Project Title and NPB project identification number: Increasing Feed Efficiency by Attenuating the Transportation Stress Response in Nursery Piglets through L-Tryptophan Supplementation (#21-092)

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Date Report Submitted: 4/2/2024

Industry Summary

The objectives of these experiments were:

1) Increase feed efficiency and growth rate of pigs by providing supplemental tryptophan pre-weaning and/or during the nursery phase.
2) Mitigate the adverse effects of early life transport stress on subsequent immune challenges by providing supplemental tryptophan during the nursery period.

To accomplish these objectives, two experiments were conducted. In Experiment 1, 480 crossbred mixed sex, single sourced pigs ((Duroc x (Yorkshire x Landrace); starting BW= 2.07 ± 0.58 kg; 5 ± 2 d of age) were used in a birth to market experiment to determine the effectiveness of pre- and/or post-weaning tryptophan (Trp) supplementation in reducing the short- and long-term reductions in feed efficiency because of immediate post-weaning transport. In Experiment 2, 384, single source, mixed sex, crossbred, weaned pigs [20 ± 3 d of age; weaning BW = 5.57 ± 1.49 kg; [Duroc x (Yorkshire x Landrace)] underwent an 8 h transportation stress, at weaning, in central Indiana, as described by Duttlinger et al. (2019), and were used in a wean to finish experiment to determine the effects of supplementing nursery pig diets with tryptophan (Trp) on subsequent growth performance following a vaccine-induced immune challenge. In Experiment 1, Trytophan improved BW and ADG of transported pigs when supplemented pre- and post-wean but did not benefit transported pigs when only supplemented post-wean, suggesting transportation induced reductions in BW and ADG can be mitigated by supplementing Trp prior to and following transportation. In Experiment 2, increasing dietary SID Trp in nursery diets to 2X the NRC (2012) recommendation improved ADG in nursery pigs and increased final market weight. Carcass weight, and back fat depth at market were greater in pigs fed supplemental Trp in the nursery, but this response was ameliorated by a vaccine induce immune challenge. Today's lean, and efficient growing pigs, may not consume enough Tryptophan on a daily basis in the nursery period. The impact of this is complicated by the fact that daily intake of many nutrients are also below requirements if diets are formulated to meet NRC (2012) nutrient concentrations and daily intake is less than the NRC prediction.

Key Findings:

- Trytophan improved BW and ADG of transported pigs when supplemented pre- and post-wean but did not benefit transported pigs when only supplemented post-wean, suggesting transportation induced reductions in BW and ADG can be mitigated by supplementing Trp prior to and following transportation
- Increasing dietary SID Trp in nursery diets to 2X the NRC (2012) recommendation improved ADG in nursery pigs and increased final market weight, carcass weight, and back fat in mature pigs during a vaccine-induced immune challenge
- Formulating diets to have NRC (2012) concentrations of SID Trp may result in inadequate daily Trp intakes as a result of lower nursery feed intakes in modern pigs

Keywords: pig, tryptophan, nursery, weaning, transportation

Scientific Abstract

Weaning of pigs is often concurrent with transportation-induced stress which can impair growth performance, feed efficiency, and immune resilience, contributing to reduced welfare and increased production losses. The objectives of these experiments were to:

1) eliminate or reduce short- and long-term, transport-induced reductions in feed efficiency and growth by providing supplemental Tryptophan (Trp) above NRC (2012) requirements pre- and/or post-weaning.
2) The objective of this experiment was to mitigate the adverse effects of early life transport stress on subsequent immune challenges by providing supplemental Tryptophan (Trp) during the nursery period.

To accomplish these objectives, two experiments were conducted. In Experiment 1, 480 pigs were blocked by sex, ancestry, and weight and used in a RCB design with a 2x2x2 factorial arrangement of pre-weaning treatment (oral gavage of Trp at 0.35, 0.45, and 0.55 g/d (increasing every 5d) pre-weaning or control), transport (transported or not at weaning), post-weaning nursery treatment (1X or 2X NRC Trp). At farrowing pigs were blocked by sex, within litter, and randomly assigned to either a pre-wean Trp or control treatment, where Trp supplementation began on d5 post-farrowing and stopped at weaning. Post-weaning Trp supplementation included a 3d oral gavage of Trp and 2X Trp in the diet for the entire 42d nursery period. At weaning, all pigs were blocked by sex, weaning weight and pre-wean trt and randomly assigned to transport and post-wean treatments. Pigs were fed four nursery phases with diets containing 1X or 2X NRC recommended concentrations of Trp. There were 10 pens/trt with 6 pigs/pen (equal barrows and gilts). Individual BW and pen feed intakes were determined every 5d pre-weaning and every 7d post-weaning. Data were analyzed as a RCB using GLM procedure in SAS 9.4 with fixed effects of pre-wean dietary treatment, transport treatment, and post-wean dietary treatment, the interactions, and random effect of replicate. In experiment 2, 384 pigs were transported for 8h at

weaning and used in a randomized complete block design with a 2x2 factorial arrangement of immune challenge (Vaccine challenged vs. Unvaccinated) and dietary treatment (Supplemented Trp vs. Non-supplemented Trp). Pigs were fed standard nursery diets, in four phases, over 35-d, with pigs receiving 1X or 2X the NRC (2012) recommended Trp concentration. There were 12 pens/trt with 8 pigs/pen (5 barrows and 3 gilts). Individual BW and pen feed intakes were determined weekly. Half the pigs on each dietary treatment were subjected to a 3-wk vaccine challenge consisting of Circovirus (Ingelvac CircoFLEX®; Boehringer Ingelheim, Ingelheim am Rhein, Germany), Mycoplasma (Respisure-One®; Zoetis Parsippany, NJ), and Influenza (Flusure XP®; Zoetis Parsippany, NJ) vaccines administered in wk 2, 3, and 4 post-wean, respectively. Data were analyzed as a RCB using GLM procedure in SAS 9.4 with fixed effects of dietary treatment and immune challenge, the interactions, and random effect of replicate. In Exp. 1, pre-weaning Trp supplementation had no effect on pre-weaning growth performance. A 3-way interaction of pre-wean treatment x transportation x post-wean treatment was observed for overall nursery BW (P=0.0148) and ADG (P=0.0175). This interaction was the result of post-weaning Trp supplementation increasing BW and ADG in all pigs, except for transported pigs that did not receive Trp pre-wean. While there were 2-way interactions of pre-wean treatment x post-wean treatment on ADFI at wk 1 (P=0.0123) and wk 6 (P=0.0297) and a 3-way interaction of pre-wean treatment x transportation x post-wean treatment at wk 2 (P=0.0092), there were no consistent or overall effects on overall ADFI or G:F. Trp improved BW and ADG of transported pigs when supplemented pre- and post-wean but did not benefit transported pigs when only supplemented post-wean, suggesting transportation induced reductions in BW and ADG can be mitigated by supplementing Trp prior to and following transportation. In Exp. 2, beginning on d7 and continuing throughout the nursery period, pigs fed diets containing 2X NRC (2012) Trp were heavier ($P< 0.05$) than pigs fed diets containing 1X Trp. Heavier BW were the result of an overall improvement in ADG for 2X Trp fed pigs ($P= 0.0140$). During wk 4 post-weaning, G:F was lower (P< 0.04) for vaccinated pigs compared to unvaccinated pigs. A 2-way interaction of diet x vaccine was observed for carcass weight (*P*-0.025) and a trend for interaction in percent lean ($P=0.053$). Trp supplementation benefits carcass weight in unvaccinated pigs but reduced carcass weight in vaccinated pigs provided 2X (NRC 2012) SID Trp. Feeding Trp at 2X NRC (2012) requirements increased market weight in vaccinated and unvaccinated pigs ($P=0.044$) and tends to increase fat ($P=0.074$) in unvaccinated pigs. Overall, for Exp. 2, increasing dietary SID Trp nursery diets to 2X the NRC (2012) recommendation improved ADG in nursery pigs and increased final market weight, carcass weight, and back fat in mature pigs during a vaccine-induced immune challenge.

Introduction

Objective 1:

Due to the expansive application of multi-site production across the United States, most weaned pigs (~4-6 kg) are transported throughout the year without food or water for durations that range from 1 to 24 h averaging more than 7 h (Harris, 2000; Lewis and Berry, 2006; Sutherland et al., 2014). Transportation at weaning is considered the most stressful and injurious event in the production system (Ma et al., 2021) leading to increased production costs and animal loss (Martínez-Miró et al., 2016). It is generally recognized that loading prior to transport significantly increases physical and psychological challenges seen by increased heart rate (Correa et al., 2010), body temperature (dalla Costa et al., 2007), and blood cortisol and lactate concentrations (Edwards et al.). Loading stress occurs because pigs undergo numerous simultaneous acute stressors including but not limited to social mixing, environmental change, and handling.

The stress response elicited from pigs during road transportation activates a cascade of detrimental downstream effects that have a negative impact on the antioxidant system, intestinal function, and productivity of the pig (Bao et al., 2008). Previous research reports transport-induced impairment in homeostasis and biochemical changes in major organs via circulating and intracellular free radical production, prolonged production of catecholamines (norepinephrine (noradrenaline) and epinephrine (adrenaline)) and increased concentrations of circulating glucocorticoids (cortisol) (Sapolsky et al., 2000; Zhang et al., 2012; Phaniendra et al., 2015b).

Under natural biological conditions, reactive oxygen species (ROS), or free radicals, are generated from oxygen as an unavoidable product of aerobic respiration, mitochondrial electron transport, gene expression and other biological processes (Djordjević, 2004; Bayir, 2005). The body is equipped to manage overproduction of ROS. Enzymatic and non-enzymatic antioxidants, like superoxide dismutase (SOD) and vitamin E, catalyze the dismutation of ROS through redox reactions which prevent ROS buildup and subsequent oxidative stress (Sies, 1991). ROS are essential for cell signaling (Lauridsen and Jensen, 2005); however an imbalance in ROS leads to oxidative stress which has deleterious effects on tissues and cellular processes (Shastri et al., 2016). Stress influences the body'sresponse to the perceived threat in either a physical, interoceptive, or biochemical manner to maintain homeostasis. In the absence of stress, the body maintains balance between the beneficial and harmful effects of free radicals through mechanisms called "redox regulation"; achieving homeostasis through regulation of oxidation and reduction reactions (Dröge, 2002; Usatyuk et al., 2006).

Stress disrupts pro-oxidant-antioxidant equilibrium, favoring pro-oxidant production (i.e. superoxide, hydroxyl, hydroperoxyl etc.) by overwhelming cellular antioxidant defenses (i.e. superoxide dismutase, catalase, glutathione peroxidase, α-tocopherol, ascorbate, etc.) causing oxidative stress (Cadenas, 1985; Sies, 1985). Weaning and transportation induced stress amplifies oxidation processes, increasing ROS production, causing tissue damage (Sailaja Rao et al., 2011). Prolonged stress maintains oxidative stress and amasses oxidative damage that

modifies cellular proteins, triggers upregulation of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) causing tight junction protein disruption and increased gut permeability (Rao, 2008; Upadhaya and Kim, 2021). Further proof of oxidative stress induced cellular damage can be seen in DNA base damage, protein and lipid oxidation products (malondialdehyde (MDA)) (Packer and Glazer, 1984), volatile hydrocarbon formation and release of glutathione disulfide from cells (Djordjević, 2004).

At weaning and transportation, the stress response is initiated via activation of the stress response pathway, or Hypothalamus-Pituitary-Adrenal (HPA) axis activation. Recognition of a stressor begins in the hypophysiotropic neurons in the medial parvocellular subdivision of the paraventricular nucleus (PVN) of the hypothalamus. Subsequent activation of these neurons stimulates the hypothalamus to secrete corticotrophin-releasing factor (CRF) (Rivier and Vale, 1983). CRF initiates a hormone cascade resulting in anterior pituitary and adrenal gland activation. The adrenal gland is responsible for glucocorticoid and catecholamine production (Perlman and Chalfie, 1977). Two regions make up the adrenal gland; the adrenal medulla which is located at the center of the gland and responsible for the production catecholamines (epinephrine and norepinephrine) and the adrenal cortex which is the outermost portion of the adrenal gland and is responsible for glucocorticoid (cortisol) production (Geor et al., 2013; Sheng et al., 2021).

Epinephrine and norepinephrine are peptide hormones that target tissues throughout the body, altering physiological and metabolic processes in response to stress. Circulating catecholamines increase blood pressure, cardiac output, dilation of air passages in the lungs, and vasodilation of skeletal muscle arteries. They also stimulate vasoconstriction in arteries of non-essential tissues necessary for immediate survival such as the gut, kidney, and skin (Tank and Wong, 2015). Catecholamines stimulate glycogenolysis and lipolysis to increase the production and delivery of glucose to essential tissues (Wingfield and Romero, 2015; Romero, 2019).

Cortisol is a glucocorticoid which is classified as a steroid hormone and is responsible for mobilization of energy stores from lipid and protein rich tissues (Papadimitriou and Priftis, 2009). Cellular responses to cortisol are dependent on the concentration of free hormone, potency, and the cell's ability to receive and transduce the hormone signal (Bamberger et al., 1996). The impact of cortisol on the body can be either *permissive*: cortisol is present before a stress event; *suppressive*: cortisol responds and hour or more after a stress event; or *stimulating*: cortisol production is activated an hour following a stress event and remains active, continually activating the HPA axis (Sapolsky et al., 2000).

Prolonged stress events, like transportation, causes cortisol to act on the body in a stimulating manner which inhibits HPA axis termination via negative feedback loops. Maintaining HPA axis activation for prolonged periods of time leads to chronic stress. Excessive or chronic stress

inhibits the body's ability to mount an adequate stress response (Smith et al., 2010a) by cortisol and ROS mediated damage or inhibition of regulatory mechanisms responsible for HPA responses (Dallman and Hellhammer, 2017).

Recent research identifies increasing L-Tryptophan (Trp), above current NRC (2012) recommended concentrations, improved neuroendocrine responses to stress, lowered plasma cortisol and norepinephrine concentrations, and improved HPA recovery time following stress in nursery pigs (Koopmans et al., 2006). Further research reported increased dietary Trp increased serotonin production in weaned pigs, resulting in reduced salivary cortisol and increased circulating Trp concentrations.

Tryptophan's structure, comprised of an indole ring and aromatic side chain, gives the amino acid antioxidant properties. Tryptophan is a potent free radical scavenger through the interaction of free radicals with the aromatic side chain (Nayak et al., 2019). Metabolites of Trp, such as melatonin, serve to protect against oxidative damage and reduce oxidation based DNA damage by scavenging and inhibiting free radical production and upregulating endogenous antioxidant defenses (Marshall et al., 1996). Research has reported that melatonin actively scavenges the free radical, hypochlorous acid (HOCl), in the brain at sufficient rates to protect against free-radical induced catalase inactivation (Marshall et al., 1996). Further research reported supplemented Trp reduced MDA and plasma urea nitrogen concentrations in weaned pigs (Mao et al., 2014).

Increasing Trp to suckling pigs inhibited secretion of cortisol and norepinephrine and increased amino acid transporters in epithelial cells via cell mTOR pathway activation (Wang et al., 2015). The benefits of increased Trp have led to improved nursery pig performance and feed efficiency (H. Wang et al., 2015) by increasing tight junction protein expression (H. Wang et al., 2015) and ghrelin expression (Zhang et al., 2007). Therefore, the study objective was to eliminate or reduce short- and long-term, transport-induced reductions in piglet feed efficiency and growth by supplementing tryptophan above NRC (2012) recommendations pre-weaning and/or during the nursery phase. We hypothesized Trp supplemented pigs would have reduced cortisol concentrations and improved feed efficiency, which would result in heavier pigs following a 6 wk nursery period.

