-inoculation EAAL measurements in current study were generally in agreement to previously reported values evaluated by Jansman et al. (2002) except for Asx, Glx, and Gly,

where lower values were measured. Also, pre-inoculation values for AID and SID for a typical corn-SBM diet agreed to values reported by Stein et al. (2007).

At 0-8 h post-inoculation, EAAL of all AA except Gly and Pro remained unaffected by $ST^{RR}$ challenge. Endogenous losses of Gly and Pro are known to be overestimated when measured using the NFD method (de Lange et al., 1989; Moughan et al., 1992). Jansman et al. (2002) explained that the probability of observing a high endogenous Pro loss increased with length of period pigs were offered NFD. In fact, pigs had been given NFD for 5 d at 0-8 h post-inoculation. Thus, it is likely that the increments of endogenous losses for Pro and Gly at 0-8 h post-inoculation are a result of abnormal AA metabolism due to prolonged NFD period, and not induced by $ST^{RR}$ challenge.

The peak EAAL values at 8-16 h post-inoculation of current study (2-4 fold increases) were considerably less than those observed at 24 h post-inoculation in previous study using the comparative slaughter technique (4-5 fold increases excluding Gly, **Chapter 5**). As the level of EAAL can be interpreted as the magnitude of acute phase immune response, pigs were relatively less affected by $ST^{RR}$ challenge in current experiment. This could be due to three reasons: differences in age/BW of pigs, *Salmonella* dosage, and collection technique.

First, pigs used in current experiment were older than previous experiment (309 vs. 54 d of age). Because young animals are more susceptible to Salmonellosis, nursery pigs are more susceptible to *Salmonella* infection than growers (Timoney et al., 1988). Also, EAAL is known to decrease with increased BW at low feed intake (Stein et al., 2007), thus smaller pigs in previous trial (17.9 ± 6.4 kg) that were fed ad libitum would have higher EAAL level than larger (76.0 ± 6.6 kg), feed restricted pigs used in current study.

Second, the actual infectious dosage levels within the GIT were different between two trials. While pigs were orally inoculated with a similar level of $ST^{RR}$ ($9.8 \times 10^9$ vs. $1.3 \times 10^{10}$ CFU), the effective infectious dosage levels at the distal ileum would have been lower

for current study as described earlier. In this study the peak RT occurred at 16 h without sustaining febrile response. In contrast, our previous study in nursery pigs resulted in elevated RT for 60 h (**Chapter 5**, data not shown), which were similar to previous RT report by Balaji et al. (2000). Finally, higher EAAL in the slaughter methods can be attributed to increased sloughing during mechanical manipulation of the distal ileum to collect digesta.

Alterations of EAAL induced by $ST^{RR}$ returned to pre-inoculation values by 56-64 h post-inoculation. Moreover, 72-80 h post-inoculation measurements seemed to be decreased for EAAL of several AA when compared to their respective pre-inoculation values. As the increases of EAAL in enteric *Salmonella* infection are known to be a result of neutrophilic inflammation (Santos et al., 2002), a gradual decrease of the EAAL indicated that intestinal epithelium began to recover at about 24 h post-inoculation. Also, following an event of marked EAAL increases (i.e., inflammatory diarrhea), it seems that there is a periodical reduction of proteins secreted into the intestinal lumen (e.g., mucins, serum albumin), resulting in decreased EAAL.

Changes in AID and SID following $ST^{RR}$ inoculation showed an opposite pattern to EAAL. Whereas the highest EAAL values were observed at 8-16 h post-inoculation, the lowest AID were measured at 8-16 h post-inoculation, and SID at 8-16 h or 24-32 h post-inoculation. Because SID were calculated based on AID and EAAL values, the lowest point seemed to coincide with the highest EAAL, which was speculated to be at 8-16 h post-inoculation. Furthermore, these results suggest a potential reduction in digestive capacity and absorption concomitant with increased intestinal epithelium damage and sloughing.

Both AID and SID gradually increased back to pre-inoculant levels by 56-64 h post-inoculation. As observed in EAAL, final measurements of AID and SID at 72-80 h seemed to be decreased for several AA when compared to their respective pre-inoculation values, indicating possible impairment to AA digestibility

## 6.6 Conclusion

Pigs orally inoculated with $10^{10}$ CFU of $ST^{RR}$ showed febrile response of Salmonellosis similar to published reports with challenge doses of $10^8$ CFU, presumably due to the lower amount of pathogen reaching the large intestine due to digesta removal from T-cannula. Daily RT displayed patterns of circadian body temperatures with post-inoculation nocturnal fever. Ileal EAAL values peaked at 8-16 h post-inoculation and reduced to pre-inoculant values by 56-64 h after *Salmonella* inoculation. The EAAL further decreased at 72-80 h post-inoculation suggesting a periodical reduction of proteins secreted into the intestinal lumen following inflammatory diarrhea. *Salmonella* infection reduced AA digestibility, with lowest AID values measured at 8-16 h post-inoculation. As SID is calculated from AID and EAAL, its low points seemed to coincide also at 8-16 h post-inoculation. Both AID and SID gradually increased back to initial levels by 56-64 h post-inoculation and showed lower values at 72-80 h, indicating impaired AA digestibility. In conclusion, results suggest that the SID concept may not be as applicable during enteric disease conditions, where EAAL is drastically elevated, as is to normal "healthy" experimental conditions.

## 6.6 Tables

Table 6.1. Diet formulation and calculated nutrient composition of N-free and control diets, as fed basis

| | N-free[1] | Control |
|---|---|---|
| Ingredients, % | | |
| Corn | - | 77.08 |
| Soybean meal (47.5%) | - | 19.40 |
| Cornstarch | 75.88 | - |
| Sucrose | 12.00 | - |
| Solca-Floc | 5.00 | - |
| Soy oil | 3.00 | 1.20 |
| Dicalcium phosphate | 1.55 | 0.68 |
| Limestone | 0.95 | 0.94 |
| Salt | 0.30 | 0.26 |
| Vitamin premix | 0.20[2] | 0.08[3] |
| Mineral premix | 0.12[4] | 0.06[5] |
| Potassium chloride | 0.55 | - |
| Magnesium sulfate | 0.10 | - |
| Sodium carbonate | 0.05 | - |
| Chromic oxide | 0.30 | 0.30 |
| Calculated nutrients | | |
| ME, kcal/kg | 3712 | 3393 |
| CP, % | - | 15.61 |
| Ca, % | 0.70 | 0.55 |
| P, %[6] | 0.29 | 0.49 |
| Lysine, %[7] | - | 0.79 |
| Methionine, %[7] | - | 0.26 |
| Threonine, %[7] | - | 0.58 |
| Arginine, %[7] | - | 0.96 |

[1] Additional vitamins supplemented per kg of diet: Vitamins B1, 47 mg; Vitamin B6, 20 mg (from Vitamin World Inc., Ronkonkoma, NY)

[2] Provided the following per kg of diet: Vitamin A, 5,281 IU; Vitamin D3, 727 IU; Vitamin E, 30 IU; Vitamin K, 5 mg; biotin, 0.22 mg; choline, 396 mg; folic acid, 0.99 mg, niacin, 22 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; and B12, 0.02 mg (from 77320014, ADM, Decatur, IL)

[3] Provided the following per kg of diet: Vitamin A, 2,347 IU; Vitamin D3, 323 IU; Vitamin E, 13 IU; Vitamin K, 2 mg; biotin, 0.10 mg; choline, 176 mg; folic acid, 0.44 mg, niacin, 10 mg; D-pantothenic acid, 7 mg; riboflavin, 2 mg; and B12, 0.01 mg (from 77320014, ADM, Decatur, IL)

[4] Provided the following as mg/kg of diet: Zn, 144 mg from ZnSO4; Fe, 144 mg from $FeSO_4$; Mn, 44 mg from $MnSO_4$; Cu, 9 mg $CuSO_4$; and Se, 0.2 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[5] Provided the following as mg/kg of diet: Zn, 72 mg from ZnSO4; Fe, 72 mg from $FeSO_4$; Mn, 22 mg from $MnSO_4$; Cu, 4 mg $CuSO_4$; and Se, 0.1 mg from $Na_2SeO_3$ (from 77069014, ADM, Decatur, IL)

[6] Available phosphorus

[7] True digestibility values

Table 6.2. Pre-inoculation means of endogenous amino acid losses (EAAL), apparent ileal amino acid digestibility (AID), and standardized ileal amino acid digestibility (SID) of ileal cannulated pigs[1]

| | EAAL[2] | AID[3] | SID[3] |
|---|---|---|---|
| Indispensable | | | |
| Arginine | 0.44 ± 0.01 | 87.2 ± 0.4 | 91.0 ± 0.4 |
| Histidine | 0.20 ± 0.02 | 87.8 ± 2.8 | 91.7 ± 2.8 |
| Isoleucine | 0.28 ± 0.01 | 82.5 ± 1.4 | 86.9 ± 1.4 |
| Leucine | 0.41 ± 0.02 | 85.9 ± 1.1 | 88.9 ± 1.1 |
| Lysine | 0.41 ± 0.03 | 85.5 ± 1.6 | 90.0 ± 1.6 |
| Methionine | 0.09 ± 0.01 | 80.7 ± 2.5 | 85.5 ± 2.5 |
| Phenylalanine | 0.26 ± 0.01 | 86.4 ± 1.2 | 89.8 ± 1.2 |
| Threonine | 0.47 ± 0.02 | 79.1 ± 2.3 | 87.0 ± 2.3 |
| Valine | 0.38 ± 0.01 | 80.0 ± 1.8 | 85.6 ± 1.8 |
| Dispensable | | | |
| Alanine | 0.41 ± 0.01 | 79.6 ± 2.5 | 85.0 ± 2.5 |
| Aspartic acid + Asparagine | 0.37 ± 0.04 | 92.8 ± 2.9 | 95.1 ± 2.9 |
| Glutamic acid + Glutamine | 0.61 ± 0.03 | 92.7 ± 1.9 | 94.7 ± 1.9 |
| Glycine | 0.78 ± 0.04 | 74.1 ± 1.0 | 87.3 ± 1.0 |
| Proline | 1.13 ± 0.13 | 85.0 ± 0.9 | 96.2 ± 0.9 |
| Serine | 0.57 ± 0.03 | 83.0 ± 2.2 | 89.8 ± 2.2 |
| Tyrosine | 0.25 ± 0.01 | 81.5 ± 1.9 | 86.6 ± 1.9 |

[1] Measured from pooled digesta collected during 24~16 h pre-inoculation, means ± SEM
[2] Measured using N-free diet method, g/kg of dry matter (DM) intake (n = 4)
[3] Digestibility, % (n = 4)

## 6.7 Figures

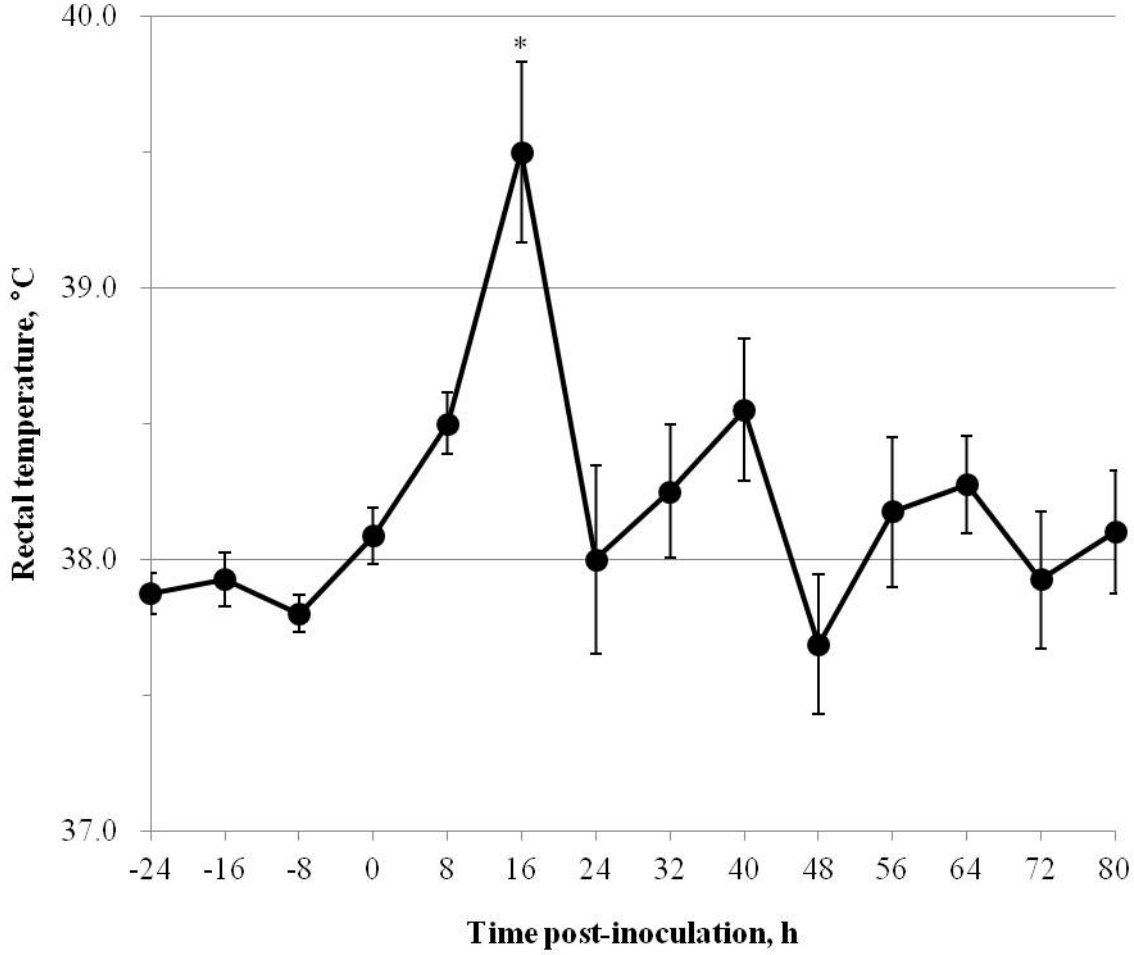


Figure 6.1. Effect of *Salmonella* Typhimurium infection on the rectal temperature of pigs. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free diet (n = 4) or a typical corn-soybean meal diet (n = 4). Values are means ± SEM. * Different from -24 h, $P < 0.0001$.

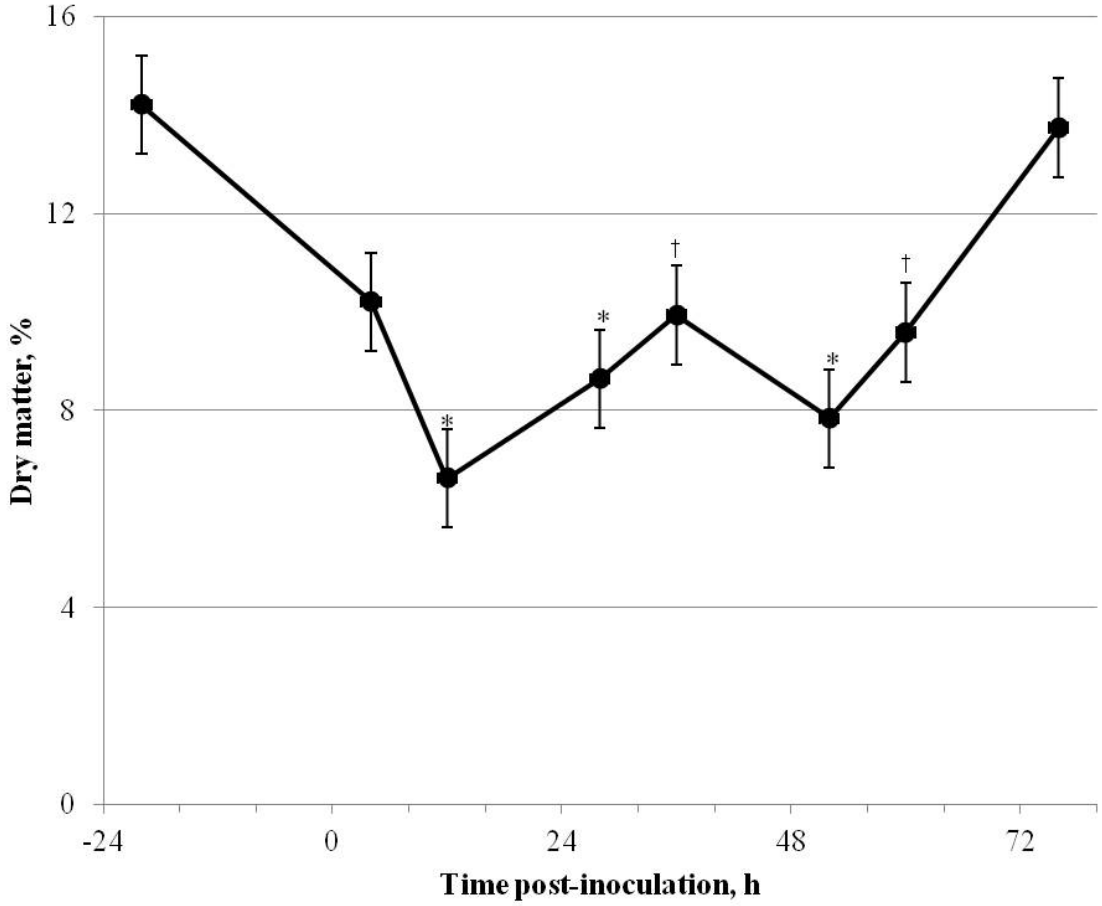


Figure 6.2. Effect of *Salmonella* Typhimurium infection on the dry matter content of ileal digesta from pigs. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free diet (n = 4) or a typical corn-soybean meal diet (n = 4). Values are means ± SEM. * Different from -24 h, $P < 0.05$. † Different from -24 h, $P < 0.10$.

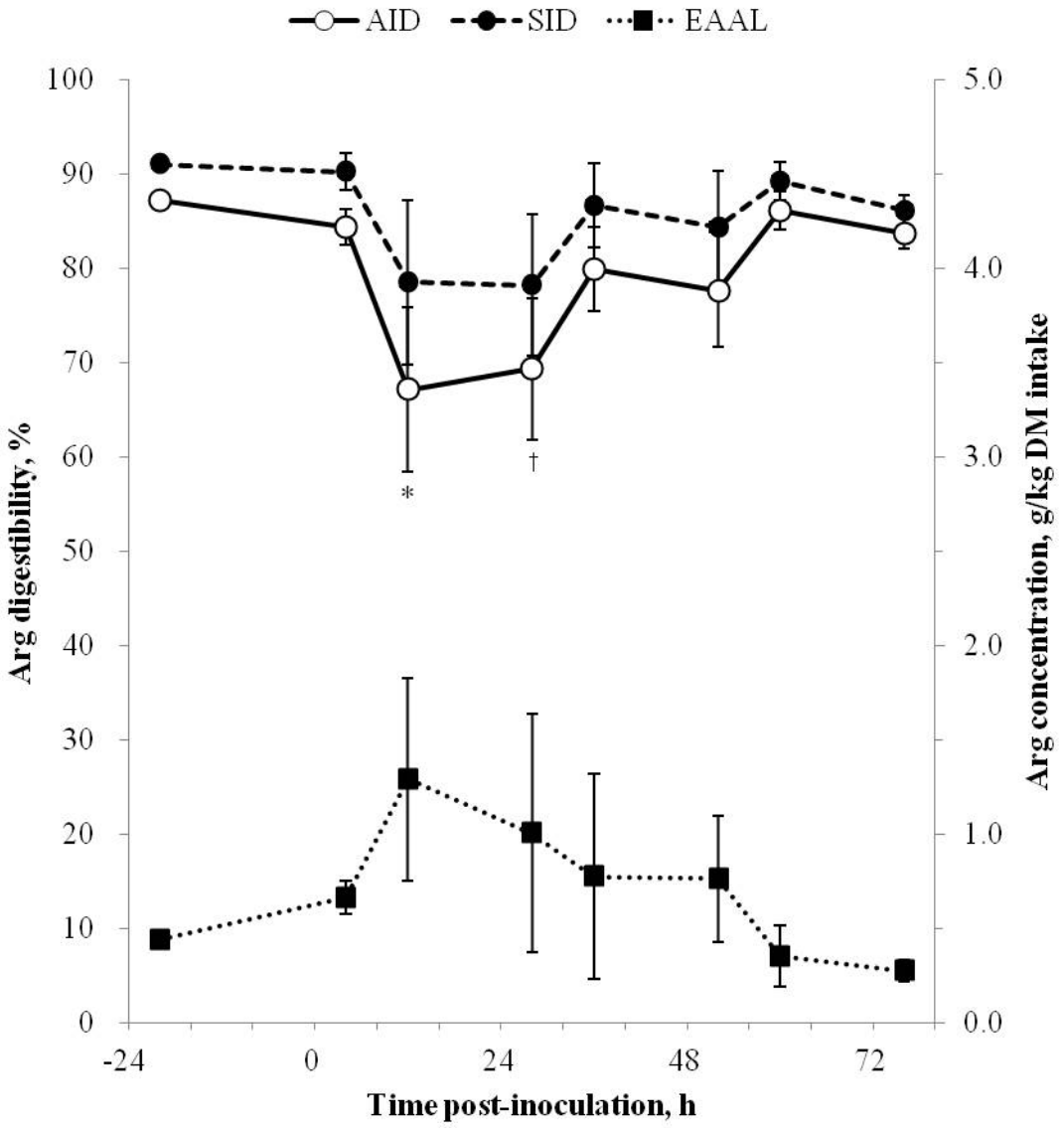


Figure 6.3. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of arginine (Arg) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P = 0.04$. † Different from -24 h, $P < 0.09$.