Objective 2:

In commercial swine production, weaning and transportation occur concurrently due to the expansive application of multi-site production systems across the United States (Poletto et al., 2010; Williams et al., 2012). The combination of weaning and transportation can be defined as an early life stressor (ELS) (C. T. Whittemore and Green, 2001). Transportation at weaning is

considered the most stressful and injurious event in the production system (Ma et al., 2021) increasing production costs and animal loss (Martínez-Miró et al., 2016). This process significantly influences the health and welfare of pigs due to piglet separation from the sow, relocation and mixing piglet groups, radical changes in diets that often reduces or eliminates feed intake during the first 48 h post-weaning, (Brooks et al., 2001) and exposure to pathogens (Adeola and Ball, 1992). Negative effects associated with weaning and transportation have been thoroughly documented and include reduced growth performance (Martínez-Miró et al., 2016) due to reduction of feed efficiency (Y. Bin Shen et al., 2012), impaired intestinal function (Olsen et al., 2005), increased susceptibility to disease (Adeola and Ball, 1992) associated with decreased intestinal health and increased gastrointestinal permeability (McLamb et al., 2013), and increased acidosis of muscle tissues (Young et al., 2005).

The stress response elicited from pigs during road transportation induces attenuation of gastrointestinal function and activation of stress pathways which occurs concurrent with gastrointestinal tract maturation (Smith et al., 2010a). At the time of weaning and transport intestinal epithelial, immune, and the enteric nervous system undergo adaptation and development (Koopman et al., 2021). This developmental disruption impairs development of intestinal mucosa (Smith et al., 2010a) causing mucosal inflammation (Vergauwen et al., 2015) leading to increased pro-inflammatory cytokines (TNF-α, IFN-g, IL-1, IL -4, IL -6, IL -8) in circulation, and decreased expression of tight junction proteins (claudin, ZO-1, and occludin) (Wu, 2013). Circulating pro-inflammatory cytokines regulate metabolic processes and immune function; however overproduction of pro-inflammatory cytokines, in response to stress, damages epithelial cell structure limiting cells ability to oxidize amino acids, inhibiting partial protein unfolding, and subsequently reducing protein synthesis (Campbell et al., 2013).

Early exposure to a prolonged stress event, like transportation, increases the likelihood of pigs developing a maladaptive stress response impacting how the system responds to future stress, in addition to persistent and/or chronic gastrointestinal disorders (Blanchard et al., 2004; Moeser et al., 2017). The impact of an ELS on growth and immune performance in piglets can be seen long after the stress event, manifesting in impaired immune responses, increased glucocorticoid production, reduced blood lymphocyte counts, and inhibition of cytokine and immunoglobulin production (Moeser et al., 2017). ELS induced homeostatic disruption of the gastrointestinal immune barrier function increases paracellular translocation of luminal bacteria, toxins, and antigens to subepithelial tissue triggering an immune response (Blikslager et al., 2007a). Post-weaning, an increase in acute phase protein (APP) synthesis occurs due to sub-clinical inflammation and immune system activation (Pomorska-Mól et al., 2012). Transport induced gastrointestinal inflammation and immune dysregulation induces upregulation of APPs: haptoglobin and fibrinogen, which are Trp rich; consequently, pigs under immune stress have decreased available Trp for body protein anabolism (de Ridder et al., 2012).

Previous research identified that increasing L-Tryptophan (Trp) by 0.8%-units maximized growth performance and increased hypothalamic serotonin that improved stress adaptation in nursery pigs (Shen et al., 2012). Further research concluded that short-term supplementation of Trp increased nursery pig performance, feed efficiency, and reduced serum cortisol concentrations following social-mixing and a relocation stress event (Shen et al., 2015). Trp is the rate-limiting substrate of serotonin production (Mao et al., 2014). In response to stress, the brain serotonergic system increases serotonin turnover in the brain (Ekkel et al., 1997; Jensen and Yngvesson, 1998). Increased serotonin turnover improves stress adaptability (Deakin and Graeff, 1991), appetite (Zhang and An, 2007), reduces stress hormone secretion (Koopmans et al., 2006), and increases immune responses (Melchior et al., 2004). Research reports oral administration of L-Trp enhanced the concentration of hypothalamic serotonin in animals (Shen et al., 2012), increased intestinal villus height (Koopmans et al., 2006), increased abundance of tight junction proteins in the duodenum and jejunum (Liang et al., 2018), promoted protein synthesis in epithelial cells (Wang et al., 2015), activated immunocytes in response to pathogen exposure (Gao et al., 2018), and decreased the concentrations of circulating stress hormones (i.e. cortisol, norepinephrine, and aldosterone) (Koopmans et al., 2006).

Extensive research has explored the effectiveness of vaccine-induced immune challenges in pigs (Salt et al., 1998; Van Den Broeck et al., 1999; Kitikoon et al., 2006). Previous research reported live attenuated vaccines, like porcine circovirus 2 (PCV2) and influenza (H1N1), can induce humoral and cellular immune responses in pigs (Chen et al., 2019). However, research reported administration of vaccines to weaned pigs following social mixing inhibited post-vaccinal immune responses in weaned pigs resulting in decreased concentrations of lymphocytes, immunoglobulin M, interferon γ, and IL-10 (De Groot et al., 2001). Thus, it can be concluded that mixing stress imposed on weaned pigs can suppress immune responses to a viral vaccine and adversely influence protection against infection (De Groot et al., 2001). Further research reported that pigs vaccinated for PCV2 remained seropositive for PCV2-specific antibodies, like viremia, at weaning suggesting that the antibodies against PCV2 are not sufficient to elucidate a protective immune response leading to reduced titers of neutralizing antibodies (Meerts et al., 2006).

From January to December (2022), approximately 126.8 million (M) pigs were weaned in the United States (Matlock, 2022). What is less clear is the costs associated with reduced pig performance, feed efficiency, and increased costs for therapeutic treatments following weaning and transportation. Therefore, it is crucial to determine if supplementing L-Tryptophan, above the NRC (2012), to recently weaned and transported pigs that undergo a secondary stress event following weaning. Therefore, the objective of this study was to mitigate the adverse effects of early life transport stress on subsequent immune challenges by providing supplemented L-Trp above NRC (2012) recommendations during the nursery period. We hypothesized that pigs

supplemented with 2X NRC (2012) SID Trp during the nursery period would have improved performance and efficiency following a 3 wk vaccine-induced immune challenge.

Objectives:

3) Increase feed efficiency and growth rate of pigs by providing supplemental tryptophan pre-weaning and/or during the nursery phase.
4) Mitigate the adverse effects of early life transport stress on subsequent immune challenges by providing supplemental tryptophan during the nursery period.

Materials & Methods:

Objective 1:

General. This study was reviewed and approved by Purdue University's Institutional Animal Care and Use Committee (IACUC #2110002209). Animal care and use standards reflected the protocol's outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020). The experiment was conducted in the farrowing barns and Purdue Swine Environmental Research Building (SERB) from November 2021 to May 2022 (winter replicate) and replicated February 2022 to August 2022 (spring replicate).

Experimental Design. A total of 480 crossbred mixed sex, single sourced pigs ((Duroc x (Yorkshire x Landrace); starting BW= 2.07 ± 0.58 kg; 5 ± 2 d of age) were used in a birth to market experiment to determine the effectiveness of pre- and/or post-weaning tryptophan (Trp) supplementation in reducing the short- and long-term reductions in feed efficiency because of immediate post-weaning transport. Pigs were cross fostered within 48 h of birth to have a minimum of 10 pigs per sow (litter size: 12 ± 4). Following cross-fostering, pigs were blocked by sex and within litter birth weight and randomly allotted to treatments in a 2x2x2 factorial design with pigs either receiving Trp supplementation or not pre-weaning, either being transported or not at weaning, and either receiving Trp supplementation or not in the nursery period. The nursery treatment structure consisted of 80 pens with 6 pigs per pen (1.83 × 2.44 m) and 10 replicates per treatment. Pigs had ad libitum access to dry feed and fresh water via one nipple waterer on the back wall of each pen and a dry self-feeder at the front of each pen.

Pre-wean Tryptophan Supplementation. On d 4 of lactation, all pigs were weighed to obtain initial body weights. Pigs were blocked by sex and within litter BW and randomly assigned either pre-wean supplementation of Trp or not. Each pig was individually ear tagged (Allflex

Livestock IntelligenceTM, Rahway, NJ) to denote treatment assignment during the pre-wean supplementation period. Beginning on d 5, Trp-supplemented pigs received 0.35 g Trp/d with 2 mL milk replacer acting as a carrier. Pigs receiving no Trp, were orally gavaged with 2 mL of milk replacer. Trp-supplemented pigs received 0.35, 0.45, and 0.55 g Trp/d from d 5-10, 11-15, and 16-21 respectively (table 2.1). Control pigs received daily oral gavages of the carrier following the same volume as Trp-supplemented individuals up to weaning (d 21). Tryptophan solutions were made the day prior to each dosing. To procure 0.35 g Trp/ 2 ml carrier, 88 g Trp was dissolved into 1 L milk replacer 12 hours prior to dosing. To meet the 0.45 g Trp/ 5 ml carrier, 90 g Trp was dissolved into 1 L milk prior to administration and 92 g Trp was dissolved into 1.5 L milk to meet the 0.55 g/ 6 mL dosage. To ensure Trp dissolved, the solution was placed on a magnetic stir plate with a stir rod set to spin at 2,000 rpm. All pigs were weighed to determine pre-wean BW and ADG beginning d 4 post-parturition and every 5 d until weaning on d 21. Morbidity and mortality were recorded daily. Pigs exhibiting clinical symptoms of illness or lameness were administered injectable antibiotics. Treatments, dose, individual, and symptoms were recorded on sow information cards above each farrowing crate.

Transportation and Weaning. Weaning was conducted on d 21 ± 3 of lactation. At this time, half of the pigs (weaning BW= 6.23 ± 2.62 kg) in each pre-wean treatment (n=120 control, n=120 treatment) underwent a 12 h transportation stress event following the transport procedures outlined by Duttlinger et al. (2019). Selected pigs were removed from sows and loaded into two gooseneck livestock trailers (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA; 2.44 × 7.32 m; Wilson Trailer Company, Sioux City, IA). Stocking density allowed 0.07 m^2 per pig and was within the range of 0.060 to 0.084 m2 per pig required for 4.54 to 9.07 kg pigs as reported in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020). Three data loggers (Elitech: RC-5+ TE; San Jose, CA; temperature accuracy: ±0.5(-20˚C/+ 40˚C) ±1.0) were evenly spaced within the trailer to measure ambient temperature in 2-minute intervals. Trailers were bedded with cedar shavings and ventilation openings were closed to prevent chilling based on external ambient temperatures at the time of transport (3.5 and 9.7◦ C respectively). During transport, trailer temperatures averaged 2.7 ± 0.9 ◦ C during the winter replicate and 10.3 ± 0.6 ◦ C during the spring replicate.

Piglets were transported as a group in the trailer for approximately 12 h and 819 km with no access to feed or water. Trailers departed simultaneously from the farrowing house at approximately 0730 h. Total transportation time was estimated based on the addition of loading and unloading (1.15 ± 0.30 h loading; 1.0 ± 0.25 h unloading), driver shift changes (0.05 ± 0.02 h), and time spent stationery while sorting pigs into their respective pens in SERB (0.45 ± 0.15 h). The transport route consisted of approximately 50% two-lane roads and 50% four-lane roads. Each route was 273 km in length, which was completed three times during the transportation stress event. Completion of one lap of the route took the driver approximately 3 h ± 15 min. Drivers switched and the truck was refueled after each 273 km route was completed.

At the conclusion of the 12 h transport, piglets arrived at SERB, were unloaded from the trailer, individually weighed, and placed in pre-assigned rooms and pens. Non-transported pigs were weaned, weighed, and moved to SERB when the transported pigs began their final 273 km lap to provide similar facility loading times. Each room, at SERB, contained 10 pens (1.83 × 2.44 m) with 6 pigs per pen (3 gilts and 3 barrows). Transported and non-transported pigs were blocked by weaning weight and randomly assigned within pre-weaning treatment to nursery treatments (Control vs. Trp Supplemented). Pigs assigned post-weaning Trp supplementation received an oral gavage of 0.55 g Trp for 3d post-weaning as pigs began to consume feed at different times and rates post-weaning. Trp supplemented pigs continue to receive an oral gavage of Trp for the first 3d of the nursery period as pigs began to consume feed at different times and rates post-weaning.

Nursery Performance Data Collection. Individual body weights and pen feed intakes were taken every 7 d during the 42 d nursery phase to determine ADG, ADFI, and G:F. Daily observations were conducted to determine general fitness. Any notable changes in health or locomotion were recorded on daily observation sheets located outside each room. Therapeutic injectable antibiotic administration was provided to any pigs showing clinical signs of illness or lameness by research and farm personnel. Treatments of individuals, including antibiotic used, dose, individual and pen identification, symptoms, and withdrawal dates were recorded.

Nursery Diets. All pigs were provided ad libitum access to clean fresh water and dry feed via one dry self-feeder and nipple waterer. During the nursery period, 4 dietary phases were fed based on a feed budget of 0.91, 2.2, 8.1kg/pig for phases 1, 2 and 3, and ad libitum phase 4 until d 42 post-weaning. Diets consisted of a corn-soybean meal base provided in meal form. Control pigs received Trp at the NRC (2012) recommended concentration and Trp- supplemented pigs receive Trp at 2X the NRC (2012) recommended concentration (tables 2.2-2.5). Control diets were balanced to meet or exceed NRC (2012) standardized ileal digestible (SID) tryptophan ratios to SID lysine. Synthetic amino acids were added, as needed, to meet or exceed NRC (2012) SID amino acid ratios up to the third limiting amino acid requirement including lysine, methionine, and threonine. Net energy was constant across diets and phases. Neomycin-oxytetracycline 100/100 D (active drug ingredients included at a concentration of 45.35 g/kg oxytetracycline hydrochloride and 45.35 g/kg Neomycin Sulfate; Phibro Animal Health; Ridgefield Park, NJ) was provided in phases 1 and 2 at an inclusion of 0.375 and 0.250%, respectively. Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) was included in phases 3 and 4 at 0.25% inclusion.

Feed Sample Collection and Analysis. A composite feed sample was collected during the creation of each phase of the nursery diets. Samples were collected from every third feed bag during the bagging process at the Animal Science Research and Education Center's Feed Mill. Feed samples were ground using a commercially available hand grinder (KRUPS® Solingen,

Germany) and stored at 20◦C until analysis. Samples were analyzed for amino acid concentration by the University of Missouri Experiment Station Laboratory (Columbia, MO USA).

Grow Finish Performance Data Collection. Pigs remained in their assigned pens following the 42-d nursery period until market and fed a common diet in 5 grower phases, each lasting 3 weeks (table 2.6). Individual BW and pen feeder weights were taken at phase changes to determine ADG, ADFI, and G:F. Surveillance for morbidity and mortality continued during the grow-finish phase. Any pigs showing clinical signs of illness or lameness were treated with an injectable antibiotic by the lead researcher and/or lead research professor; antibiotic, dose, individual and pen identification, symptoms, and withdrawal dates were recorded.