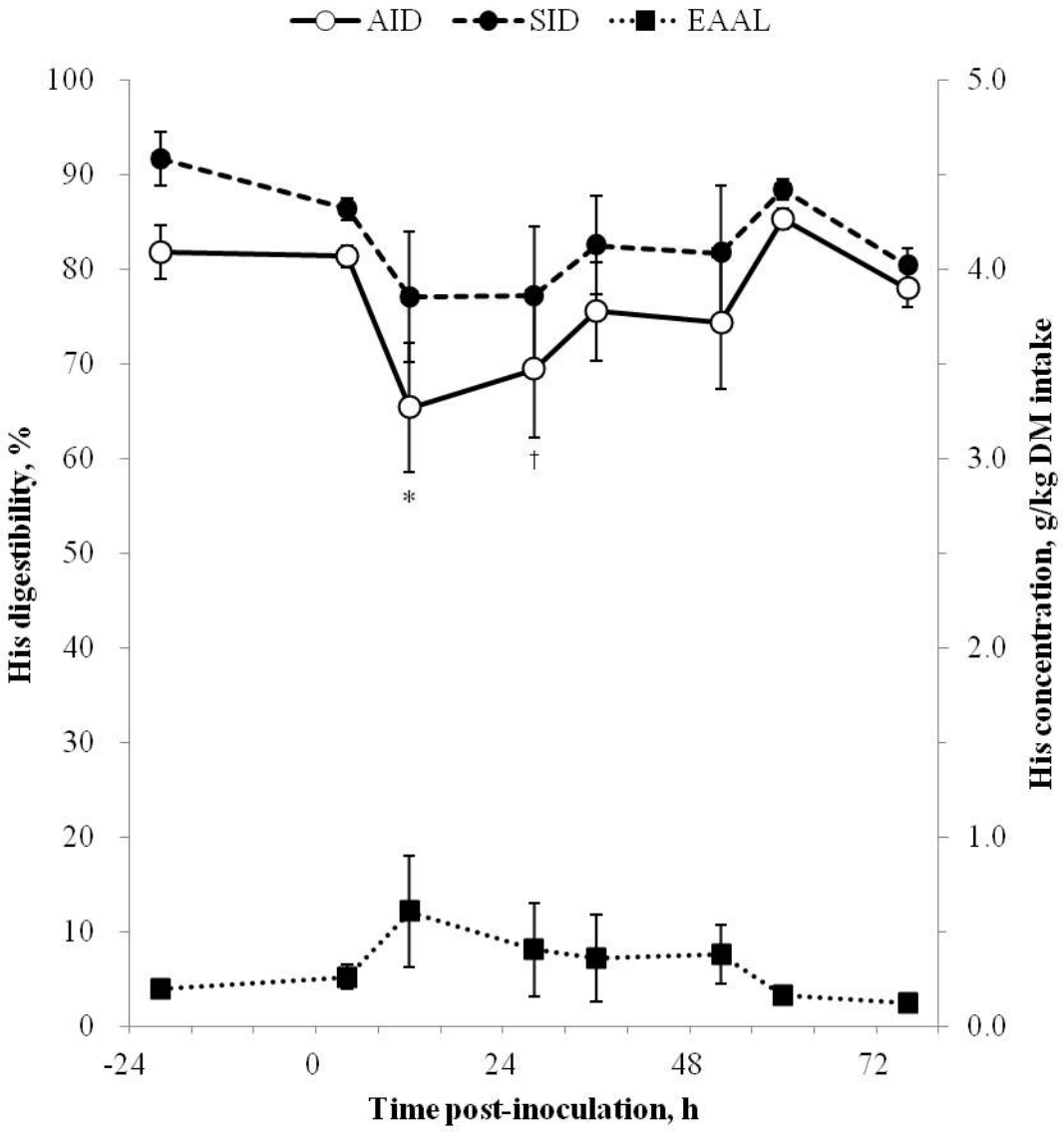


Figure 6.4. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of histidine (His) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P = 0.02$. † Different from -24 h, $P = 0.08$.

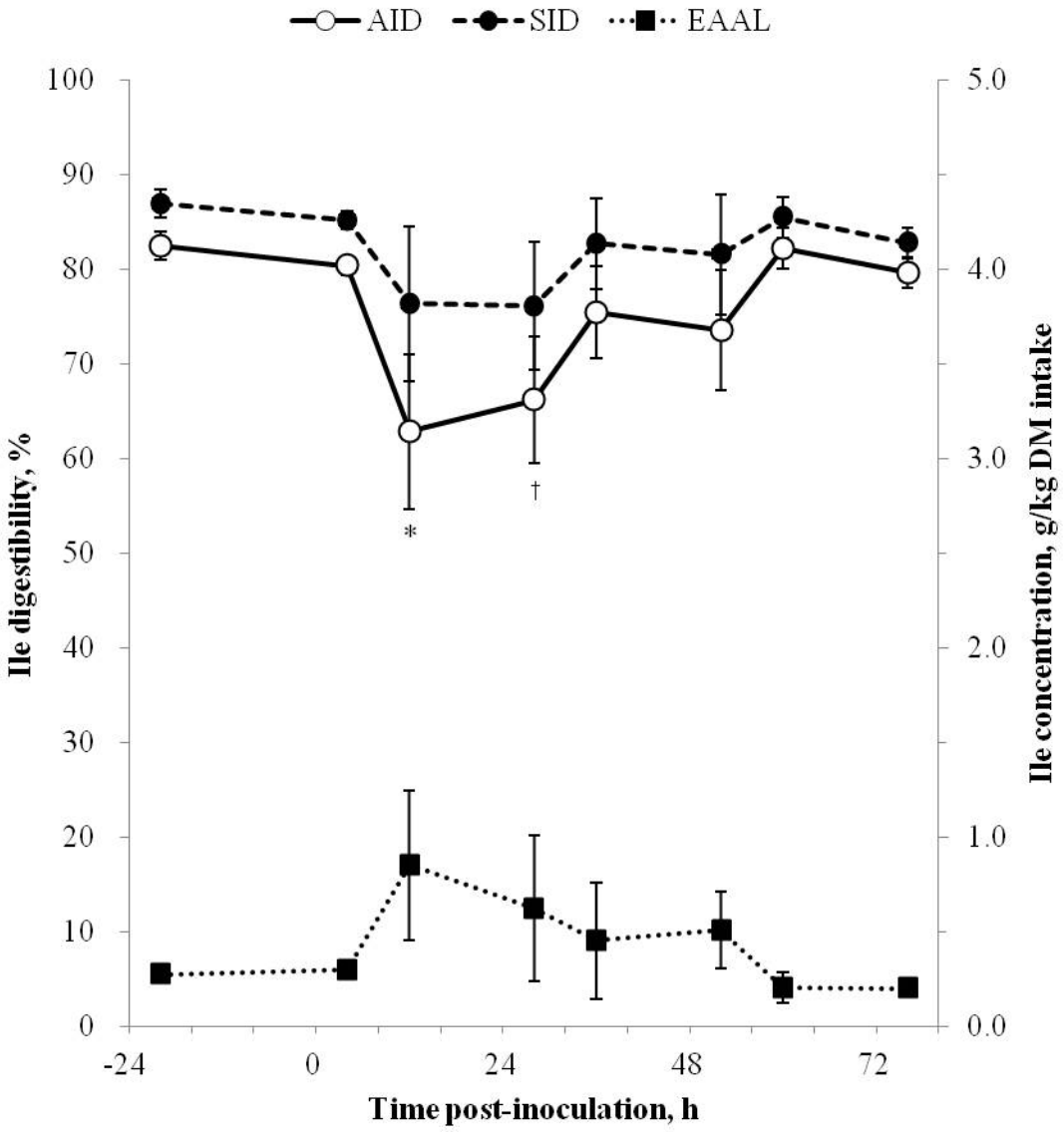


Figure 6.5. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of isoleucine (Ile) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P < 0.03$. † Different from -24 h, $P < 0.10$.

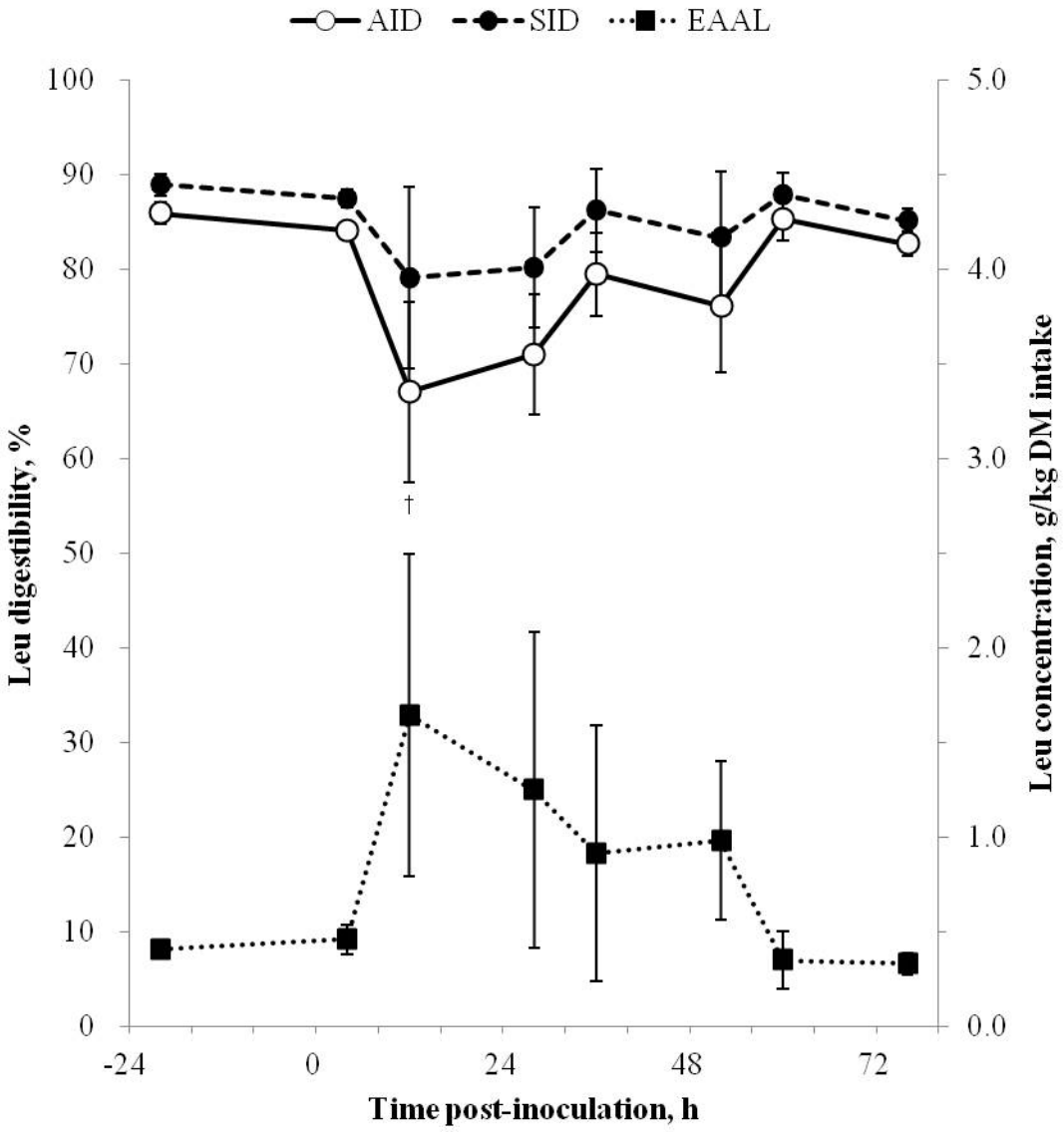


Figure 6.6. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of leucine (Leu) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. † Different from -24 h, $P < 0.06$.

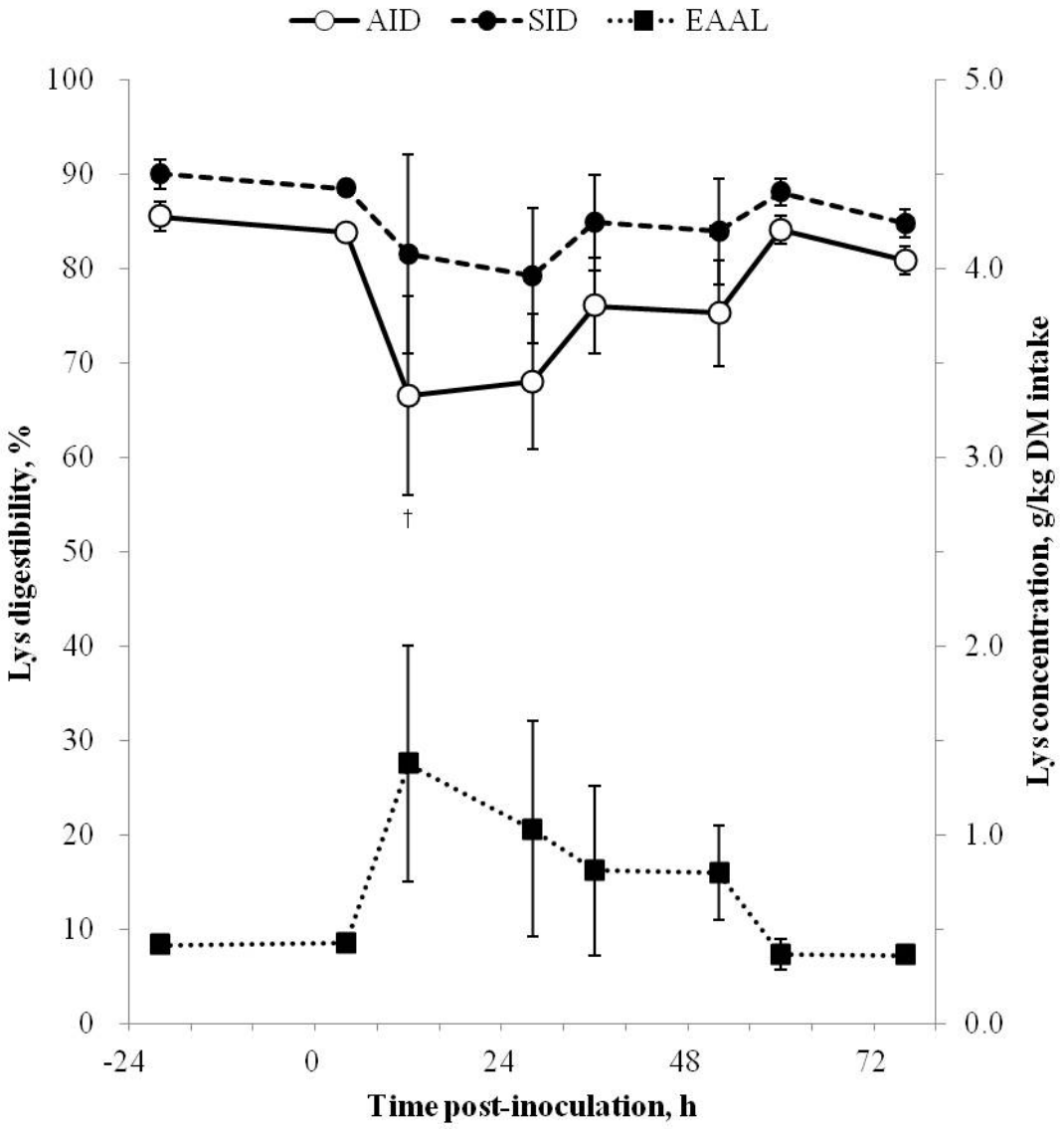


Figure 6.7. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of lysine (Lys) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. † Different from -24 h, $P < 0.09$.

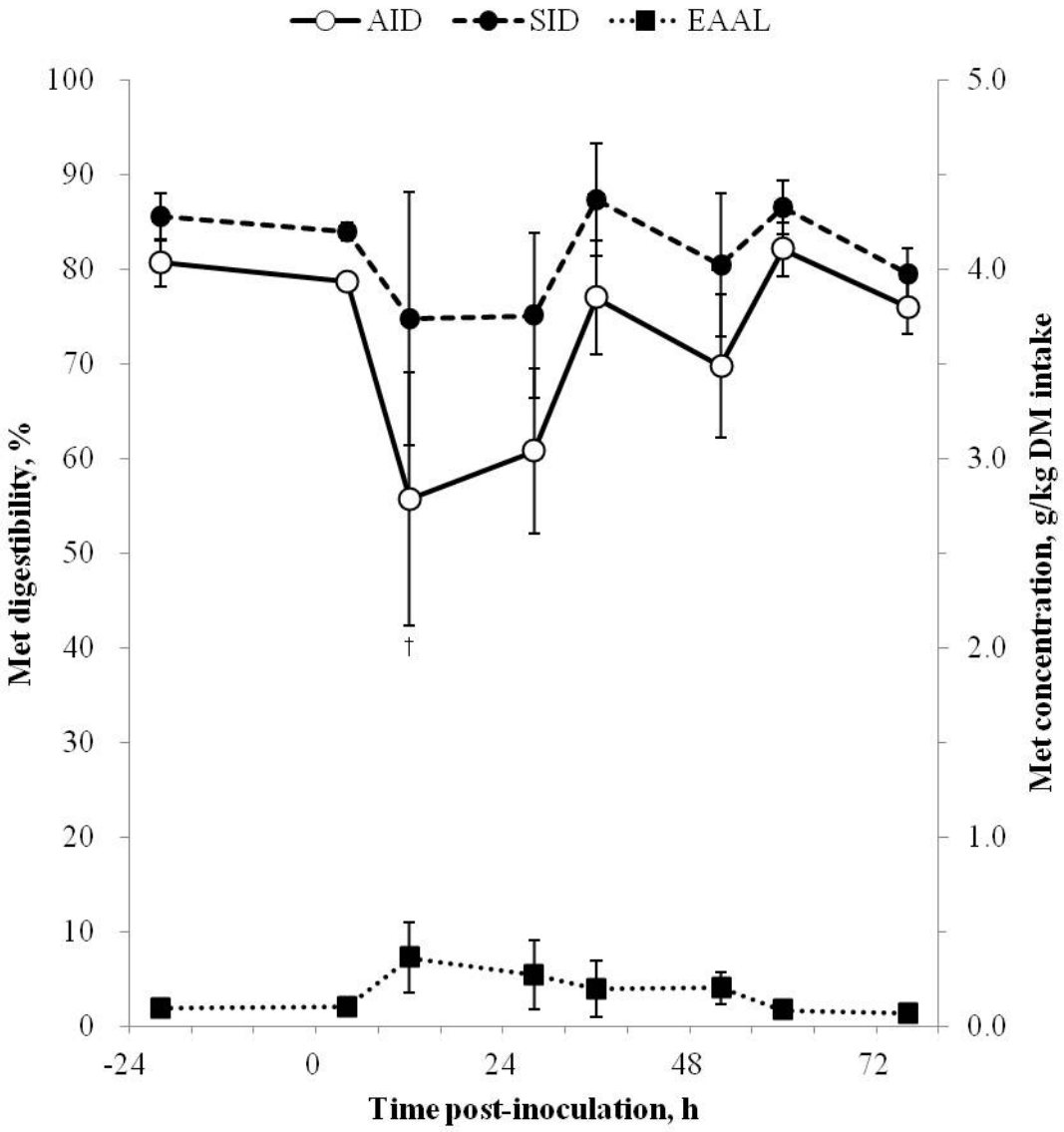


Figure 6.8. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of methionine (Met) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. † Different from -24 h, $P < 0.08$.