Live Ultrasound Scanning, Market, and Carcass Data Collection. At d 165 all pigs were ultrasonically scanned (Aloka 500; Hitachi Aloka Medical Ltd., Tokyo, Japan) to measure back fat depth at the 10th rib in addition to loin depth and loin eye area. Market ready pigs and feeders from each pen were individually weighed to determine final ADG, ADFI, and G:F. Pigs in each replicate were marketed across two market shipments to mimic industry practices. Pigs in the heaviest blocks of the winter replicate were marketed at d 178 while light replicates were shipped on d 193. Pigs, in the heaviest block, were marketed on a privately owned gooseneck trailer (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA) with a stocking density of 41 pigs to Tyson Foods Inc (Logansport, IN). Light replicates were similarly shipped on a two-tier commercial livestock semitruck to Indiana Packers Corporation (IPC) (Delphi, IN) with a stocking density of 169 pigs. Similar shipping procedures were followed for the spring replicate with pigs marketing on d 176 and d 183. Two market loads were shipped on a two-tier commercial livestock semitruck to IPC (Delphi, IN) with a stocking density of 126 and 116 pigs respectively.

Pigs were individually tattooed with pen numbers the morning of each scheduled shipment. Carcass data was collected from Tyson and IPC including lean percentage, average fat depth, average loin depth, and hot carcass weight. Lean percentage was calculated following the Indiana Packers Corporation (2015) formula (Indiana Packers Corporation, 2015). Fat depth and loin depth were measured using the Fat-O-Meat'erTM optical probe (Fat-O-Meat'er, SFK Technology S/F, Herlev, Denmark).

Statistical Analysis. Data were analyzed as a random complete block design (RCB) using the GLM procedure in SAS 9.4 (SAS Institute INC., Cary, NC). Pen was the experimental unit with fixed effects of pre-wean dietary treatment, transport treatment, and post-wean dietary treatment, the 2-way and 3-way interactions, and random effect of replicate. Initial piglet body weight (d -15) was run as a covariate. All data met assumptions of normality and independence with ± 2 standard deviations. Post-hoc comparison of means were determined using Duncan's multiple

range test. Data are presented as least square means and considered significant at $P \leq 0.05$ with a tendency towards a trend outlined at $0.05 < P \leq 0.10$.

Objective 2:

General. This experiment was conducted in the nursery barns of Purdue University's Animal Science Research and Education Center Swine Unit in West Lafayette, IN. The experimental protocol was reviewed and approved by Purdue University's Institutional Animal Care and Use Committee (IACUC #2110002209) and animal care and use standards were based on the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (2020). The study was conducted from June 21 to December 7, 2022.

Experimental Design. A total of 384, single source, mixed sex, crossbred, weaned pigs [20 ± 3 d of age; weaning BW = 5.57 ± 1.49 kg; [Duroc x (Yorkshire x Landrace)] underwent an 8 h transportation stress, at weaning, in central Indiana, as described by Duttlinger et al. (2019), and were used in a wean to finish experiment to determine the effects of supplementing nursery pig diets with tryptophan (Trp) on subsequent growth performance following a vaccine-induced immune challenge. The day preceding weaning and transportation, all pigs were individually weighed, blocked by BW and sex and randomly allotted to 48 pens with 8 pigs per pen and 12 pens per treatment. Pens were randomly assigned treatments in a 2x2 factorial design of dietary Trp supplementation (1X vs. 2X NRC (2012)) in the nursery and immune challenge (Control vs. Vaccine Challenge).

Weaning, Transportation, and Nursery-Phase. At weaning (20 ± 3 d of age) pigs were removed from sows and immediately loaded into 2 gooseneck livestock trailers (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA; 2.44 × 7.32 m; Wilson Trailer Company, Sioux City, IA) for transportation. Trailer dimensions allowed 0.07 m^2 of space per pig within the range of 0.060 to 0.084 m^2 per pig required for 4.54 to 9.07 kg pigs as listed in *the Guide for the Care and Use of Agricultural Animals in Research and Teaching* (2020). Three data loggers (Elitech: RC-5+ TE; San Jose, CA; temperature accuracy: ±0.5(-20˚C/+ 40˚C) ±1.0) were evenly spaced within each trailer to measure ambient temperature T_A in 2-min intervals. During transport, T_A averaged 26.7 ± 0.3 ˚C with a low of 12.9 ± 0.4 ˚C and high of 34.1 ± 1.6 ˚C. Trailers were bedded with cedar shavings and ventilation openings were opened to allow airflow through the trailer based on T_A (25.6˚C) at the time of transport.

Pigs were transported as a group for approximately 8 h and 546 km without feed or water. Trailers departed 1 h apart to accommodate weaning, loading, and unloading time. Total transport time was estimated based on the addition of loading time (1.25 ± 0.15 h), time spent in stationary trailer (0.75 ± 0.25 h), unloading time (1.0 ± 0.15 h), and the sorting time into their

respective pens in the nursery facility (1.0 ± 0.15 h). The average time to wean, load, and begin transport per trailer was 2 ± 0.5 h. Following the transportation protocol outlined by Duttlinger et al. (2019) the transport route was approximately 50% two-lane roads and 50% four-lane roads with a total route distance of 273 km completed 2 times during the transport phase. Total trip duration, per lap, averaged 3 ± 0.25 h; drivers switched, and the truck was refueled following the completion of each 273 km lap. At the conclusion of the 8 h transport, pigs were unloaded from each trailer and placed in their assigned pen (nursery 1= 1.42 × 1.22 m; nursery 2= 1.42 × 1.52 m) within the nursery houses. Each nursery consisted of 32 pens with 16 pens per row. Nursery house temperatures were set at 30˚C at weaning; temperatures were reduced in a stepwise fashion by 2.2˚C every 7 d. Post transport pigs were allotted to pens consisting of 8 pigs per pen (5 barrows and 3 gilts). Pigs were blocked by BW and sex with siblings balanced across treatments. Access to clean water and dry feed was provided *ad libitum* via one stainless steel dry nursery self-feeder and cup waterer.

Nursery Diets. Nursery diets were formulated to meet or exceed all nutritional requirements (NRC, 2012). Diets were corn-soybean-meal based and fed in meal form. Pigs were fed diets that contained 1X or 2X the NRC (2012) standardized ileal digestible tryptophan requirement across 4 nursery phases. Each phase was based on a feed budget of 0.9, 2.2, and 8.1 kg/pig for phase 1, 2, and 3 respectively, with phase 4 then being provided *ad libitum* until d 35 post-weaning (tables 3.1-1.4). Control diets were balanced to meet NRC (2012) SID tryptophan to SID lysine ratios. Treatment diets contained 2X the NRC (2012) recommended concentration of SID tryptophan with an inclusion rate of 0.25, 0.235, 0.218, and 0.209% (tables 3.1-3.4), respectively for phases 1-4. Net energy weas kept constant across treatments for each phase. Neomycin-oxytetracycline (45.35 g/kg oxytetracycline hydrochloride and 45.35 g/kg Neomycin Sulfate; Phibro Animal Health; Ridgefield Park, NJ) was provided in phases 1 and 2 of the nursery diet at an inclusion of 0.375 and 0.250%, respectively. Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) was included in phases 3 and 4 at 0.25% inclusion.

Feed Sample Collection and Analysis. A composite sample (~0.22 kg per subsample) of each nursery diet was collected during the bagging process at the Animal Science Research and Education Center's Feed Mill. Samples were ground using a commercial hand grinder for subsequent analysis. Feed was analyzed for amino acid concentration by the University of Missouri Experiment Station Laboratory (Columbia, MO).

Vaccine Challenge. On d 14 post-weaning, immune-challenged pigs began a 3-wk vaccine regimen consisting of vaccines for circovirus (Ingelvac CircoFLEX®; Boehringer Ingelheim, Ingelheim am Rhein, Germany), mycoplasma (Respisure-One®; Zoetis Parsippany, NJ), and influenza (Flusure XP®; Zoetis Parsippany, NJ) administered intramuscularly in the neck during wk 2, 3, and 4 at a dose of 2 ml/pig/week, respectively. Control pigs received 2 ml/pig sterile saline (0.9% NaCl solution) injected intramuscularly into the deck during the 3-wk immune-

challenge to account for injection stress. All injections were administered by trained personnel using an XFY livestock injector gun with an adjustable continuous syringe. BW and immune challenge were conducted concurrently; total time spent handling each pig was approximately 1 ± 0.5 min.

Performance Data Collection. Individual pig and feeder weights were taken every 7 d during the 35-d nursery period to determine ADG, ADFI, and G:F. Pigs were observed daily for clinical abnormalities and general fitness. Therapeutic antibiotic administration was provided, for the duration of the trial, to any animal exhibiting clinical symptoms of illness or lameness by personnel trained to identify pigs in need of antibiotics. Therapeutic treatment, dose, individual and pen identification, exhibited symptoms, and withdrawal dates were recorded.

Grow-Finish Phase. On d 35 post-weaning pigs were moved to a grow-finish facility where they remained in their respective pens until market. Movement from the nursery to the grow-finish facility required minimum transportation of approximately 96 m via a walk-on open air hydraulic trailer (1.83 × 4.57 m; Hi-Lo Trailers Worldwide, Transfer, PA). Transport time from the nursery house to the grow-finish facility was approximately 15 minutes; total transport time was estimated based on the addition of loading time (5.0 ± 2.0 min), time spent stationary in trailer (4.0 ± 1.0 min), moving between houses (1.0 ± 0.5 min), and time spent unloading trailer (2.0 ± 1.0 min). Pigs were herded out of the nursery by trained farm staff, using herding boards (Hog Slat, Inc; Newton Grove, NC) and loaded onto a hydraulic trailer. Stocking density of the trailer was 48 pigs per load. Pigs were blocked by vaccine-treatment, BW, and sex. Diets fed during grow-finish consisted of a common corn-soybean meal-based diet fed in 6 phases (table 3.5).

Live Ultrasound Scanning, Market, and Carcass Data Collection. Prior to shipping, market ready pigs were individually weighed, and backfat (BF) and total loin depth (TLD) at the 10^{th} rib were determined by ultrasound (Sonosite, Bothwell, WA). Pigs were marketed in two shipments. Pigs in the heaviest replicates were marketed at d 173 while pigs on the light replicates were marketed on d 184 to mimic industry practices and to have similar weights at market. Market pigs were shipped on a two-tier commercial livestock semitruck with a stocking density of 163 pigs and 151 pigs, respectively. Pigs from each pen were individually tattooed with a common pen number, to collect carcass data, and shipped approximately 48 km to Indiana Packers Corporation (IPC; Delphi, IN). Carcass data were collected from IPC including net grade, lean percentage, back fat, and loin depth. Lean percentage was calculated following the Indiana Packers Corporation (2015) formula (Indiana Packers Corporation, 2015). Fat depth and loin depth were measured using the Fat-O-Meat'er™ optical probe (Fat-O-Meat'er, SFK Technology S/F, Herlev, Denmark).

Statistical Analysis. Data were analyzed as a randomized complete block design using the PROC GLM procedure in SAS 9.4 (SAS Institute INC., Cary, NC) with pen as the experimental

unit and fixed effects of dietary treatment and immune challenge, the interactions, and random effect of replicate. All data met the assumption of normality and independence with ±2 standard deviations. Data are presented as least square means. Data were considered significant at $P \leq 0.05$ and a tendency was defined at $0.05 < P \leq 0.10$.

Results

Objective 1:

Pre-wean Body Weight and Average Daily Gain. There were no differences observed for pre-wean treatment on piglet body weight ($P = 0.6890 \pm 0.624$; table 2.10) or average daily gain ($P = 0.6890 \pm 0.003$; table 2.10).

Nursery Body Weight and Average Daily Gain. Initial body weight and weaning weight were used as covariates (d -15 and d 0). A 3-way interaction of pre-wean x transport x post-wean treatment was observed for BW in wk 3 ($P = 0.022 \pm 0.435$; table 2.11), wk 5 ($P = 0.005 \pm 1.084$; table 2.11), and for the overall nursery period ($P = 0.030 \pm 1.598$; table 2.11). A trend for a 3-way interaction was observed in wk 2 ($P = 0.062 \pm 0.188$; table 2.11) and wk 4 ($P = 0.054 \pm 0.689$; table 2.11). Transport reduced body weight (wk 3) in pre-wean SID Trp supplemented pigs by 4.8% compared to pigs receiving no supplemental SID Trp pre-wean. Feeding pigs 2X SID Trp post-wean increased body weight in transported, pre-wean SID Trp supplemented pigs but had no effect in non-transported pigs supplemented with SID Trp pre-wean (table 2.11). In contrast, supplementing 2X SID Trp post-wean increased body weight in non-transported pigs receiving no supplemental SID Trp pre-wean but decreased body weight in transported pigs with no pre-wean SID Trp. Transport reduced body weight (wk 5) in post-wean pigs fed 2X SID Trp, but not in pigs receiving supplemental SID Trp pre-wean (table 2.11). Feeding pigs 2X SID Trp post-wean decreased body weight after transport in pigs that were not supplemented with Trp pre-wean, but increased body weight in non-transported pigs by 5.8%. In contrast, post-wean supplemented 2X SID Trp decreased body weight in non-transported pigs when fed supplemental SID Trp pre-wean (table 2.11). Overall, 2X SID Trp supplementation post-wean is beneficial in transported pigs supplemented with SID Trp pre-wean, but results in decreased body weight in transported pigs with no pre-wean SID Trp supplementation.

A 3-way interaction of pre-wean x transport x post-wean treatment was observed for ADG in wk 5 ($P = 0.008 \pm 0.006$; table 2.11) and for the overall nursery period ($P = 0.032 \pm 0.001$; table 2.11). A trend for a 3-way interaction was observed in wk 2 ($P = 0.097 \pm 0.002$; table 2.11) and wk 3 ($P = 0.062 \pm 0.003$; table 2.11). 2X SID Trp supplemented post-weaning reduced ADG (wk 5) in transported and non-transported pigs when provided supplemented SID Trp pre-wean. In contrast, non-transported pigs receiving no pre-wean supplemented SID Trp had improved

ADG when supplemented 2X SID Trp post-wean, but reduced ADG in transported pigs fed post-wean 2X SID Trp. Overall, supplemented 2X SID Trp improved ADG in transported pigs by 4.5%, following a 42 d nursery period, when also supplemented pre-wean but resulted in a 2.3% reduction in ADG for transported pigs with no pre-wean SID Trp supplementation.

A 2-way interaction of transport x post-wean treatment was observed for ADG in wk 4 (P= 0.033 ± 0.002; table 2.11) with a trend for a 2-way interaction of pre-wean x transport treatment on ADG in wk 6 (P= 0.067 ± 0.011; table 2.11). Transport improved ADG (wk 4) in pigs with no post-wean SID Trp supplementation, but results in reduced ADG when pigs are provided SID Trp post-wean.

Nursery Average Daily Feed Intake and Feed Efficiency. There were no effects of dietary or transport treatment on G:F during the 42 d nursery period. A 3-way interaction of pre-wean x transport x post-wean treatment was observed for ADFI in wk 2 (P= 0.016 ± 0.003; table 2.12). Post-wean 2X SID Trp improved ADFI in transported pigs by 4.2% when pigs also received pre-wean SID Trp but not in non-transported pigs. Feeding pigs 2X SID Trp post-wean reduced ADFI in transported pigs that were not supplemented SID Trp pre-wean but improved ADFI by 3.1% in non-transported pigs receiving no supplemental SID Trp pre-wean.