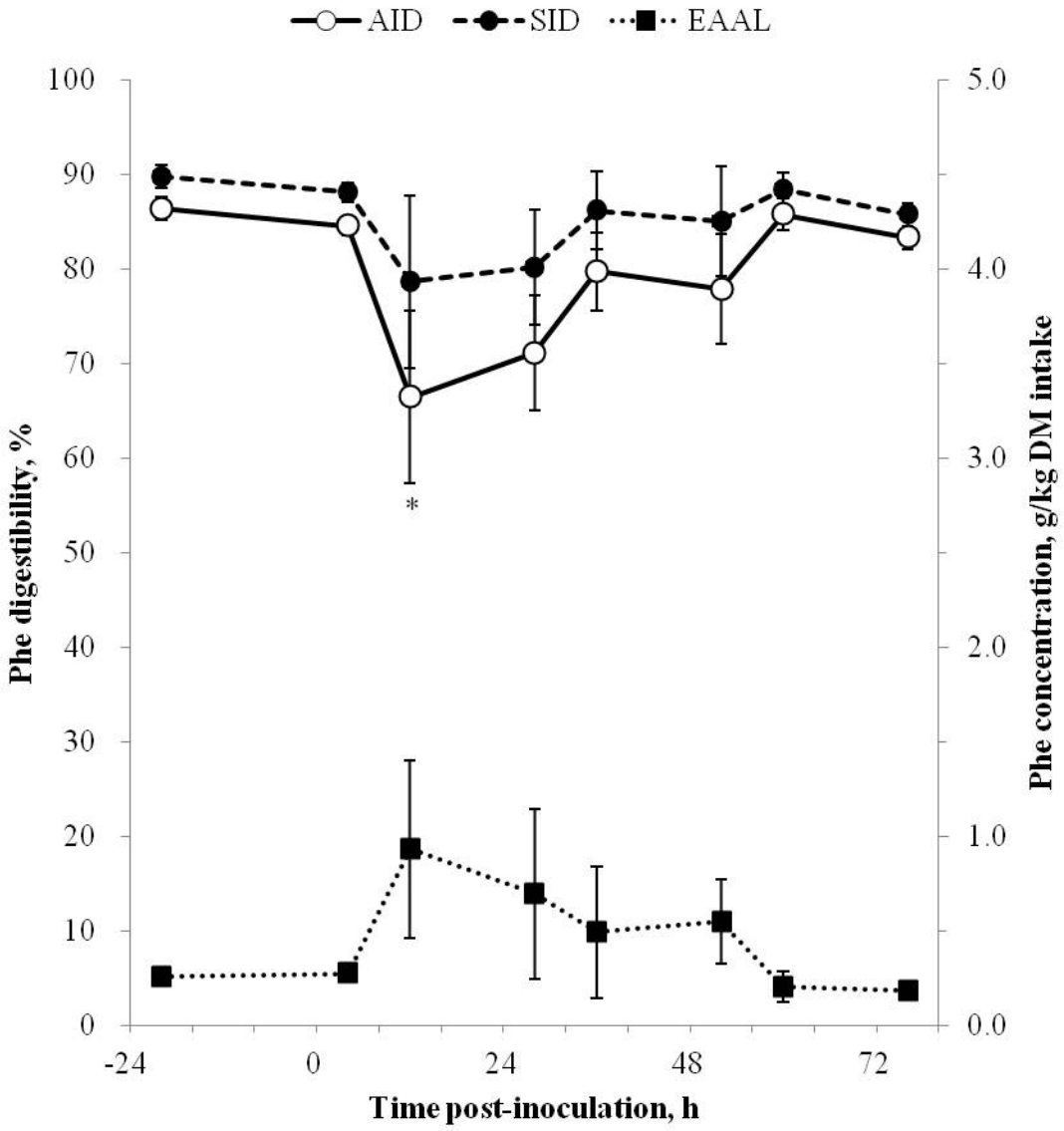


Figure 6.9. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of phenylalanine (Phe) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P < 0.03$.

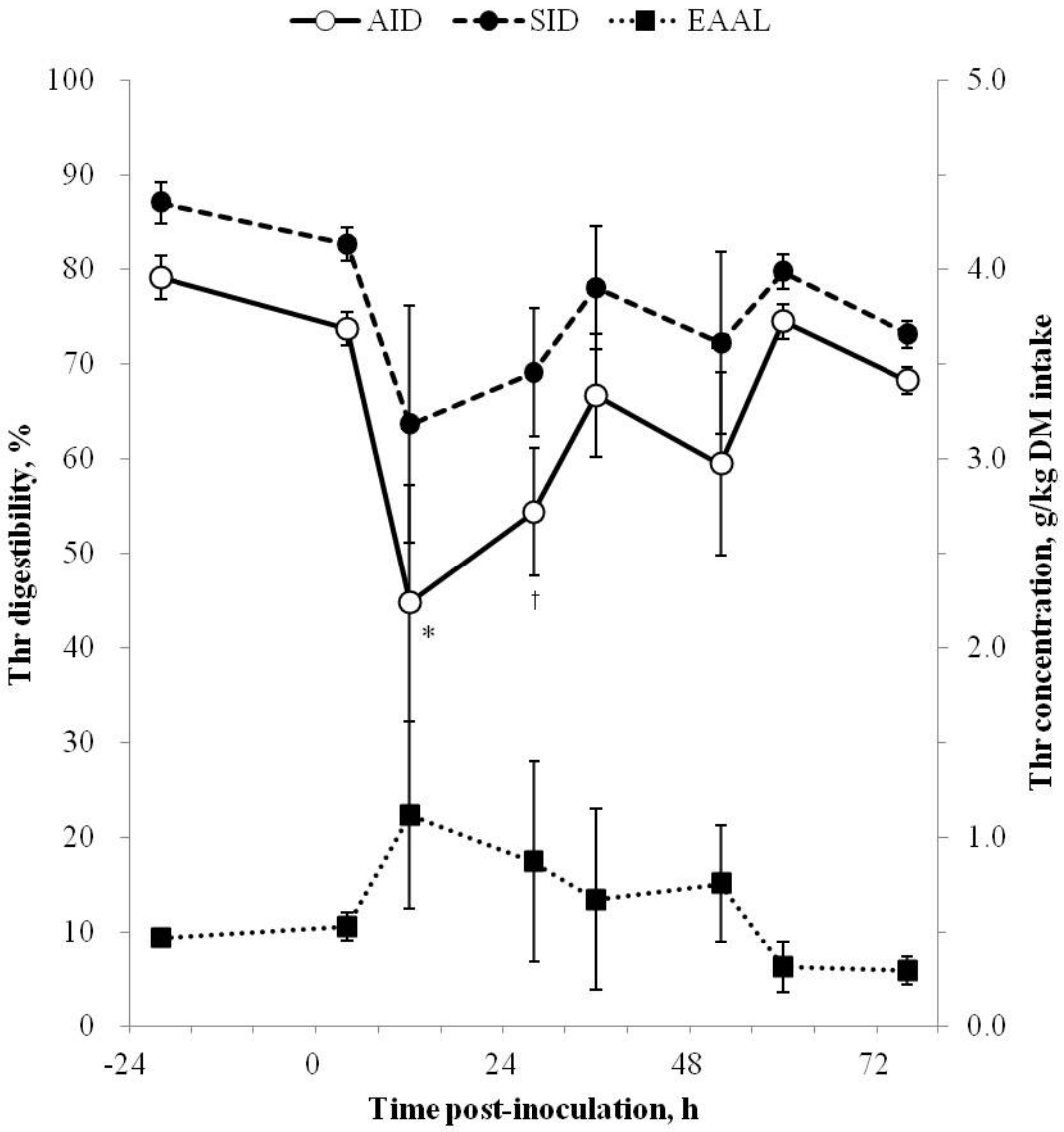


Figure 6.10. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of threonine (Thr) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P < 0.01$. † Different from -24 h, $P < 0.10$.

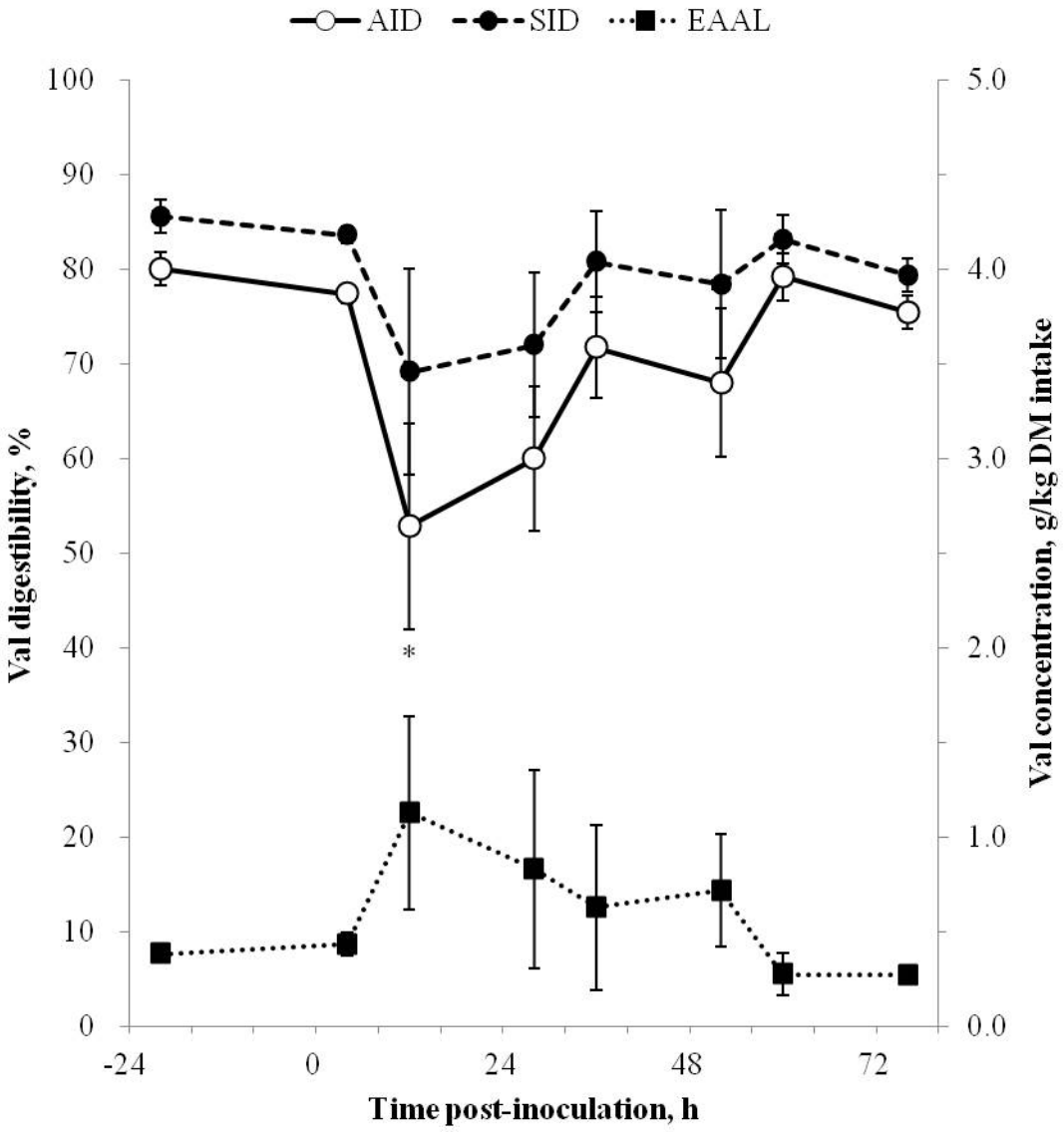


Figure 6.11. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of valine (Val) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P = 0.01$.