A 2-way interaction of pre-wean x post-wean treatment was observed for ADFI for wk 1 (P= 0.008 ± 0.002; table 2.12) and wk 6 (P= 0.034 ± 0.015; table 2.12) with a trend for a 2-way interaction of transport x post-wean treatment observed in wk 4 (P= 0.072 ± 0.040; table 2.12). Post-wean supplemented SID Trp reduced ADFI (wk 1) in pigs not supplemented with pre-wean SID Trp but had no effect in post-wean supplemented pigs fed 2X SID Trp that also received pre-wean SID Trp supplementation. 2X SID Trp supplementation post-wean increased ADFI (wk 6) in pigs that received no supplemented SID Trp pre-wean, improving ADFI in wk 6 by 5.2%; but resulted in reduced ADFI in post-wean 2X SID Trp supplemented pigs provided pre-wean SID Trp. Supplementing SID Trp pre-wean improved ADFI (wk 6) in transported pigs but had no effect in non-transported pigs. Overall ADFI was reduced in pigs that were transported at weaning (P= 0.050 ± 0.003; table 2.12).

Average Daily Tryptophan Consumption during the Nursery Phase. Results of the feed amino acid analysis confirm SID Trp concentrations were successfully doubled in treatment diets compared to control diets across all nursery phases (Tables 2.7-2.8). While dietary concentrations of Trp were adequate based on NRC (2012) recommendations, daily consumption of Trp was lower than recommended during wk 1, 3, 4, and 6 of the nursery period as a result of lower than predicted (NRC, 2012) feed intake (Table 2.9). Extrapolated per kilogram of BW gain, Trp intake was adequate in Control-fed pigs during weeks, 1, 2, 5, and 6, but deficient during wk 3 and 4. However, this does become a chicken vs. the egg question: Is Trp adequate

per kg of gain as a result of low growth rates, or are growth rates limited by low daily feed and therefore nutrient intakes?

Grow-Finish Body Weight and Average Daily Gain. There was a 3-way interaction of pre-wean x transport x post-wean treatment observed for BW in wk 9 (P= 0.025 ± 1.617; table 2.13) in the grow finish period. Post-wean 2X SID Trp supplementation improved body weight in transported pigs supplemented with SID Trp pre-wean but resulted in reduced body weight in transported pigs with no pre-wean SID Trp supplementation. Feeding pigs 2X SID Trp post-wean improved body weight in non-transported pigs receiving no supplemented SID Trp pre-wean.

A 2-way interaction of transport x post-wean treatment was observed for BW in wk 12 (P= 0.002 ± 3.332; table 2.13) and wk 15 (P= 0.042 ± 5.771; table 2.13). A trend for a 2-way interaction of pre-wean x transport on ADG in wk 9 (P= 0.054 ± 0.010; table 2.13) and wk 12 (P= 0.098 ± 0.002; table 2.13) were noted. Transport reduced body weight (wk 12) in post-wean 2X SID Trp supplemented pigs; in contrast, body weight improved in transported pigs not provided post-wean 2X SID Trp. Similarly, transport reduces body weight (wk 15) in post-wean 2X SID Trp supplemented pigs by 3.3%, but improved body weight in transported pigs not provided 2X SID Trp post-wean. In general, transported pigs had a 4.0% improvement in ADG (wk 18) compared to non-transported pigs at weaning (P= 0.048 ± 0.004; table 2.13). A trend for transported pigs having improved ADG in wk 24 (P= 0.070 ± 0.009; table 2.13) was also noted.

Grow-Finish Average Daily Feed Intake and Feed Efficiency. A trend for a 3-way interaction of pre-wean x transport x post-wean treatment for ADFI in wk 15 (P= 0.051 ± 0.020; table 2.14), and trend for a 2-way interaction of transport x post-wean treatment on ADFI in wk 15 (P= 0.088 ± 0.020; table 2.14) were noted. A 2-way interaction of pre-wean x transport treatment was observed for ADFI in wk 15 (P= 0.011 ± 0.016; table 2.14). Pre-wean SID Trp supplementation reduced ADFI (wk 15) in transported pigs but improved ADFI in transported pigs provided no pre-wean SID Trp. In general, pigs that were transported at weaning had a 5.5% improvement in ADFI in wk 15 (P= 0.044 ± 0.016; table 2.14). Transported pigs tended to have improved ADFI in wk 18 (P= 0.066 ± 0.024; table 2.14). It was also noted that post-wean 2X SID Trp supplemented pigs tended to have improved ADFI in wk 21 (P= 0.098 ± 0.024; table 2.14) and 24 (P= 0.093 ± 0.048; table 2.14) of the grow-finish period.

A 2-way interaction of pre-wean x transport treatment was observed for G:F in wk 15 (P= 0.026 ± 0.001; table 2.14) and a trend for a 2-way interaction of transport x post-wean treatment on G:F in wk 18 (P= 0.091 ± 0.001; table 2.14). In general, transported pigs supplemented with pre-wean SID Trp maintained G:F while G:F was reduced by 5.7% in non-transported pigs that were not provided supplemented SID Trp pre-wean.

Final Market Weight, Average Daily Gain, and Feed Efficiency. Overall, dietary and/or transport treatment had no effect on final market weights (P= 0.894 ± 19.97; table 2.15) ADG (P= 0.800 ± 0.032; table 2.15), ADFI (P= 0.680 ± 0.138; table 2.15) or G:F (P= 0.109 ± 0.005; table 2.15). However, pigs that were transported at weaning tended to be heavier than non-transported pigs (P= 0.072 ± 19.97; table 2.15). At the culmination of the experiment pigs that were transported, at weaning, improved ADG in both the light (P= 0.026 ± 0.001; table 2.15) and heavy (P= 0.029 ± 0.001; table 2.15) shipments.

Winter Replicate. A tendency for a 3-way interaction of pre-wean x transport x post-wean treatment was observed for market weight (P= 0.061 ± 13.15; table 2.17) in the winter replicate. In general, pigs that were transported at weaning were approximately 5.2 kg heavier than pigs that were not transported (P= 0.034 ± 13.15; table 2.17).

Spring Replicate. There were no differences observed across treatments for market weight (P= 0.252 ± 25.83; table 2.18).

Live Ultrasound Characteristics. Supplementing SID Trp pre-wean increased back fat in market pigs (P= 0.019 ± 2.50; table 2.16) resulting in 1.18 mm more back fat in pre-wean Trp supplemented pigs compared to pigs that did not receive pre-wean SID Trp. There were no differences between treatments for overall loin depth (P= 0.676 ± 6.743; table 2.16) or loin eye area (P= 0.596 ± 0.859; table 2.16) at market.

Winter Replicate. In the winter replicate, a 2-way interaction of pre-wean x post-wean treatment on back fat (P= 0.001 ± 3.59; table 2.17) and a 2-way interaction of pre-wean x transport treatment (P= 0.009 ± 4.32; table 2.17) was observed in market pigs. Providing Trp supplementation pre-wean increased back fat in pigs that were transported at weaning but reduce back fat in transported pigs that were not supplemented with pre-wean SID Trp. Further, pre-wean Trp supplementation increased back fat in transported pigs but results in reduced back fat in transported pigs that were not provided supplemental Trp pre-wean. Combining final carcass price with average carcass weights, non-transported pigs supplemented Trp pre-weaning but were not provided 2X SID Trp post-wean had the greatest revenue per pig ($113.73) followed by control pigs that were transported ($112.18). Control pigs and pigs that did not receive pre-wean Trp but were transported and fed 2X SID Trp post-wean generated the lowest revenue ($83.58 and $85.60 respectively). In general, pigs provided supplemental Trp pre-wean had deeper loins and larger loin eye areas where pigs that were not provided pre-wean Trp had reduced back fat and smaller loin eyes.

Spring Replicate. Overall, there were no differences observed across treatments for back fat (P= 0.904 ± 2.24; table 2.18) or loin eye area (P= 0.770 ± 6.34; table 2.18). A 2-way interaction of pre-wean x transport treatment for loin depth (P= 0.044 ± 4.25; table 2.18) as well as a 2-way interaction of pre-wean x post-wean treatment for loin depth (P= 0.033 ± 4.25; table 2.18) was

observed. Feeding pigs 2X SID Trp post-wean increases loin depth in transported pigs, but results in reduced loin depths in non-transported pigs provided supplemental 2X SID Trp post-wean. Further, providing supplemental 2X SID Trp post-wean increased loin depth in pigs that received SID Trp pre-wean. In contrast, pigs fed 2X SID Trp post-wean had decreased loin depth when no pre-wean SID Trp was provided. Combining final carcass price with average carcass weights, non-transported pigs supplemented Trp pre-weaning but were not provided 2X SID Trp post-wean had the greatest revenue per pig ($113.69) followed by pigs receiving no pre- or post-wean Trp that were transported ($113.15). Pre-wean Trp supplemented pigs that were transported and did not receive 2X SID Trp post-wean generated the lowest revenue ($110.33). Comparing treatment carcass values between the winter and spring replicate, carcass value was highest in non-transported pigs provided pre-wean Trp but not fed 2X SID Trp post-wean.

In general, transported pigs that were supplemented with pre-wean Trp had reduced back fat by market. A 2-way interaction of pre-wean x post-wean treatment was observed for loin depth (P= 0.033 ± 4.25; table 2.18) and a 2-way interaction of transport x post-wean treatment on loin depth (P= 0.044 ± 4.25; table 2.18).; where supplementing SID Trp prior to or following transport increases loin depths in market ready pigs.

Carcass Characteristics. An error recording carcass data occurred at the plant in the winter replicate resulting in missing data; therefore, only carcass characteristics from the spring replicate are reported. Overall, there was a 3-way interaction of pre-wean x transport x post-wean on final carcass price per kg (P< 0.001 ± 0.001) where non-transported pigs supplemented Trp pre-weaning but were not provided 2X SID Trp post-wean had the greatest revenue. There were no differences observed across treatments for carcass back fat (P= 0.972 ± 0.029; table 2.19) or loin depth (P= 0.717 ± 6.867; table 2.19). A 2-way interaction of transport x post-wean treatment was observed on percent lean (P= 0.035 ± 0.531; table 2.19) for marketed pigs. Transport reduces percent lean in pigs supplemented with 2X SID Trp post-wean but improves percent lean in non-transported pigs provided 2X SID Trp post-wean.

Results, Objective 2:

Feed Composition and Average Tryptophan Consumption. Analyzed Trp concentrations of the feed were generally in agreement with calculated values (Table 3.6). Chemical analysis of feed samples reported Trp concentrations in treatment diets showed Trp supplemented diets were doubled (Table 3.7-3.8). Vaccinated pigs fed 1X NRC (2012) SID Trp diets generally consumed less Trp (g) per day compared to unvaccinated pigs throughout the duration of the 35-d nursery period (table 3.9). Vaccinated pigs fed 2X NRC (2012) SID Trp diets consumed 0.05 g more daily SID Trp than unvaccinated pigs supplemented SID Trp in wk 1 (table 3.9). At the culmination of a 35-d nursery period, vaccinated pigs fed 2X NRC (2012) SID Trp diets

consumed 1.96 g more Trp per day compared to vaccinated pigs fed 1X SID Trp (table 3.8) but consumed 0.14 g less SID Trp compared to unvaccinated pigs fed 2X NRC (2012) SID Trp.

Body Weight and Average Daily Gain. There were no interactions of diet x vaccine observed for body weight or ADG. Supplemented SID Trp at 2X the NRC (2012) requirement improved body weight in vaccinated pigs in wk 2 (P= 0.0240 ± 0.081; table 3.10), wk 3 (P= 0.0476 ± 0.241; table 3.10), wk 4 (P= 0.0121 ± 0.360; table 3.10), and overall (P= 0.0079 ± 0.693; table 3.10). SID Trp supplemented pigs had improved ADG in wk 4 (P= 0.0489 ± 0.002; table 3.10) compared to pigs there received no supplemental SID Trp. Pigs that were supplemented with SID Trp improved overall ADG (P= 0.0175 ± 0.001; table 3.9) at the end of a 35 d nursery period when fed 2X NRC (2012) SID Trp regardless of vaccine-challenge.

Pigs had reduced ADG in wk 5 (P= 0.0164 ± 0.002; table 3.10) following the 3-week vaccine induce-immune challenge, regardless of dietary SID Trp. In general, vaccinated pigs tended to have reduced overall ADG (P= 0.0737 ± 0.001; table 3.10) at the end of the nursery period. However, vaccine did not influence piglet body weight.

Average Daily Feed Intake and Feed Efficiency. No interactions of diet x vaccine were observed for G:F or ADFI. Vaccination reduced G:F in pigs in wk 5 (P= 0.0201 ± 0.002; table 3.11); however, vaccination had no overall effect on pigs' G:F (P= 0.5380 ± 0.002; table 3.11) or ADFI (P= 0.7171 ± 0.002; table 3.11) during the 35 d nursery. Pigs fed 2X NRC (2012) SID Trp tended to have improved ADFI in wk 2 (P= 0.0563 ± 0.081; table 3.11) and wk 3 (P= 0.0947 ± 0.008; table 3.11) compared to pigs fed diets containing 1X NRC (2012) SID Trp.

Final Market Weight and Carcass Weight. A 2-way interaction of diet x vaccine was observed for hot carcass weight (P= 0.025 ± 0.831; table 3.12). Pigs undergoing a vaccine-induced immune challenge that were supplemented SID Trp had reduced carcass weight compared to pigs that did not receive supplemental SID Trp. In contrast, unvaccinated pigs that received 2X SID Trp had heavier carcass weights, but pigs that were not fed SID Trp or vaccinated had lighter carcasses compared to all other pigs. At the culmination of the experiment, pigs that were fed 2X SID Trp were heavier at market (P= 0.044 ± 12.708; table 3.12). When average carcass weights were combined with final carcass price, vaccinated pigs that were not supplemented 2X SID Trp generated the greatest revenue ($170.83) followed by 2X SID Trp supplemented pigs that were not vaccinated ($169.07); suggesting that carcass value can be maintained when pigs are fed 2X SID Trp during the nursery period. However pigs that were supplemented Trp and vaccinated generated $1.66 less than unvaccinated pigs supplemented 2X SID Trp. Finally, pigs that were not supplemented Trp or vaccinated generated the lowest revenue ($164.58).

Live Ultrasound Characteristics. There were no effects, or interactions, of dietary or vaccine treatments on back fat (mm) (P= 0.932 ± 2.123; table 3.12), loin depth (mm) (P= 0.663 ± 0.409; table 3.12), or total loin depth (mm) (P= 0.777 ± 3.801; table 3.12) across treatments.

*Carcass Characteristics*A 2-way interaction of diet x vaccine was observed for carcass fat (*P*= 0.019 ± 0.000; table 3.12) with a trend for a 2-way interaction of diet x vaccine for lean percent (*P*= 0.053 ± 0.000; table 3.12). This resulted from pigs having 1.01 mm more fat at market when supplemented Trp in the absence of vaccine challenge compared to pigs provided 1X NRC (2012) SID Trp concentrations. Vaccinated pigs tended to have 0.67 mm deeper loins (*P*= 0.096 ± 0.02; table 3.12) compared to pigs that were not vaccinated in the nursery

Table 2.1. Tryptophan concentration and carrier volume for pre-wean supplementation

Days pre-wean	Trp Concentration (g)	Carrier Volume (mL)
d 5-10	0.35	2.0
d 11-15	0.45	4.0
d 16- 24[1]	0.55	6.0

[1]Pigs allotted to post-wean Trp supplementation continued oral supplementation of 0.55 g Trp for 3 d post-wean.

Table 2.2. Phase 1 nursery diet composition

	Phase 1[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	41.56	41.29
SBM, 47.5% CP	13.86	13.86
Soybean oil	5.00	5.00
Limestone	0.971	0.971
MonoCal	0.232	0.232
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix[4]	0.05	0.05
Phytase[5]	0.10	0.10
Salt	0.13	0.13
Plasma Protein	4.50	4.50
Spray dried blood meal	1.50	1.50
Soy concentrate	4.00	4.00
Fish Meal	3.50	3.50
Dried Whey	23.00	23.00
Lysine-HCL	0.259	0.259
DL-Methionine	0.170	0.170
L-Threonine	0.047	0.047
L-Tryptophan	-	0.27
Neo-OxyTet 100/100[6]	0.375	0.375
Zinc Oxide	0.375	0.375
Calculated Composition		
NE, kcal/kg	2723	2716
CP, %	22.78	22.99
SID Lys, %	1.55	1.55
SID Trp, %	0.25	0.50
Ca, %	0.85	0.85
Total P, %	0.64	0.64
Avail. P, %	0.45	0.85

[1]Pigs fed based on a feed budget of 0.9 kg/pig. [2]vitamin premix provided per kilogram (kg) of diet: vitamin a, 5,512 iu; vitamin d_3, 551 iu; vitamin e, 37 iu; vitamin k, 1.8 mg; vitamin b_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]trace mineral (tm) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]selenium premix provided at 0.03 mg per kilogram (kg) of diet. [5]phytase activity level 600.1 pu/kg (phyzyme, danisco animal health; dupont, st. Louis, mo).

[6]neomycin-oxytetracycline 100/100 d (active drug ingredients included at a concentration of 45.35 g/kg oxytetracycline hydrochloride and 45.35 g/kg neomycin sulfate; phibro animal health; ridgefield park, nj) provided at 4.54 mg/kg.

Table 2.3. Phase 2 nursery diet composition

	Phase 2[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	42.04	41.78
SBM, 47.5% CP	17.91	17.91
DDGS - Rens. 7% fat	5.00	5.00
Soybean oil	4.00	4.00
Limestone	1.097	1.097
MonoCal	0.144	0.144
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix[4]	0.05	0.05
Phytase[5]	0.10	0.10
Salt	0.30	0.30
Plasma Protein	2.50	2.50
Spray dried blood meal	1.00	1.00
Soy concentrate	4.00	4.00
Fish Meal	3.50	3.50
Dried Whey	17.00	17.00
Lysine-HCL	0.223	0.223
DL-Methionine	0.125	0.125
L-Threonine	0.043	0.043
L-Tryptophan	-	0.260
Neo-OxyTet 100/100[6]	0.250	0.250
Zinc Oxide	0.350	0.350
Calculated Composition		
NE, kcal/kg	2638	2631
CP, %	23.32	23.52
SID Lys, %	1.45	1.45
SID Trp, %	0.23	0.46
Ca, %	0.85	0.85
Total P, %	0.61	0.61
Avail. P, %	0.40	0.40

[1]Pigs fed based on a feed budget of 2.2 kg/pig. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Selenium Premix provided at 0.03 mg per kilogram (kg) of

diet. [5]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO).[6]Neomycin-oxytetracycline 100/100 D (active drug ingredients included at a concentration of 45.35 g/kg oxytetracycline hydrochloride and 45.35 g/kg Neomycin Sulfate; Phibro Animal Health; Ridgefield Park, NJ) provided at 4.54 mg/kg.

Table 2.4. Phase 3 nursery diet composition

	Phase 3[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	44.58	44.34
SBM, 47.5% CP	29.12	29.12
DDGS- Rens. 7% fat	7.50	7.50
Swine grease	3.00	3.00
Limestone	0.941	0.941
MonoCal	0.305	0.305
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix[4]	0.05	0.05
Phytase[5]	0.10	0.10
Salt	0.53	0.53
Fish Meal	4.00	4.00
Dried Whey	8.60	8.60
Lysine-HCL	0.231	0.231
DL-Methionine	0.083	0.083
L-Threonine	0.058	0.058
L-Tryptophan	-	0.242
Carbadox[6]	0.250	0.250
Zinc Oxide	0.280	0.280
Calculated Composition		
NE, kcal/kg	2527	2521
CP, %	23.43	23.62
SID Lys, %	1.35	1.35
SID Trp, %	0.22	0.44
Ca, %	0.80	0.80
Total P, %	0.63	0.63
Avail. P, %	0.38	0.38

[1]Pigs fed based on a feed budget of 8.16 kg/pig. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Selenium Premix provided at 0.03 mg per kilogram (kg) of diet. [5]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis,

MO). [6]Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.5. Phase 4 nursery diet composition

	Phase 4[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	51.16	50.93
SBM, 47.5% CP	31.97	31.97
DDGS - Rens. 7% fat	10.00	10.00
Swine grease	3.00	3.00
Limestone	1.151	1.151
MonoCal	0.775	0.775
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix[4]	0.05	0.05
Phytase[5]	0.10	0.10
Salt	0.56	0.56
Lysine-HCL	0.290	0.290
DL-Methionine	0.099	0.099
L-Threonine	0.116	0.116
L-Tryptophan	-	0.229
Carbadox[6]	0.250	0.250
Copper sulphate	0.100	0.100
Calculated Composition		
NE, kcal/kg	2482	2476
CP, %	22.59	22.77
SID Lys, %	1.25	1.25
SID Trp, %	0.21	0.42
Ca, %	0.70	0.70
Total P, %	0.60	0.60
Avail. P, %	0.33	0.33

[1]Pigs fed *ad libitum* to d 42. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Selenium Premix provided at 0.03 mg per kilogram (kg) of diet. [5]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). [6]Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.6. Grow-finish diet composition.

	Diets					
Item	GF1	GF2	GF3	GF4	GF5	GF6
Ingredient, % as fed						
Corn	51.55	53.05	60.07	64.17	71.39	73.55
SBM, 47% CP	28.50	23.30	16.60	12.50	10.60	8.55
DDGS - Rens.	15.00	20.00	20.00	20.00	15.00	15.00
Swine Grease	2.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.42	1.40	1.34	1.26	1.14	1.17
MonoCal Phos.	0.24	0.08	0.00	0.00	0.03	0.00
Vitamin Prx[1]	0.20	0.15	0.15	0.15	0.13	0.10
Trace Mineral. Prx[2]	0.10	0.09	0.08	0.07	0.05	0.05
Selenium Prx[3]	0.05	0.05	0.05	0.05	0.03	0.03
Phytase[4]	0.10	0.10	0.10	0.10	0.05	0.05
Salt	0.35	0.35	0.30	0.30	0.25	0.25
Lysine-HCL	0.22	0.20	0.20	0.20	0.15	0.15
DL-Methionine	0.03	-	-	-	-	-
L-Threonine	0.03	-	-	-	-	-
L-Tryptophan	-	-	-	-	-	-
Lincomycin[5]	0.10	0.10	0.04	-	-	-
Calculated Composition						
ME, Kcal/kg	3439.9	3335.5	3348.6	3344.5	3358.5	3372.0
NE, Kcal/kg	2467.0	2468.6	2508.8	2509.4	2547.3	2594.0
CP, %	22.28	18.46	16.10	16.71	15.03	12.04
SID Lys, %	1.10	0.975	0.80	0.701	0.60	0.551
Ca, %	0.70	0.65	0.60	0.55	0.50	0.50
Phos, %	0.51	0.47	0.45	0.41	0.39	0.36
Av.P, %	0.32	0.30	0.28	0.26	0.24	0.20

[1]Pigs fed *ad libitum*; dietary phase chances occurred every 3 wk. [2]GF1 vitamin premix provided per kilogram (kg) of diet: vitamin A, 5450.9 IU; vitamin D_3, 2180.4 IU; vitamin E, 29.1 IU; vitamin K, 4.4 mg; vitamin B_2, 0.04 µg; riboflavin, 10.9 mg; pantothenic acid, 36.3 mg; niacin, 36.3. GF2-4 vitamin premix provided per kilogram (kg) of diet: vitamin A, 5039.1 IU; vitamin D_3, 2015.6 IU; vitamin E, 26.9 IU; vitamin K, 4.0 mg; vitamin B_2, 0.04 µg; riboflavin, 10.1 mg; pantothenic acid, 33.6 mg; niacin, 60.5 mg. GF5 vitamin premix provided per kilogram (kg) of diet: vitamin A, 3633.9 IU; vitamin D_3, 1453.6 IU; vitamin E, 19.4 IU; vitamin K, 2.9 mg; vitamin B_2, 0.03 µg; riboflavin, 7.3 mg; pantothenic acid, 24.2 mg; niacin, 43.6. GF6 vitamin premix provided per kilogram (kg) of diet: vitamin A, 2645.5 IU; vitamin D_3, 254.6 IU; vitamin E, 17.6 IU; vitamin K, 0.9 mg; vitamin B_{12}, 0.02 µg; riboflavin, 3.5 mg; pantothenic acid, 8.8

mg; niacin, 13.2. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: 50 mg Fe; 50 mg Zn; 6.2 mg Mn; 4.66 mg Cu; 0.19 mg I. [4]Selenium Premix provided at 0.3 mg per kilogram (kg) of diet. [5]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). [6]Lyconmycin (Lincomix, Zoetis, Parsippany, NJ) provided at 110,000ppm.

Table 2.7. Analyzed amino acid composition of nursery diets (winter replicate)[1]

	Diet							
	PH1		PH2		PH3		PH4	
Amino Acid, W/W%	1X	2X	1X	2X	1X	2X	1X	2X
Taurine §	0.17	0.18	0.17	0.17	0.18	0.18	0.15	0.17
Hydroxyproline	0.08	0.10	0.09	0.11	0.12	0.13	0.02	0.00
Aspartic Acid	2.08	2.24	1.98	2.17	2.19	2.20	1.94	2.19
Threonine	0.85	0.94	0.83	0.91	0.92	0.89	0.81	0.92
Serine	0.80	0.88	0.78	0.87	0.84	0.87	0.78	0.90
Glutamic Acid	3.66	3.62	3.43	3.71	3.88	3.88	3.63	4.01
Proline	1.18	1.15	1.14	1.20	1.19	1.21	1.16	1.23
Lanthionine §	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01
Glycine	0.88	0.94	0.89	0.95	0.99	1.01	0.83	0.91
Alanine	1.11	1.21	1.13	1.20	1.15	1.17	1.05	1.12
Cysteine	0.34	0.38	0.34	0.38	0.33	0.35	0.30	0.32
Valine	1.13	1.29	1.12	1.20	1.14	1.14	1.02	1.12
Methionine	0.38	0.46	0.40	0.44	0.44	0.44	0.36	0.39
Isoleucine	0.97	0.97	0.92	0.99	1.05	1.05	0.94	1.04
Leucine	1.92	2.09	1.91	2.04	1.94	1.94	1.83	1.97
Tyrosine	0.68	0.69	0.64	0.71	0.68	0.70	0.61	0.70
Phenylalanine	1.05	1.11	1.00	1.08	1.07	1.07	1.00	1.11
Hydroxylysine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ornithine §	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	1.47	1.66	1.45	1.48	1.44	1.45	1.22	1.41
Histidine	0.58	0.68	0.57	0.62	0.57	0.58	0.52	0.58
Arginine	1.27	1.26	1.17	1.29	1.36	1.38	1.21	1.38
Tryptophan	0.37	0.58	0.26	0.44	0.28	0.45	0.25	0.43
Total	21.03	22.49	20.28	22.02	21.82	22.15	19.69	21.95
Crude protein*	22.78	23.31	22.69	22.87	22.35	22.45	21.91	22.11

[1]Chemical analysis conducted by University of Missouri Experiment Station Laboratory (Columbia, MO). [2]W/W%= grams per 100 grams of sample. § Non-proteinogenic amino acids. *Crude protein= %N x6.25. Results are expressed on an "as is" basis unless otherwise indicated.

Table 2.8. Analyzed amino acid composition of nursery diets (spring replicate)[1]

	Diet							
	PH1		PH2		PH3		PH4	
Amino Acid, W/W%	1X	2X	1X	2X	1X	2X	1X	2X
Taurine §	0.18	0.17	0.19	0.18	0.18	0.18	0.16	0.17
Hydroxyproline	0.09	0.09	0.05	0.09	0.14	0.11	0.00	0.05
Aspartic Acid	2.22	2.09	2.18	2.23	2.14	2.19	2.05	2.12
Threonine	1.01	0.94	1.30	0.99	1.17	0.90	0.87	0.88
Serine	0.85	0.88	0.86	0.91	0.89	0.84	0.92	0.81
Glutamic Acid	3.57	3.45	3.69	3.75	3.81	3.88	3.77	3.84
Proline	1.15	1.11	1.18	1.19	1.20	1.21	1.15	1.16
Lanthionine §	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	0.95	0.90	0.97	0.98	0.97	0.99	0.85	0.89
Alanine	1.21	1.15	1.19	1.21	1.16	1.18	1.07	1.08
Cysteine	0.38	0.36	0.37	0.37	0.33	0.34	0.31	0.33
Valine	1.30	1.18	1.21	1.24	1.10	1.15	1.00	1.10
Methionine	0.42	0.42	0.46	0.44	0.40	0.43	0.36	0.38
Isoleucine	0.99	0.90	1.00	1.01	1.02	1.06	0.94	1.01
Leucine	2.09	1.98	2.03	2.07	1.94	1.99	1.88	1.90
Tyrosine	0.68	0.68	0.70	0.70	0.68	0.71	0.68	0.69
Phenylalanine	1.12	1.06	1.09	1.11	1.07	1.09	1.05	1.08
Hydroxylysine	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02
Ornithine §	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	1.68	1.55	1.53	1.57	1.43	1.48	1.36	1.42
Histidine	0.68	0.64	0.63	0.65	0.57	0.58	0.55	0.57
Arginine	1.28	1.23	1.30	1.33	1.34	1.37	1.31	1.37
Tryptophan	0.27	0.46	0.26	0.45	0.27	0.44	0.25	0.43
Total	22.16	21.29	22.23	22.51	21.86	22.16	20.57	21.32
Crude protein*	23.01	21.78	22.69	22.88	23.89	23.81	22.52	22.20

[1]Chemical analysis conducted by University of Missouri Experiment Station Laboratory (Columbia, MO). [2]W/W%= grams per 100 grams of sample. § Non-proteinogenic amino acids. *Crude protein= %N x6.25. Results are expressed on an "as is" basis unless otherwise indicated.

Table 2.9. Average daily tryptophan consumption[1]

		Control				Trp			
		No Transport		Transport		No Transport		Transport	
	NRC, 2012	Control	Trp	Control	Trp	Control	Trp	Control	Trp
g/d									
wk 1	0.7	0.42	0.7	0.36	0.69	0.34	0.83	0.41	0.7
wk 2	1.08	1.12	2.34	1.11	2.65	1.13	2.44	1.17	2.49
wk 3	1.91	1.22	2.59	1.21	2.71	1.17	2.70	1.22	2.69
wk 4	1.91	1.53	3.13	1.56	3.53	1.61	3.40	1.39	3.74
wk 5	1.91	2.14	4.41	1.93	4.46	1.91	4.64	2.15	4.31
wk 6	2.5	2.15	4.87	2.39	5.44	2.42	5.70	2.36	5.34
g/kg									
wk 1	3.33	4.51	6.60	2.96	6.90	3.16	8.12	3.34	6.29
wk 2	3.24	3.71	9.23	3.85	8.29	4.07	7.95	3.72	8.64
wk 3	3.26	2.85	6.47	2.88	6.34	2.93	6.43	3.11	6.42
wk 4	3.26	2.90	6.19	3.06	6.75	3.11	6.11	2.63	6.72
wk 5	3.26	3.41	7.50	3.28	7.05	3.43	8.12	3.44	7.17
wk 6	3.29	3.34	7.50	3.47	7.04	3.64	8.09	3.35	7.33

[1]Averages calculated based on individual feed intake per 7 d.