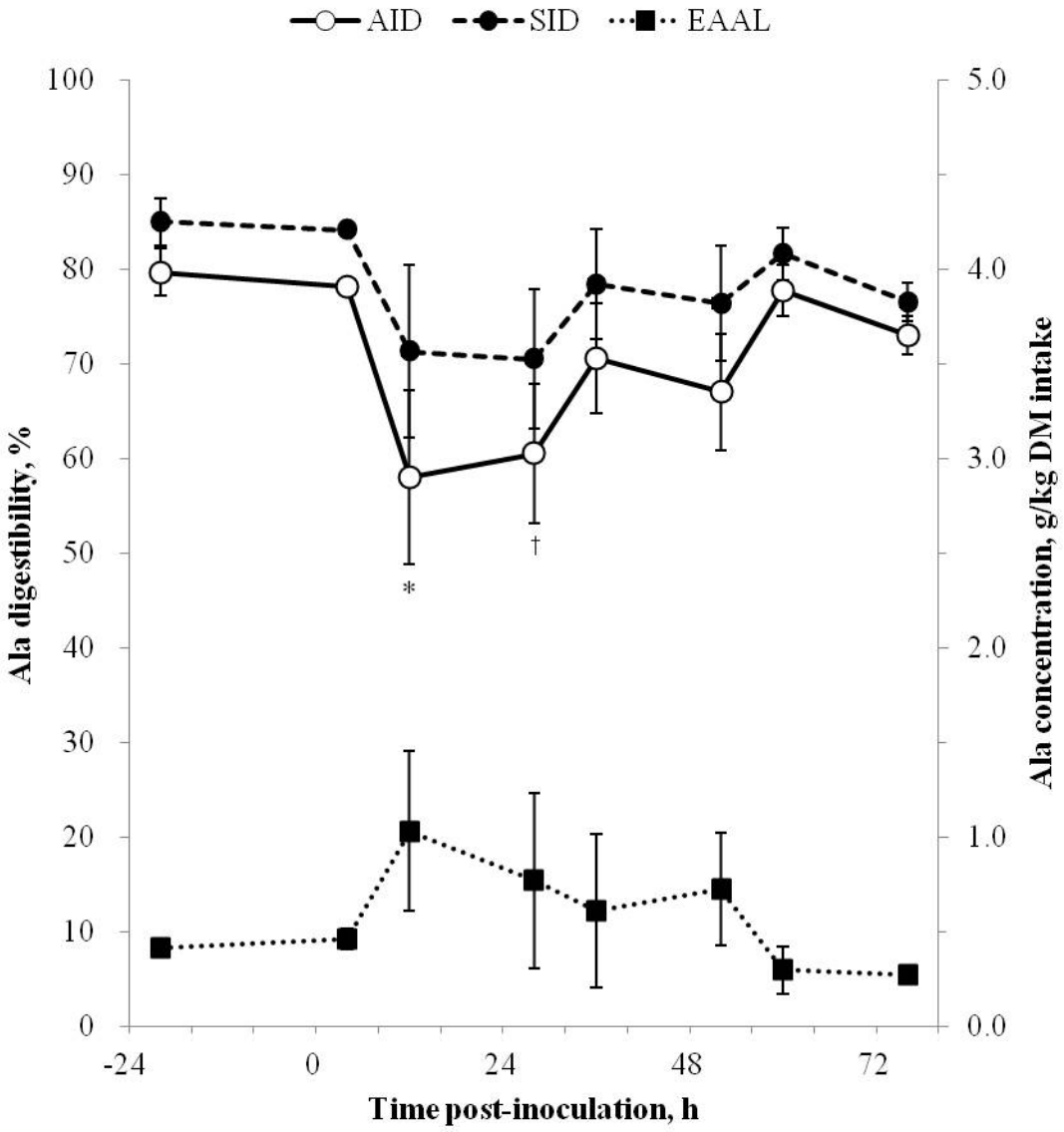


Figure 6.12. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of alanine (Ala) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P < 0.05$. † Different from -24 h, $P = 0.10$.

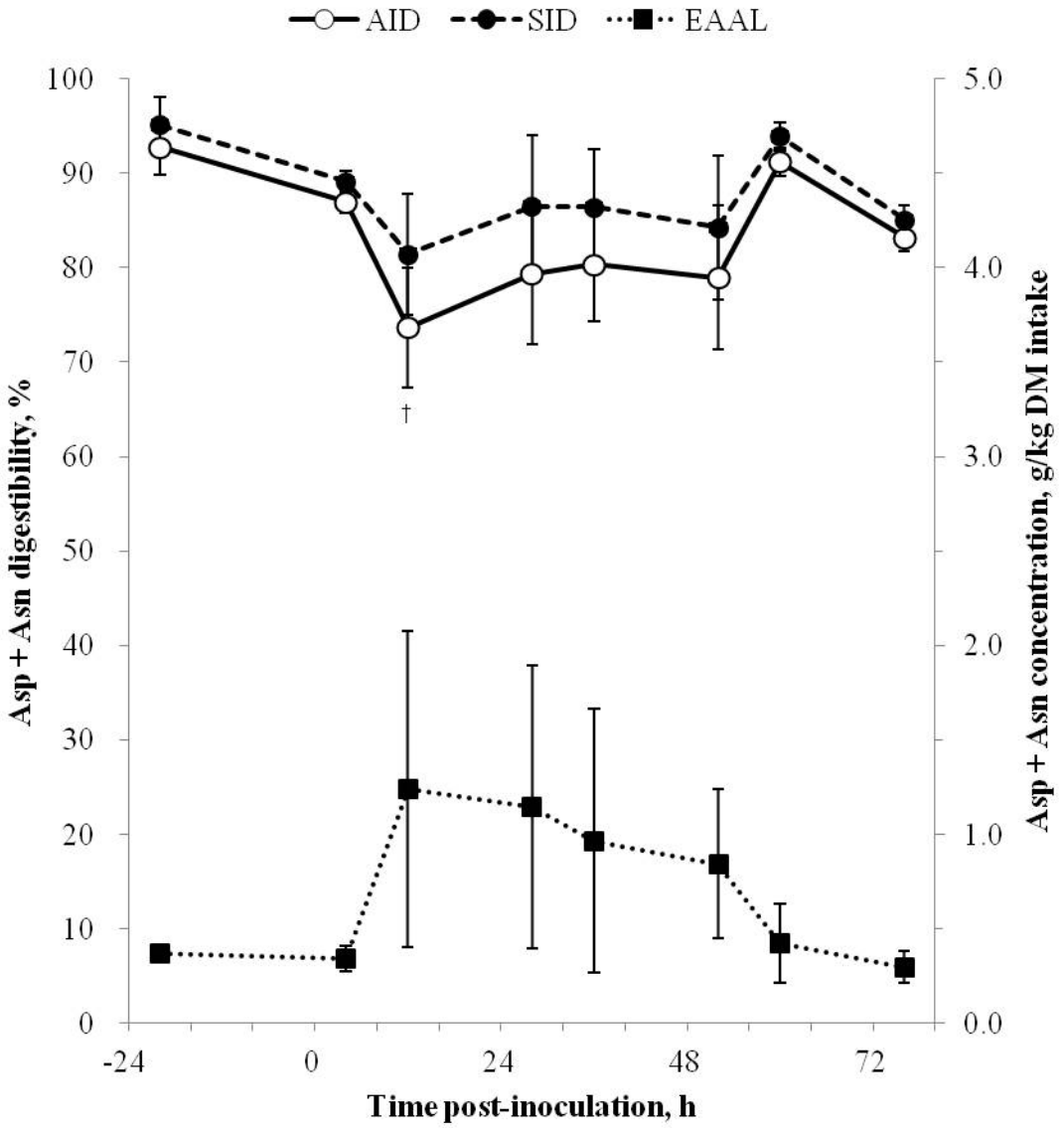


Figure 6.13. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of aspartic acid and asparagine (Asp + Asn) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. † Different from -24 h, $P < 0.09$.

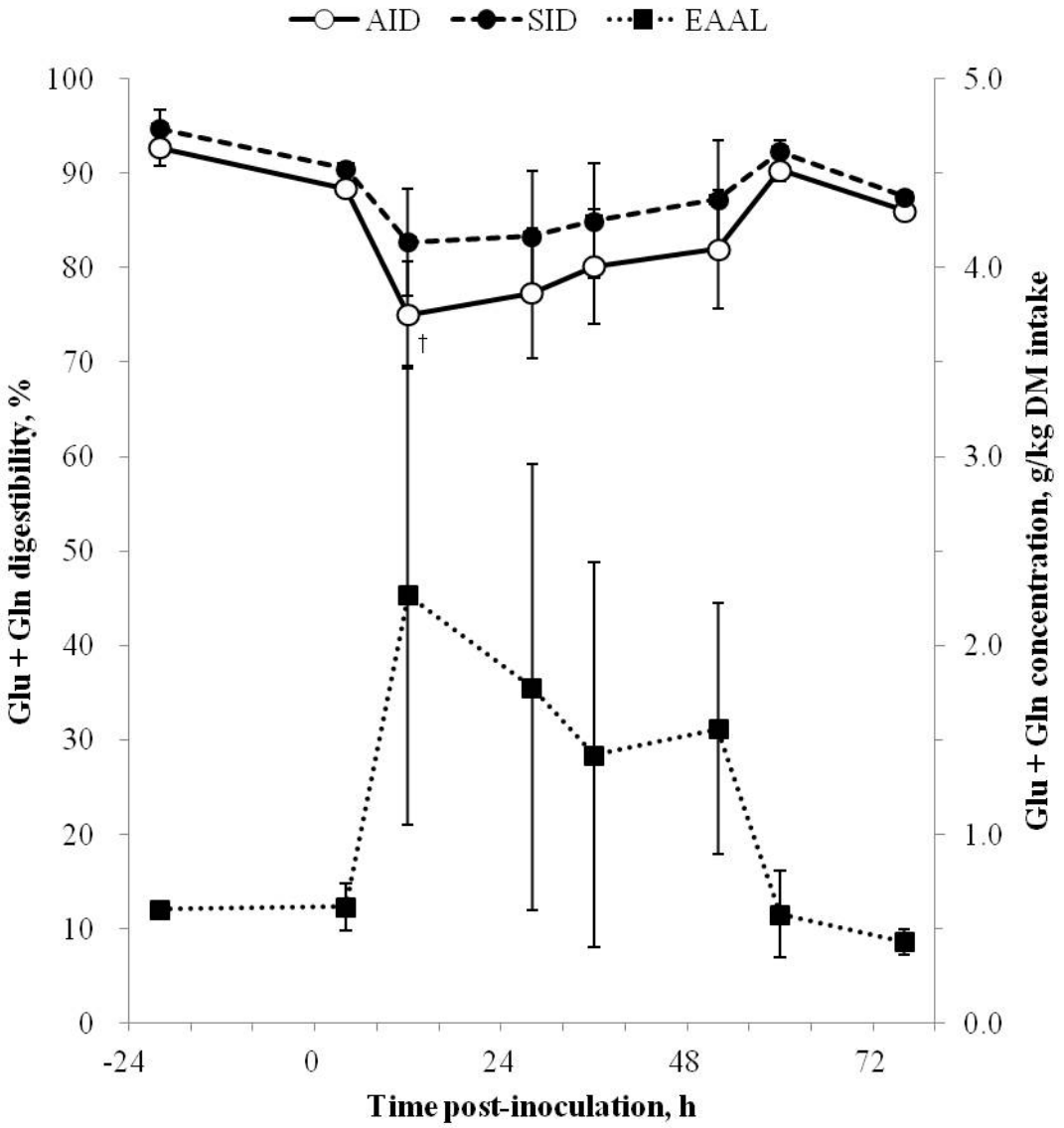


Figure 6.14. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of glutamic acid and glutamine (Glu + Gln) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. † Different from -24 h, $P < 0.06$.