[2]Average Trp consumption (g/d) determined by the National Research Council.

Table 2.10. Main effect of pre-wean Tryptophan supplementation on piglet performance[1]

	Pre-wean treatment			
	Control	Trp	SEM	*P*-value
Weight (kg)[2]				
d -15[3]	2.02	2.02	-	-
d -10	3.25	3.24	0.097	0.8226
d -5	4.66	4.66	0.308	0.9565
d 0	6.07	6.10	0.624	0.6890
ADG (kg)[2]				
d -10	0.25	0.24	0.002	0.2790
d -5	0.28	0.28	0.004	0.8346
d 0	0.28	0.29	0.004	0.1562
overall	0.27	0.27	0.003	0.6890

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Negative (-) d represent days leading up to weaning (d21). [3]Experiment began d-15; initial treatment day used as covariate.

Table 2.11. Effect of pre-wean by transport by post-wean on nursery performance[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
BW (kg)																
wk 1	7.02	7.06	7.20	6.92	7.01	7.03	7.11	7.05	0.052	0.552	0.192	0.259	0.688	0.777	0.323	0.488
wk 2	9.09	9.21	9.47	8.95	9.17	9.07	9.09	9.22	0.188	0.405	0.384	0.432	0.934	0.519	0.694	0.062
wk 3	11.87	12.13	12.48	11.63	12.14	12.17	11.89	12.40	0.435	0.649	0.307	0.975	0.840	0.140	0.618	0.022
wk 4	15.34	15.85	16.39	15.37	15.75	16.10	15.68	16.16	0.689	0.550	0.694	0.589	0.442	0.166	0.176	0.054
wk 5	19.32	20.56	20.41	19.25	20.16	20.09	20.00	20.39	1.084	0.448	0.292	0.600	0.709	0.925	0.089	0.005
wk 6	24.41	25.62	25.76	24.38	25.21	25.59	24.74	25.30	1.598	0.870	0.091	0.435	0.444	0.571	0.113	0.030
ADG (kg)[2]																
wk 1	0.13	0.12	0.13	0.10	0.12	0.12	0.12	0.11	0.001	0.689	0.148	0.287	0.745	0.727	0.413	0.717
wk 2	0.30	0.31	0.31	0.29	0.30	0.29	0.28	0.31	0.002	0.459	0.643	0.760	0.987	0.463	0.903	0.097
wk 3	0.40	0.42	0.43	0.38	0.43	0.44	0.40	0.45	0.003	0.060	0.263	0.410	0.668	0.120	0.709	0.062
wk 4	0.50	0.53	0.55	0.53	0.52	0.56	0.54	0.53	0.002	0.446	0.462	0.188	0.142	0.803	0.033	0.849
wk 5	0.58[ab]	0.67[a]	0.59[ab]	0.54[b]	0.63[ab]	0.57[ab]	0.64[ab]	0.62[ab]	0.006	0.410	0.395	0.586	0.013	0.132	0.139	0.008
wk 6	0.71	0.74	0.76	0.73	0.72	0.78	0.68	0.70	0.011	0.440	0.143	0.344	0.067	0.416	0.470	0.955
overall	0.43	0.46	0.46	0.43	0.45	0.46	0.44	0.45	0.001	0.850	0.081	0.420	0.427	0.548	0.107	0.032

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.12. Effect of pre-wean by transport by post-wean on nursery efficiency[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
ADFI (kg)[2]																
wk 1	0.18[a]	0.13[ab]	0.16[ab]	0.12[b]	0.13[ab]	0.14[ab]	0.13[ab]	0.13[ab]	0.002	0.110	0.494	0.032	0.632	0.008	0.697	0.816
wk 2	0.49	0.52	0.51	0.46	0.50	0.47	0.47	0.49	0.003	0.236	0.126	0.534	0.585	0.990	0.752	0.016
wk 3	0.54	0.56	0.55	0.52	0.58	0.58	0.55	0.57	0.005	0.128	0.100	0.822	0.886	0.786	0.872	0.354
wk 4	0.96	1.03	0.96	0.89	0.85	1.03	0.95	0.91	0.040	0.620	0.388	0.431	0.489	0.428	0.072	0.580
wk 5	0.96	0.97	0.92	0.90	1.03	0.96	0.96	0.99	0.021	0.181	0.178	0.725	0.592	0.749	0.521	0.361
wk 6	1.16	1.13	1.23	1.12	1.16	1.18	1.06	1.14	0.015	0.346	0.409	0.724	0.070	0.034	0.922	0.224
overall	0.72	0.72	0.72	0.67	0.71	0.73	0.69	0.71	0.003	0.908	0.050	0.907	0.911	0.154	0.353	0.306
G:F																
wk 1	0.85	0.98	0.77	0.88	0.93	0.85	0.89	0.89	0.068	0.786	0.235	0.461	0.412	0.143	0.544	0.797
wk 2	0.66	0.63	0.66	0.67	0.64	0.66	0.64	0.66	0.005	0.738	0.464	0.777	0.417	0.345	0.551	0.539
wk 3	0.74	0.76	0.78	0.74	0.74	0.76	0.73	0.78	0.005	0.827	0.527	0.381	0.849	0.106	0.542	0.141
wk 4	0.62	0.61	0.61	0.66	0.67	0.64	0.65	0.62	0.015	0.487	0.944	0.732	0.446	0.314	0.582	0.647
wk 5	0.64	0.72	0.64	0.63	0.63	0.61	0.67	0.63	0.013	0.341	0.991	0.982	0.150	0.243	0.168	0.328
wk 6	0.61	0.65	0.62	0.66	0.62	0.67	0.67	0.61	0.013	0.942	0.592	0.479	0.890	0.297	0.432	0.249
overall	0.61	0.64	0.64	0.65	0.64	0.64	0.64	0.65	0.001	0.815	0.481	0.247	0.464	0.229	0.635	0.310

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.13. Effect of pre-wean by transport by post-wean on grow-finish performance[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
Weight (kg)																
wk 9	24.41	25.61	25.76	24.33	25.22	25.59	24.74	25.30	1.617	0.862	0.087	0.461	0.487	0.565	0.105	0.025
wk 12	41.48	42.84	43.70	41.01	42.55	44.30	42.27	42.50	3.332	0.205	0.142	0.665	0.124	0.076	0.002	0.111
wk 15	61.16	62.88	63.74	61.70	62.06	63.64	62.44	62.84	5.771	0.784	0.652	0.401	0.382	0.479	0.042	0.235
wk 18	79.61	81.24	82.82	81.52	81.40	82.81	81.53	82.14	8.460	0.417	0.480	0.116	0.356	0.626	0.157	0.373
wk 21	99.5	101.6	102.3	102.3	100.9	102.3	102.5	102.2	13.31	0.551	0.193	0.334	0.491	0.716	0.207	0.784
wk 24	116.1	117.6	120.3	119.7	117.6	119.6	119.0	119.1	17.64	0.677	0.120	0.398	0.170	0.814	0.383	0.972
ADG (kg)[2]																
wk 9	0.71	0.74	0.76	0.74	0.72	0.79	0.68	0.70	0.010	0.407	0.139	0.326	0.054	0.398	0.550	0.931
wk 12	0.81[b]	0.82[b]	0.84[ab]	0.80[b]	0.83[b]	0.89[a]	0.84[ab]	0.81[b]	0.002	0.028	0.279	0.893	0.098	0.073	0.000	0.649
wk 15	0.95	0.96	0.94	0.99	0.94	0.92	0.95	0.96	0.004	0.227	0.241	0.371	0.596	0.255	0.433	0.961
wk 18	0.88	0.89	0.91	0.94	0.93	0.90	0.91	0.92	0.004	0.482	0.048	0.757	0.210	0.392	0.440	0.717
wk 21	0.96	0.99	0.91	0.99	0.93	0.95	0.99	0.96	0.007	0.852	0.552	0.350	0.131	0.159	0.871	0.182
wk 24	0.81	0.78	0.85	0.89	0.84	0.86	0.85	0.88	0.009	0.245	0.070	0.513	0.198	0.636	0.250	0.329
overall	0.85	0.86	0.87	0.89	0.86	0.88	0.87	0.87	0.001	0.686	0.272	0.126	0.137	0.862	0.841	0.305

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.14. Effect of pre-wean by transport by post-wean on grow-finish efficiency[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
ADFI *(kg)*																
wk 9	1.36	1.30	1.39	1.30	1.35	1.36	1.26	1.30	0.018	0.521	0.378	0.408	0.140	0.102	0.967	0.608
wk 12	1.64	1.68	1.65	1.69	1.59	1.75	1.72	1.64	0.020	0.940	0.868	0.161	0.995	0.839	0.088	0.051
wk 15	2.13	2.13	2.25	2.26	2.22	2.23	2.21	2.20	0.016	0.385	0.044	0.939	0.011	0.913	0.721	0.852
wk 18	2.54[b]	2.62[ab]	2.65[ab]	2.72[a]	2.58[ab]	2.70[ab]	2.70[ab]	2.66[ab]	0.024	0.507	0.066	0.098	0.282	0.620	0.239	0.279
wk 21	2.65	2.72	2.67	2.81	2.66	2.73	2.76	2.81	0.048	0.544	0.210	0.093	0.711	0.563	0.636	0.507
wk 24	2.81	2.83	2.87	2.98	2.74	2.86	2.88	2.90	0.054	0.562	0.255	0.176	0.964	0.844	0.550	0.169
overall	2.21	2.25	2.28	2.32	2.22	2.29	2.28	2.29	0.013	0.723	0.159	0.117	0.520	0.956	0.887	0.386
G:F																
wk 9	0.54	0.57	0.56	0.59	0.55	0.60	0.59	0.54	0.012	0.977	0.732	0.404	0.655	0.395	0.488	0.250
wk 12	0.56	0.53	0.55	0.53	0.56	0.54	0.53	0.54	0.003	0.705	0.949	0.299	0.556	0.212	0.667	0.504
wk 15	0.34	0.34	0.34	0.35	0.36	0.33	0.34	0.35	0.001	0.083	0.575	0.545	0.026	0.297	0.206	0.978
wk 18	0.35	0.34	0.34	0.35	0.36	0.33	0.34	0.35	0.001	0.571	0.546	0.376	0.638	0.567	0.091	0.299
wk 21	0.36	0.36	0.34	0.36	0.35	0.35	0.36	0.34	0.001	0.407	0.598	0.710	0.357	0.294	0.788	0.492
wk 24	0.30	0.28	0.30	0.30	0.31	0.31	0.30	0.31	0.002	0.198	0.575	0.797	0.518	0.561	0.627	0.996
overall	0.42	0.42	0.42	0.43	0.43	0.42	0.43	0.42	0.001	0.966	0.783	0.947	0.930	0.562	0.767	0.540

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.15. Effect of pre-wean by transport by post-wean on market performance and efficiency[1]

Pre-wean	Control				Trp				SEM	*P*-value						
Transport	No Transport		Transport		No Transport		Transport									
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
Weight *(kg)*																
market	126.1	126.5	127.1	128.3	130.0	129.8	127.3	130.3	19.97	0.933	0.072	0.340	0.193	0.667	0.873	0.894
ADG *(kg)*																
market	0.80	0.71	0.78	0.82	0.78	0.74	0.70	0.83	0.032	0.592	0.702	0.793	0.599	0.494	0.073	0.800
light block	0.73	0.72	0.74	0.75	0.73	0.74	0.74	0.75	0.001	0.903	0.026	0.259	0.259	0.684	0.684	0.618
heavy block	0.81	0.80	0.83	0.84	0.81	0.82	0.82	0.83	0.001	0.886	0.029	0.420	0.209	0.789	0.624	0.721
ADFI *(kg)*																
market	2.37	2.44	2.44	2.44	2.42	2.42	2.44	2.57	0.138	0.659	0.619	0.521	0.729	0.946	0.691	0.680
light block	2.31	2.39	2.43	2.37	2.38	2.35	2.37	2.61	0.157	0.612	0.376	0.571	0.706	0.618	0.731	0.297
heavy block	2.64	2.64	2.45	2.74	2.56	2.70	2.74	2.38	0.059	0.953	0.731	0.824	0.838	0.287	0.603	0.170
G:F																
market	0.35	0.30	0.32	0.34	0.33	0.31	0.29	0.32	0.005	0.346	0.644	0.639	0.523	0.565	0.565	0.109
light block	0.37	0.36	0.36	0.37	0.37	0.36	0.36	0.36	0.000	0.458	0.974	0.573	0.840	0.827	0.549	0.658
heavy block	0.33	0.33	0.33	0.33	0.33	0.32	0.32	0.33	0.000	0.594	0.911	0.519	0.757	0.818	0.391	0.852

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment

Table 2.16. Overall live scans and carcass characteristics

Prewean diet[a]	Control				Trp											
Transport[b]	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Postwean diet[c]	Con	Trp	Con	Trp	Con	Trp	Con	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
Live Scan Characteristics (mm)																
Back Fat	12.6	12.5	12.0	12.1	13.7	12.7	13.1	13.0	2.50	0.019	0.333	0.427	0.678	0.440	0.489	0.563
Loin Depth	55.7	55.2	55.2	56.6	56.4	56.8	55.2	56.7	9.02	0.368	0.862	0.292	0.387	0.720	0.260	0.776
Loin Eye Area (cm^2)	45.5	45.1	44.8	45.4	45.3	44.5	44.4	46.1	10.95	0.859	0.915	0.714	0.706	0.834	0.240	0.596
Carcass Characteristics[1]																
Lean (%)	54.4	54.5	54.9	54.4	53.6	54.3	54.5	54.3	0.585	0.123	0.202	0.996	0.619	0.336	0.147	0.834
Back Fat (mm)	2.04[ab]	1.97[ab]	1.89[b]	2.06[ab]	2.20[a]	2.07[ab]	1.94[ab]	2.05[ab]	0.030	0.173	0.159	0.716	0.351	0.568	0.033	0.982
Loin Depth (mm)	61.6	60.1	60.7	62.1	60.8	61.6	59.1	61.4	6.743	0.640	0.820	0.399	0.374	0.334	0.190	0.676

[1] spring replicate only.

Table 2.17. Effect of pre-wean by transport by post-wean on winter replicate live scan characteristics[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
BW (kg)																
Market	124.8	130.0	131.2	129.6	128.7	128.8	129.7	132.0	13.15	0.456	0.034	0.203	0.695	0.779	0.327	0.061
Scan Characteristics																
Back Fat (mm)	14.38[a]	12.63[ac]	12.83[abc]	11.91[c]	11.79[c]	13.31[abc]	12.97[abc]	13.9[ab]	1.32	0.888	0.734	0.881	0.009	0.001	0.866	0.342
Loin Depth (mm)	55.41	53.43	53.23	54.49	57.23	54.33	57.30	55.00	4.32	0.009	0.883	0.032	0.486	0.100	0.156	0.326
Loin Eye Area (cm^2)	40.75	41.27	43.73	42.62	44.05	44.06	44.04	44.61	3.59	0.001	0.051	0.995	0.126	0.632	0.657	0.368

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.18. Effect of pre-wean by transport by post-wean on spring replicate live scan characteristics[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
Weight (kg)																
Market	127.13	123.62	128.81	130.22	125.57	128.65	126.61	127.10	25.83	0.774	0.237	0.820	0.182	0.385	0.718	0.252
Scan Characteristics (mm)																
Back Fat	12.31	12.25	13.35	13.80	12.44	12.80	11.61	12.25	2.24	0.179	0.530	0.468	0.045	0.757	0.682	0.904
Loin Depth	56.94[ab]	53.54[b]	57.91[a]	58.00[a]	56.74[ab]	56.99[ab]	57.18[ab]	59.45[a]	4.25	0.139	0.003	0.763	0.338	0.033	0.044	0.578
Loin Eye Area (cm^2)	47.38	46.06	47.48	46.22	47.92	46.36	48.79	48.21	6.34	0.203	0.357	0.150	0.446	0.892	0.746	0.770

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [2]Values with different superscripts within a row are statistically different $P \leq 0.05$. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 2.19. Effect of pre-wean by transport by post-wean on spring replicate carcass characteristics[1]

Pre-wean	Control				Trp											
Transport	No Transport		Transport		No Transport		Transport		SEM	*P*-value						
Post-wean	Control	Trp	Control	Trp	Control	Trp	Control	Trp		Pre-wean[a]	Trans.[b]	Post-wean[c]	a*b	a*c	b*c	a*b*c
Carcass Characteristics																
Lean (%)	54.56	54.74	54.40	53.62	54.51	54.75	54.80	53.97	0.531	0.444	0.067	0.204	0.392	0.980	0.035	0.905
Back Fat (mm)	1.97	2.00	2.02	2.24	1.97	1.94	1.97	2.11	0.029	0.294	0.046	0.093	0.572	0.517	0.105	0.972
Loin Depth (mm)	60.18	62.45	60.60	61.59	59.90	60.81	61.95	60.37	6.867	0.593	0.727	0.441	0.541	0.243	0.264	0.717

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Significant values of $P \leq 0.05$ and trends of $0.05 < P \leq 0.10$ with 95% confidence. [a] pre-wean treatment; [b] transport-treatment; [c] post-wean treatment.

Table 3.1. Phase 1 nursery diet composition

	Phase 1[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	41.56	41.29
SBM, 47.5% CP	13.86	13.86
Soybean oil	5.00	5.00
Limestone	0.971	0.971
MonoCal	0.232	0.232
Vitamin premix [2]	0.25	0.25
Trace mineral premix [3]	0.125	0.125
Selenium premix	0.05	0.05
Phytase[4]	0.10	0.10
Salt	0.13	0.13
Plasma Protein	4.50	4.50
Spray dried blood meal	1.50	1.50
Soy concentrate	4.00	4.00
Fish Meal	3.50	3.50
Dried Whey	23.00	23.00
Lysine-HCL	0.259	0.259
DL-Methionine	0.170	0.170
L-Threonine	0.047	0.047
L-Tryptophan	-	0.270
Neo-OxyTet 100/100[5]	0.375	0.375
Zinc Oxide	0.375	0.375
Calculated Composition		
NE, kcal/kg	2723	2716
CP, %	22.78	22.99
SID Lys, %	1.55	1.55
SID Trp, %	0.25	0.50
Ca, %	0.85	0.85
Total P, %	0.64	0.64
Avail. P, %	0.45	0.85
Chemical Composition		
Lys, %	1.69	1.72
Trp, %	0.30	0.59

1Pigs fed based on a feed budget of 0.9 kg/pig. 2Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B12, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. 3Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg.4Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). 5 Neomycin-oxytetracycline 100/100 g/lb (Phibro Animal Health; Ridgefield Park, NJ) provided at 4.54 mg/kg.

Table 3.2. Phase 2 nursery diet composition

	Phase 2[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	42.04	41.78
SBM, 47.5% CP	17.91	17.91
DDGS - Rens. 7% fat	5.00	5.00
Soybean oil	4.00	4.00
Limestone	1.097	1.097
MonoCal	0.144	0.144
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix	0.05	0.05
Phytase[4]	0.10	0.10
Salt	0.30	0.30
Plasma Protein	2.50	2.50
Spray dried blood meal	1.00	1.00
Soy concentrate	4.00	4.00
Fish Meal	3.50	3.50
Dried Whey	17.00	17.00
Lysine-HCL	0.223	0.223
DL-Methionine	0.125	0.125
L-Threonine	0.043	0.043
L-Tryptophan	-	0.260
Neo-OxyTet 100/100[5]	0.250	0.250
Zinc Oxide	0.350	0.350
Calculated Composition		
NE, kcal/kg	2638	2631
CP, %	23.32	23.52
SID Lys, %	1.45	1.45
SID Trp, %	0.23	0.46
Ca, %	0.85	0.85
Total P, %	0.61	0.61
Avail. P, %	0.40	0.40
Chemical Composition		
Lys, %	1.53	1.45
Trp, %	0.27	0.47

[1]Pigs fed based on a feed budget of 2.2 kg/pig. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12},

0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). [5] Neomycin-oxytetracycline 100/100 g/lb (Phibro Animal Health; Ridgefield Park, NJ) provided at 4.54 mg/kg.

Table 3.3. Phase 3 nursery diet composition

	Phase 3[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	44.58	44.34
SBM, 47.5% CP	29.12	29.12
DDGS - Rens. 7% fat	7.50	7.50
Swine grease	3.00	3.00
Limestone	0.941	0.941
MonoCal	0.305	0.305
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix	0.05	0.05
Phytase[4]	0.10	0.10
Salt	0.53	0.53
Fish Meal	4.00	4.00
Dried Whey	8.60	8.60
Lysine-HCL	0.231	0.231
DL-Methionine	0.083	0.083
L-Threonine	0.058	0.058
L-Tryptophan	-	0.242
Carbadox[5]	0.250	0.250
Zinc Oxide	0.280	0.280
Calculated Composition		
NE, kcal/kg	2527	2521
CP, %	23.43	23.62
SID Lys, %	1.35	1.35
SID Trp, %	0.22	0.44
Ca, %	0.80	0.80
Total P, %	0.63	0.63
Avail. P, %	0.38	0.38
Chemical Composition		
Lys, %	1.47	1.46
Trp, %	0.27	0.43

[1]Pigs fed based on a feed budget of 8.1 kg/pig. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco

Animal Health; Dupont, St. Louis, MO). [5] Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 3.4. Phase 4 nursery diet composition

	Phase 4[1]	
Item	1X	2X
Ingredient, % as fed		
Corn	51.16	50.93
SBM, 47.5% CP	31.97	31.97
DDGS - Rens. 7% fat	10.00	10.00
Swine grease	3.00	3.00
Limestone	1.151	1.151
MonoCal	0.775	0.775
Vitamin premix[2]	0.25	0.25
Trace mineral premix[3]	0.125	0.125
Selenium premix	0.05	0.05
Phytase[4]	0.10	0.10
Salt	0.56	0.56
Lysine-HCL	0.290	0.290
DL-Methionine	0.099	0.099
L-Threonine	0.116	0.116
L-Tryptophan	-	0.229
Carbadox[6]	0.250	0.250
Copper sulphate	0.100	0.100
Calculated Composition		
NE, kcal/kg	2482	2476
CP, %	22.59	22.77
SID Lys, %	1.25	1.25
SID Trp, %	0.21	0.42
Ca, %	0.70	0.70
Total P, %	0.60	0.60
Avail. P, %	0.33	0.33
Chemical Composition		
Lys, %	1.38	1.40
Trp, %	0.25	0.45

[1]Pigs fed *ad libitum* to d 35. [2]Vitamin premix provided per kilogram (kg) of diet: vitamin A, 5,512 IU; vitamin D_3, 551 IU; vitamin E, 37 IU; vitamin K, 1.8 mg; vitamin B_{12}, 0.03 µg; riboflavin, 7.5 mg; pantothenic acid, 18 mg; niacin, 27.5 mg. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.0 mg; copper, 11.3 mg; iodine, 0.46 mg. [4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). [5] Neomycin-oxytetracycline 100/100 g/lb (Phibro Animal

Health; Ridgefield Park, NJ) provided at 4.54 mg/kg. [6] Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 3.5. Grow-finish diet composition

	Diets					
Item	GF1	GF2	GF3	GF4	GF5	GF6
Ingredient, % as fed						
Corn	51.55	53.05	60.07	64.17	71.39	73.55
SBM, 47% CP	28.50	23.30	16.60	12.50	10.60	8.55
DDGS - Rens.	15.00	20.00	20.00	20.00	15.00	15.00
Swine Grease	2.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.42	1.40	1.34	1.26	1.14	1.17
MonoCal Phos.	0.24	0.08	0.00	0.00	0.03	0.00
Vitamin Prx[1]	0.20	0.15	0.15	0.15	0.13	0.10
Trace Mineral. Prx[2]	0.10	0.09	0.08	0.07	0.05	0.05
Selenium Prx[3]	0.05	0.05	0.05	0.05	0.03	0.03
Phytase[4]	0.10	0.10	0.10	0.10	0.05	0.05
Salt	0.35	0.35	0.30	0.30	0.25	0.25
Lysine-HCL	0.22	0.20	0.20	0.20	0.15	0.15
DL-Methionine	0.03	-	-	-	-	-
L-Threonine	0.03	-	-	-	-	-
L-Tryptophan	-	-	-	-	-	-
Lincomycin[5]	0.10	0.10	0.04	-	-	-
Calculated Composition						
ME, Kcal/kg	3439.9	3335.5	3348.6	3344.5	3358.5	3372.0
NE, Kcal/kg	2467.0	2468.6	2508.8	2509.4	2547.3	2594.0
CP, %	22.28	18.46	16.10	16.71	15.03	12.04
SID Lys, %	1.10	0.975	0.80	0.701	0.60	0.551
Ca, %	0.70	0.65	0.60	0.55	0.50	0.50
Phos, %	0.51	0.47	0.45	0.41	0.39	0.36
Av.P, %	0.32	0.30	0.28	0.26	0.24	0.20

[1]Pigs fed *ad libitum*; dietary phase chances occurred every 3 wk. [2]GF1 vitamin premix provided per kilogram (kg) of diet: vitamin A, 5450.9 IU; vitamin D_3, 2180.4 IU; vitamin E, 29.1 IU; vitamin K, 4.4 mg; vitamin B_2, 0.04 µg; riboflavin, 10.9 mg; pantothenic acid, 36.3 mg; niacin, 36.3. GF2-4 vitamin premix provided per kilogram (kg) of diet: vitamin A, 5039.1 IU; vitamin D_3, 2015.6 IU; vitamin E, 26.9 IU; vitamin K, 4.0 mg; vitamin B_2, 0.04 µg; riboflavin, 10.1 mg; pantothenic acid, 33.6 mg; niacin, 60.5 mg. GF5 vitamin premix provided per kilogram (kg) of diet: vitamin A, 3633.9 IU; vitamin D_3, 1453.6 IU; vitamin E, 19.4 IU; vitamin K, 2.9 mg; vitamin B_2, 0.03 µg; riboflavin, 7.3 mg; pantothenic acid, 24.2 mg; niacin, 43.6. GF6 vitamin premix provided per kilogram (kg) of diet: vitamin A, 2645.5 IU; vitamin D_3, 254.6 IU; vitamin E, 17.6 IU; vitamin K, 0.9 mg; vitamin B_{12}, 0.02 µg; riboflavin, 3.5 mg; pantothenic acid, 8.8 mg; niacin, 13.2. [3]Trace mineral (TM) premix provided per kilogram (kg) of diet: 50 mg Fe; 50 mg Zn; 6.2 mg Mn; 4.66 mg Cu; 0.19 mg I. [4]Selenium Premix provided at 0.3 mg per kilogram

(kg) of diet. [5]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health; Dupont, St. Louis, MO). [6]Lyconmycin (Lincomix, Zoetis, Parsippany, NJ) provided at 110,000ppm.

Table 3.6. Analyzed and calculated tryptophan content of nursery diets

	Diet, Phase 1			
	Control		2X SID Trp	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated
Tryptophan	0.30	0.37	0.59	0.54
	Diet, Phase 2			
	Control		2X SID Trp	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated
Tryptophan	0.27	0.26	0.47	0.52
	Diet, Phase 3			
	Control		2X SID Trp	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated
Tryptophan	0.27	0.24	0.43	0.48
	Diet, Phase 4			
	Control		2X SID Trp	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated
Tryptophan	0.25	0.23	0.45	0.46

Table 3.7. Analyzed amino acid composition of basal diets for phases 1-4[1]

Amino Acid, W/W%[2]	Dietary Phase 1	2	3	4
Taurine §	0.18	0.18	0.18	0.15
Hydroxyproline	0.10	0.13	0.10	0.00
Aspartic Acid	2.33	2.21	2.27	2.05
Threonine	1.01	0.93	0.93	0.86
Serine	0.95	0.92	0.87	0.81
Glutamic Acid	3.75	3.77	4.01	3.78
Proline	1.20	1.22	1.21	1.17
Lanthionine §	0.02	0.02	0.02	0.00
Glycine	0.99	0.97	1.02	0.86
Alanine	1.24	1.22	1.19	1.07
Cysteine	0.40	0.38	0.35	0.32
Valine	1.31	1.21	1.18	1.07
Methionine	0.51	0.47	0.45	0.40
Isoleucine	1.00	1.01	1.08	0.99
Leucine	2.13	2.07	1.99	1.86
Tyrosine	0.70	0.71	0.70	0.64
Phenylalanine	1.15	1.11	1.11	1.04
Hydroxylysine	0.02	0.02	0.02	0.02
Ornithine §	0.01	0.02	0.02	0.02
Lysine	1.69	1.53	1.47	1.38
Histidine	0.69	0.63	0.60	0.56
Arginine	1.33	1.31	1.43	1.29
Tryptophan	0.30	0.27	0.27	0.25
Total	23.01	22.31	22.47	20.79
Crude protein*	23.36	22.82	22.05	20.50

[1]Chemical analysis conducted by University of Missouri Experiment Station Laboratory (Columbia, MO). [2]W/W%= grams per 100 grams of sample. § Non-proteinogenic amino acids. *Crude protein= %N x6.25. Results are expressed on an “as is” basis unless otherwise indicated.

Table 3.8. Analyzed amino acid composition of 2X SID Trp diets for phases 1-4[1]

	Dietary Phase			
Amino Acid, W/W%[2]	1	2	3	4
Taurine §	0.18	0.18	0.18	0.17
Hydroxyproline	0.06	0.10	0.12	0.01
Aspartic Acid	2.37	2.08	2.20	2.07
Threonine	0.97	0.87	0.89	0.86
Serine	0.90	0.80	0.81	0.78
Glutamic Acid	3.91	3.59	3.89	3.80
Proline	1.20	1.15	1.22	1.19
Lanthionine §	0.00	0.00	0.00	0.00
Glycine	1.00	0.93	1.01	0.87
Alanine	1.24	1.16	1.19	1.09
Cysteine	0.42	0.35	0.33	0.33
Valine	1.35	1.18	1.16	1.09
Methionine	0.48	0.41	0.44	0.39
Isoleucine	1.07	0.98	1.07	1.00
Leucine	2.15	1.98	1.97	1.91
Tyrosine	0.71	0.65	0.70	0.66
Phenylalanine	1.17	1.05	1.08	1.05
Hydroxylysine	0.02	0.02	0.03	0.02
Ornithine §	0.02	0.02	0.02	0.02
Lysine	1.72	1.45	1.46	1.40
Histidine	0.69	0.60	0.58	0.57
Arginine	1.36	1.23	1.37	1.31
Tryptophan	0.59	0.47	0.43	0.41
Total	23.58	21.25	22.15	20.84
Crude protein*	23.39	22.50	23.48	21.78

[1]Chemical analysis conducted by University of Missouri Experiment Station Laboratory (Columbia, MO). [2]W/W%= grams per 100 grams of sample. § Non-proteinogenic amino acids. *Crude protein= %N x6.25. Results are expressed on an "as is" basis unless otherwise indicated.