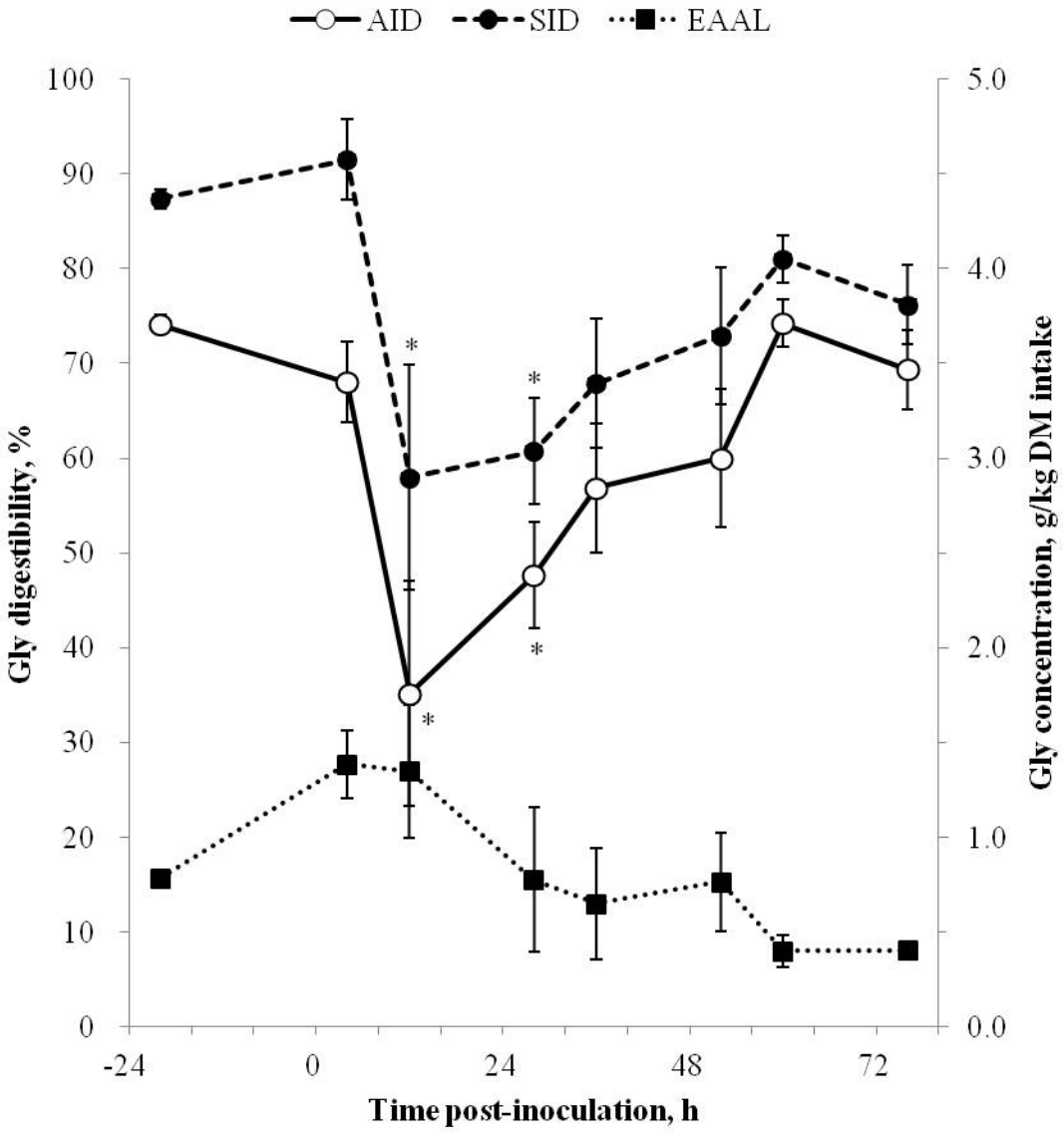


Figure 6.15. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of glycine (Gly) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P \leq 0.01$.

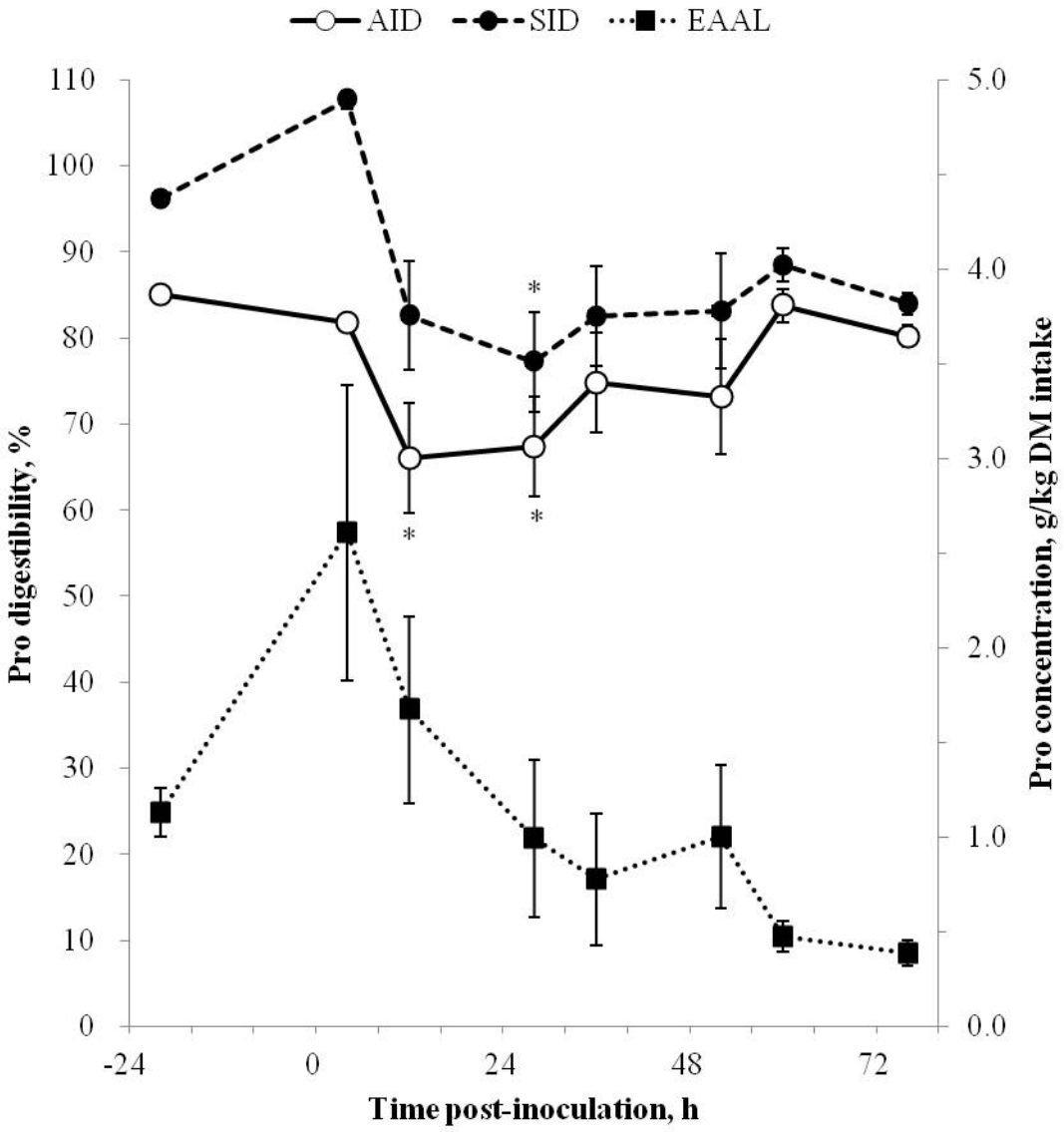


Figure 6.16. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of proline (Pro) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P \leq 0.05$.

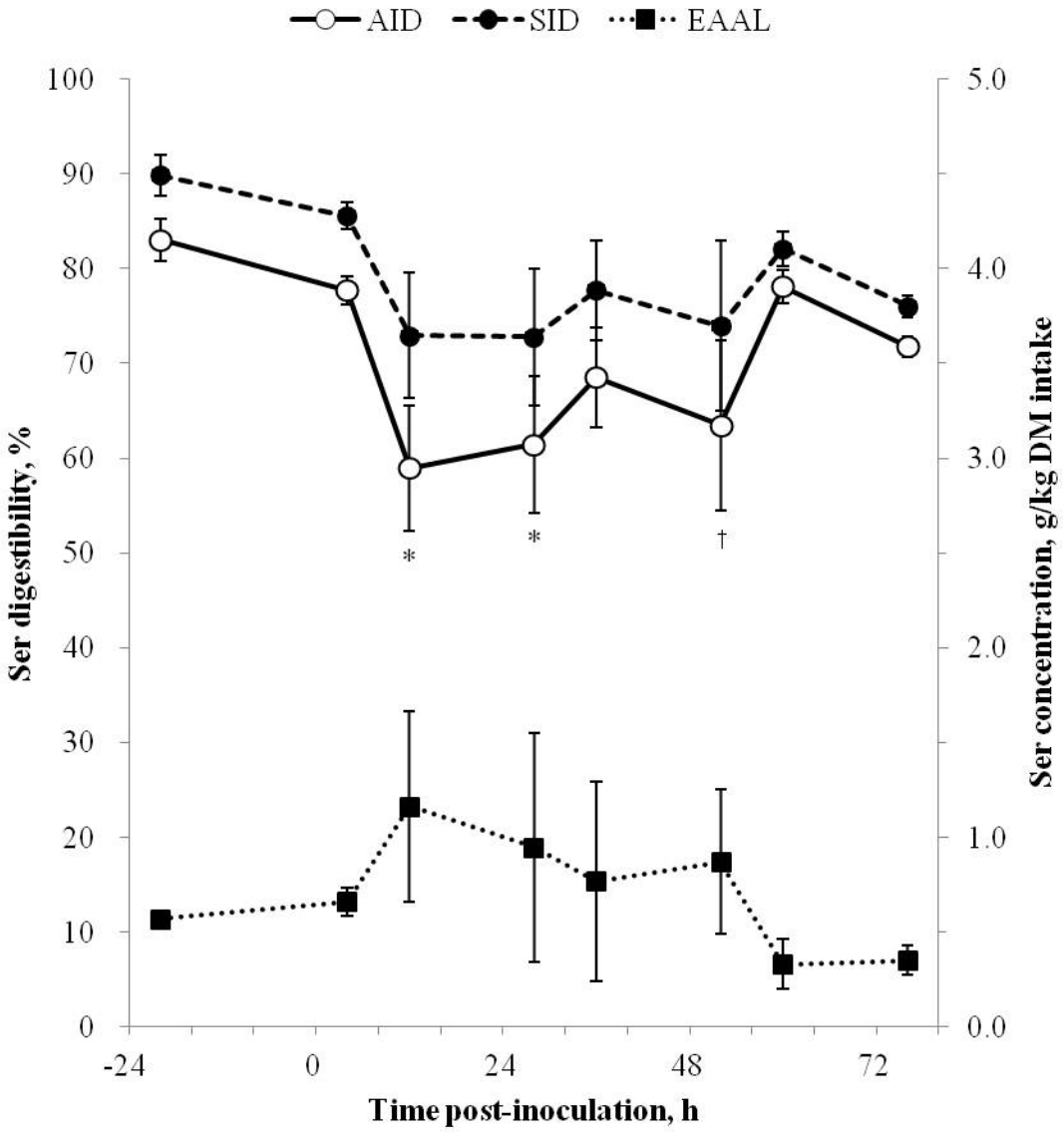


Figure 6.17. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of serine (Ser) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P < 0.04$. † Different from -24 h, $P < 0.08$.

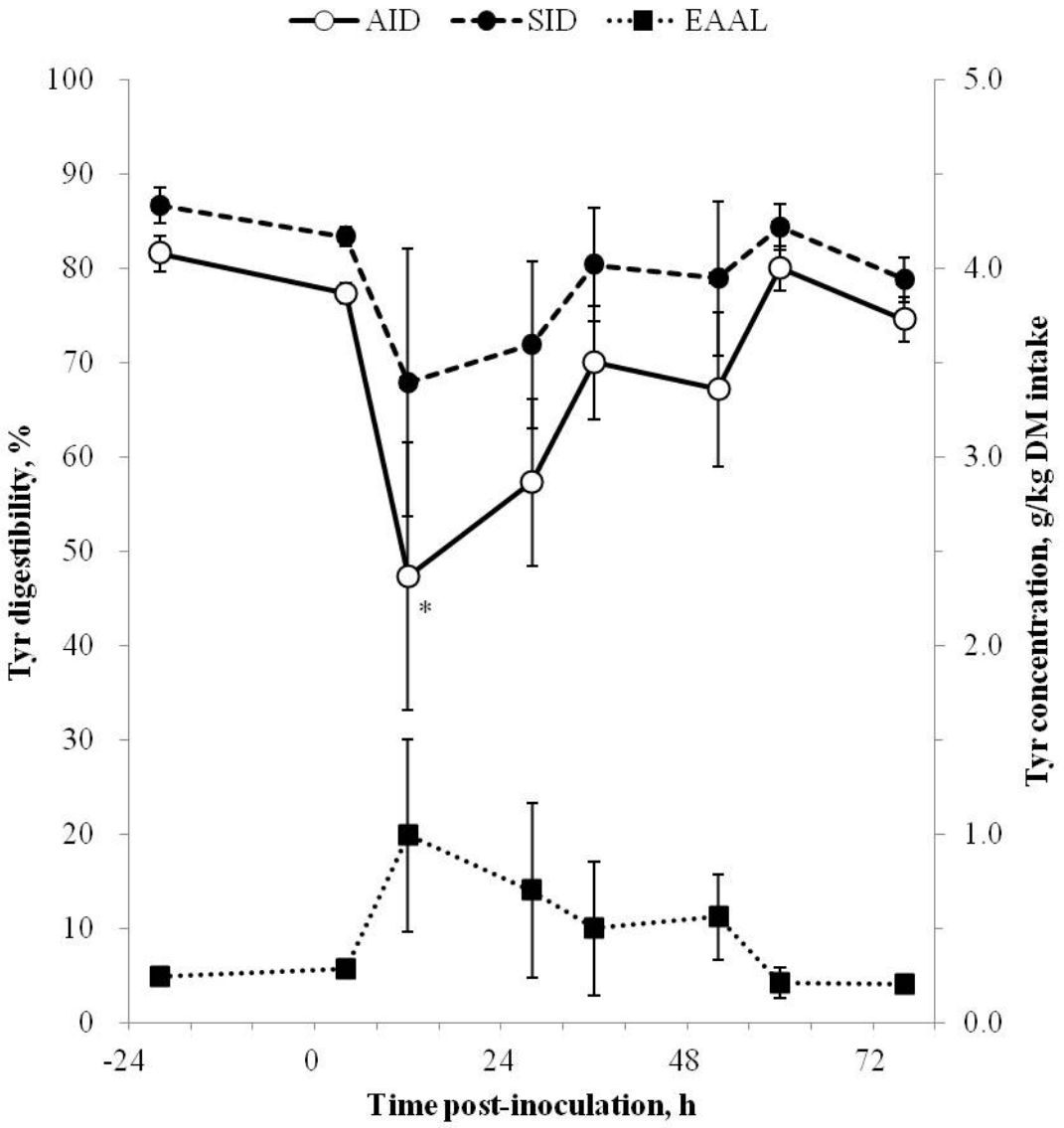


Figure 6.18. Changes in apparent ileal amino acid (AA) digestibility (AID, %), endogenous AA losses (EAAL, g/kg of dry matter intake), and standardized ileal AA digestibility (SID, %) of tyrosine (Tyr) in pigs infected with *Salmonella* Typhimurium. Ten ml of inoculant containing $1.3 \times 10^{10}$ CFU of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin ($ST^{RR}$) were mixed to diets and offered to pigs at 0 h. Pigs fitted with a simple-T cannula were given N-free (n = 4) or a typical corn-soybean meal diet (n = 4) for EAAL or AID measurement, respectively. Standardized ileal AA digestibility was calculated from AID and EAAL. Each observation represents a pooled digesta sample of 8-h collection period. Values are means ± SEM. * Different from -24 h, $P = 0.01$.

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# CHAPTER 7

## Overall conclusions

The studies conducted in this dissertation were designed to test two exogenous factors that were hypothesized to affect the amino acid (AA) digestibility of monogastric animals. First, we examined the effect of a novel mixture of carbohydrases (i.e., mixture of cellulase, hemicellulase, β-glucanase, and xylanase) on energy and AA digestibility in diets containing high inclusion of distillers dried grains with solubles (DDGS) in poultry (**Chapter 3**) and swine (**Chapter 4**).

The addition of a novel carbohydrase mixture improved the growth rate of broilers that were fed an energy-restricted diet with 20% corn DDGS inclusion. While significant improvements in AA digestibility were observed, the improvements in growth performance of broilers were likely accounted for an increase in energy utilization induced by the novel carbohydrase mixture. Supplementation of a novel carbohydrase mixture to a swine diet with high corn DDGS content did not improve energy utilization but increased AA digestibility and tended to reduce urinary nitrogen. Collectively, results indicate improvements in AA digestibility when carbohydrases are supplemented to monogastric diets with high corn DDGS content. However, as the primary mode of action of carbohydrases is to reduce viscosity of digesta, it seems that exogenous carbohydrase supplementations are more effective when applied to poultry than swine, due to the fact that the viscosity of digesta in pigs is considerably less than in poultry in respect to greater water content in pig digesta.

Second, we quantified the impact of *Salmonella enteric* serovar Typhimurium (*S.* Typhimurium) infection on apparent ileal AA digestibility (AID), endogenous AA losses (EAAL), and its derived standardized ileal AA digestibility (SID), using both comparative

slaughter technique (**Chapter 5**) and ileal-cannulated pigs (**Chapter 6**).

An inflammatory diarrhea induced by oral inoculation of *S*. Typhimurium reduced the growth and AID of pigs at 24 h post-inoculation. The EAAL for all AA were significantly increased during the acute phase immune response. By 72 h post-inoculation, the EAAL of *Salmonella* challenged pigs returned to normal levels, but the AID and SID were lower. A marked increase in endogenous Gly loss suggested a disruption in the enterohepatic recycling of bile acids. When measured in ileal-cannulated pigs, both AID and SID reached their lowest levels at 8-16 h post-inoculation. The AA digestibilities gradually recovered to pre-inoculation levels at 56-64 h but were decreased at 72-80 h post-inoculation. Ileal EAAL values peaked at 8-16 h post-inoculation and were reduced to pre-inoculant values by 56-64 h after *Salmonella* inoculation. The EAAL further decreased at 72-80 h post-inoculation suggesting a periodical reduction of proteins secreted into the intestinal lumen following inflammatory diarrhea. A lowered effective infectious dose in ileal-cannulated pigs was speculated, presumably due to the lower amount of pathogen reaching the large intestine as a result of digesta removal via the T-cannula. In conclusion, results suggest AA digestibility is reduced during the acute phase immune response but gradually recovered by 56-64 h of enteric infection. Moreover, the SID concept may not be as applicable during enteric disease conditions, where EAAL is drastically elevated, as it is in normal "healthy" experimental conditions.

## Biography

Hanbae Lee was born on December 19, 1979 in Daejeon, South Korea. He graduated from Chung-nam High School in Daejeon in 1998. Hanbae attended Seoul National University and completed his Bachelors of Science degree in Animal Science and Biotechnology, with concentrations in dietary chromium picolinate supplementation to pigs in February, 2002. After graduating from the university, he fulfilled his 28 month obligatory military service and was honorably discharged as first lieutenant in July, 2004. In July, 2006, Hanbae completed his Masters of Science degree in Animal Science at Seoul National University under the direction of Dr. Yoo Yong Kim. For his M.S., he evaluated feed additives as antibiotic alternatives in weaning pigs. He worked for the Korea National Open University as a teaching assistant from July 2006 to July 2008. Hanbae continued his pursuit for higher education by joining Dr. Jeffery Escobar at Virginia Tech in August, 2008. An important aspect of his life is family – his wife, Jungwon and his son, Jongwoo. He also enjoys playing basketball and intends to play until his knees give away.