Table 3.9. Average daily tryptophan consumption[1]

		Control		2X SID Trp	
	NRC (2012)	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
g/day					
wk 1	0.7	0.53	0.48	1.08	1.08
wk 2	1.08	0.80	0.77	1.87	1.81
wk 3	1.91	1.09	1.02	2.56	2.42
wk 4	1.91	1.23	1.42	3.04	3.08
wk 5	1.91	1.67	1.63	3.73	3.59
g/kg					
wk 1	3.33	3.09	2.97	6.33	5.98
wk 2	3.24	3.19	2.98	6.91	6.98
wk 3	3.26	3.03	2.93	6.93	6.54
wk 4	3.26	2.74	3.31	6.33	6.85
wk 5	3.26	3.10	3.33	6.90	6.90

[1]Averages calculated based on individual feed intake per 7 d.

[2] Average Trp consumption (g/d) determined by the National Research Council

Table 3.10. Effect of diet by vaccine on growth performance of nursery pigs[1]

Diet (DT)	Control		2X SID Trp		SEM	*P*-value		
Vaccine (VC)[2]	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated		DT	VC	DT x VC
Weight (kg)								
wk 0[3]	5.58	5.64	5.66	5.73	-	-	-	-
wk 1	6.91	6.91	7.02	7.15	0.031	0.2637	0.5537	0.2391
wk 2	8.67	8.71	8.97	8.98	0.081	0.0240	0.6299	0.8960
wk 3	11.22	11.14	11.57	11.56	0.241	0.0476	0.4567	0.8089
wk 4	14.35	14.18	14.95	14.75	0.360	0.0121	0.1432	0.9283
wk 5	17.92	17.69	18.83	18.42	0.693	0.0079	0.1096	0.6895
ADG (kg)								
wk 1	0.17	0.16	0.17	0.18	0.000	0.4207	0.9249	0.2276
wk 2	0.25	0.26	0.27	0.26	0.001	0.0656	0.9603	0.2304
wk 3	0.36	0.35	0.37	0.37	0.003	0.3419	0.6384	0.9755
wk 4	0.45	0.43	0.48	0.45	0.002	0.0489	0.1771	0.5515
wk 5	0.54	0.49	0.54	0.52	0.002	0.2143	0.0164	0.4661
overall	0.36	0.34	0.37	0.36	0.001	0.0175	0.0737	0.9840

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Results considered statistically significant at *P*-values of $P<0.05$ and a tendency at $0.05 < P \leq 0.10$ with 95% confidence. [2]Vaccine challenged pigs were challenged with circovirus (Ingelvac CircoFLEX®; Boehringer Ingelheim, Ingelheim am Rhein, Germany), mycoplasma (Respisure-One®; Zoetis Parsippany, NJ), and influenza (Flusure XP®; Zoetis Parsippany, NJ) administered intramuscularly in the neck during wk 2, 3, and 4 at a dose of 2 ml/pig/week, respectively. [3]d 0 represents pre-weaning body weights measured at d 20 and was used as a covariant.

Table 3.11. Effects of diet by vaccine on nursery efficiency[1]

Diet (DT)	Control		2X SID Trp		SEM	*P*-value		
Vaccine (VC)[2]	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated		DT	VC	DT x VC
ADFI (kg)								
wk 1	0.21	0.19	0.20	0.20	0.001	0.7507	0.3021	0.3550
wk 2	0.34	0.33	0.37	0.35	0.001	0.0563	0.2887	0.6063
wk 3	0.50	0.47	0.53	0.52	0.008	0.0947	0.2676	0.6899
wk 4	0.57	0.62	0.63	0.63	0.009	0.2188	0.3873	0.3920
wk 5	0.79	0.78	0.81	0.78	0.006	0.7753	0.3883	0.7757
overall	0.48	0.48	0.51	0.50	0.002	0.0946	0.5152	0.7171
G:F								
wk 1	0.84	0.89	0.84	0.88	0.016	0.7417	0.2156	0.8585
wk 2	0.72	0.76	0.74	0.75	0.004	0.7728	0.1742	0.3183
wk 3	0.75	0.75	0.71	0.72	0.023	0.4537	0. 8904	0.4382
wk 4	0.84	0.71	0.77	0.73	0.026	0.6197	0.0886	0.2779
wk 5	0.68	0.63	0.68	0.67	0.002	0.1628	0.0201	0.1281
overall	0.74	0.72	0.73	0.73	0.002	0.8579	0.3582	0.5380

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Results considered statistically significant at *P*-values of $P<0.05$ and a tendency at $0.05 < P \leq 0.10$ with 95% confidence. [2]Vaccine challenged pigs were challenged with circovirus (Ingelvac CircoFLEX®; Boehringer Ingelheim, Ingelheim am Rhein, Germany), mycoplasma (Respisure-One®; Zoetis Parsippany, NJ), and influenza (Flusure XP®; Zoetis Parsippany, NJ) administered intramuscularly in the neck during wk 2, 3, and 4 at a dose of 2 ml/pig/week, respectively.

Table 3.12. Effects of diet by vaccine on market performance, live scan, and carcass characteristics[1]

Diet	Control		2X SID Trp		SEM	*P*-value		
Vaccine challenge[2]	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated		DT	VC	DT x VC
Weight (kg)[3]								
Market	117.06	119.35	122.03	120.72	12.708	0.044	0.756	0.252
Carcass	92.54^{a}	94.63^{ab}	96.63^{b}	94.21^{ab}	0.831	0.046	0.813	0.025
Loin Characteristics (mm)								
Back Fat	11.60	11.57	11.67	11.82	2.123	0.884	0.956	0.932
Total Loin Depth	61.19	61.23	61.87	62.34	0.409	0.118	0.601	0.663
Loin Depth	50.19	49.67	50.21	50.52	3.801	0.766	0.944	0.777
Carcass Quality[3]								
Lean, *%*	0.56^{ab}	0.56^{ab}	0.55^{a}	0.56^{b}	0.000	0.913	0.151	0.053
Fat *(mm)*	18.29^{a}	18.80^{ab}	19.30^{b}	18.54^{a}	0.000	0.074	0.461	0.019
Total Loin Depth *(mm)*	64.38	65.05	64.61	67.34	0.002	0.185	0.096	0.262

[1]Values are expressed as least square means with pooled standard error of mean (SEM). Results considered statistically significant at *P*-values of $P<0.05$ and a tendency at $0.05 < P \leq 0.10$ with 95% confidence. [2]Vaccine challenged pigs were challenged with circovirus (Ingelvac CircoFLEX®; Boehringer Ingelheim, Ingelheim am Rhein, Germany), mycoplasma (Respisure-One®; Zoetis Parsippany, NJ), and influenza (Flusure XP®; Zoetis Parsippany, NJ) administered intramuscularly in the neck during wk 2, 3, and 4 at a dose of 2 ml/pig/week, respectively. [3]Values with different superscripts within a row are statistically different $P<0.05$;

Discussion

Objective 1:

Early life stressors, like weaning and transport, cause physiological stress on the developing gastrointestinal tract of pigs, which manifests as perturbations to growth performance and feed efficiency and increased concentrations of circulating cortisol and norepinephrine (Moeser et al., 2017). Therefore, the objective of the present study was to eliminate or reduce short- and long-term, transport-induced reductions in feed efficiency and growth by providing supplemental Tryptophan (Trp) above NRC (2012) pre- and/or post-weaning.

Pre-wean supplementation of Trp during the lactation period had no effect on ADG in piglets. We believe this may have been caused by daily handling and dosing stress. Previous research reports a direct relationship between piglet age and stress management on performance, where piglets handled during the lactation period were lighter at weaning and in the nursery period compared to unhandled piglets (De Oliveira et al., 2015). While all pigs were handled daily prior to weaning, the concentration of supplemented Trp may not have been adequate to account for the imposed handling stress to report a measurable difference between treatments. However, further research is required to determine if different concentrations of Trp mitigate fluctuating cortisol concentrations resultant from imposed stressors.

In the present study, transported pigs allotted to pre- and post-wean Trp treatments were heavier throughout the study. We observed 3-way interactions of pre-wean x transport x post-wean treatment on weekly ADG and overall body weight. Post-wean supplemented SID Trp reduced ADFI (wk 1) in pigs not supplemented with pre-wean SID Trp but had no effect in post-wean supplemented pigs that also received pre-wean SID Trp supplementation. We hypothesize supplementing Trp while the gastrointestinal tract was developing provided additional available SID Trp for protein synthesis and upregulation of tight junction proteins (H. Wang et al., 2015).

These results highlight the attenuative effects supplemented SID Trp has on the impact of weaning and transport on growth performance and feed efficiency. While limited research is available pertaining to the effects of pre-wean SID Trp supplementation on piglet performance, other authors (Y.B. Shen et al., 2012; Rodrigues et al., 2022) have noted dietary SID Trp mediated improvements in growth performance and feed efficiency during the nursery period. We believe this could be due to pre-wean SID Trp supplementation increasing amino acid transporter production, via mTOR activation, in younger pigs due to higher concentrations of available SID Trp in plasma (H. Wang et al., 2015). Other studies have shown supplementing dietary Trp (0.2-0.4%) activated mTOR signaling and increased the abundance of zonula occludins -1, -3, and claudin-1 in the small intestine (Tossou et al., 2016; Liang et al., 2018). Pigs supplemented with Trp post-wean had improved ADFI when also provided pre-wean Trp,

suggesting pre-wean supplementation of Trp stimulated appetite in pigs following a transport-induced stress event.

We observed carry over effects of pre- and or/post-weaning supplemented SID Trp during the grow finish period and for market characteristics. Feeding 2X SID Trp during the nursery period improved subsequent grow-finish body weight and ADG when pigs were transported and provided supplemental SID Trp prior to and following weaning. However, transported pigs that were not provided pre-wean Trp had reduced body weight. We also observed that feeding 2X NRC (2012) SID Trp during the nursery phase improves body weight in non-transported grower pigs. Other authors have found similar results, reporting 50- 80 kg pigs achieved optimum performance efficiency when supplemented dietary SID Trp was increased from 0.15 to 0.225 SID Trp:Lys and from 0.184 to 0.201 SID Trp:Lys in 80-110 kg pigs (Liu et al., 2019).

Post-wean pigs supplemented 2X SID Trp were heavier at market, subsequently increasing carcass weight. The increased carcass weight was due to increased back fat, causing deeper loins, in pigs supplemented with SID Trp pre- and post-wean. However, there were no differences between treatments for overall loin depth or loin eye area. Past research reinforces our results; Page et al. (1993) reported increasing SID Trp by 0.12%-units relative to SID Lys to 55 kg pigs increased fat thickness at the 10^{th} rib and reduced loin eye area but had no effect on percent lean (Page et al., 1993). Leading us to conclude, increasing SID Trp in the diet increased fat deposition at market resulting in heavier market and hot carcass weights.

Delivery of SID Trp to the brain is dependent on the concentration of plasma Trp and the ratio of Trp to other large neutral amino acids in blood (LNAA) (Koopmans et al., 2012). By increasing dietary SID Trp we hypothesize increased concentrations of plasma Trp will outcompete other LNAA at the blood brain barrier, therefore increasing available Trp for serotonin production. Research reports increasing plasma Trp:LNAA concentrations by 3.9-fold in pigs (50 kg) when Trp was supplemented in the diet at 7 g/kg (Koopmans et al., 2005). Further, feeding diets with a 3.9-fold increased Trp:LNAA reduced long-term post-stress cortisol concentrations but had no effect on plasma norepinephrine (Koopmans et al., 2005)

Objective 2:

Performance data shows a consistent increase in body weight, resulting from higher average daily gain, in nursery pigs provided 2X Trp in the diet. Pigs fed a diet with 2X the NRC (2012) recommend concentrations of Trp came onto feed following weaning faster, resulting in a reduced fasting period common post weaning. These results highlight the impact of weaning and transport stress on growth performance. Other authors (Henry et al., 1996; Y.B. Shen et al., 2012; Shen et al., 2015) have noted similar results of improved performance and feed efficiency

following weaning. We believe this is because Tryptophan is a precursor for a wide array of metabolites associated with protein synthesis, stress attenuation, and appetite (Keszthelyi et al., 2009).

Tryptophan is the fourth limiting amino acid in typical swine diets. Swine diets, predominantly composed of corn, contain low available Trp content. In this study, corn inclusion was reduced while the concentration of L-Trp increased in the diet. As corn was reduced, a greater proportion of free Trp was available to pigs. We suspect that the improvement in pig performance was the result of the increase in free available Trp. Tryptophan is a large neutral amino acid (LNAA) which is absorbed via the B^0AT1 transporter on the apical membrane of enterocytes. The B^0AT1 is a competitive transporter signifying Trp competes with other LNAA (valine, leucine, isoleucine, tyrosine, and phenylalanine) for absorption (Keszthelyi et al., 2009). Excluding lysine, Trp has the lowest affinity for the transport system limiting its rate of uptake. Considering transporter competition and limited Trp content in early-phase nursery diets, we believe recently weaned pigs are not receiving adequate concentrations of Trp.

The analyzed values of Trp chiefly corresponded with calculated values. The analyzed values of supplemented diets containing 2X Trp were consistently double the control diets, following a step wise reduction in Trp across phases 1 to 4. The observed differences in analyzed versus calculated Trp values were minimal, indicating that the additional Trp in the diet was successfully integrated during batch creation. At the culmination of a 35-d nursery period, Trp supplementation increased daily Trp (g) consumption by 1.96 g in vaccinated pigs compared to pigs fed 1X NRC (2012) SID Trp, however vaccination reduced SID Trp consumption by 0.19 g/d in pigs fed 2X SID Trp compared to unvaccinated pigs provided SID Trp.

Weaning induces deleterious metabolic and immunological responses in piglets which directly affects intestinal barrier function (McLamb et al., 2013). Increased gut permeability introduces the naïve immune system to harmful microbes and feed-associated antigens causing inflammation and leaky gut which manifests as diarrhea and reduced performance. Microbial induction of immune responses leads to increased expression of pro-inflammatory cytokines like TNF- α and IL-1β. With the knowledge that proinflammatory cytokines mediate immune and metabolic processes, we believe that inducing transportation and immune stressors on weaned pigs reduced amino acid oxidation and protein synthesis. This conclusion was supported by our results where vaccine challenged pigs fed a standard nursery diet were the lightest at the end of the nursery period. However further analysis of plasma cytokine concentrations is necessary to determine the effects of a vaccine-induced immune challenge on immune response and pig performance.

Intestinal and immune homeostasis can be maintained under stress conditions with the addition of Trp. Tryptophan metabolism produces endogenous and microbial aryl hydrocarbon receptor

(AhR) ligands, a natural-ligand activated immunosuppressive transcription factor which regulates gene expression (Stockinger et al., 2011; Munn and Mellor, 2013). AhR regulates intestinal homeostasis via Trp-AhR activation pathway improving intestinal mucosal barrier function (Sun et al., 2019) and participating in immune responses (Qiu et al., 2013). Trp-AhR activation induces production TGF-β1, IL-6, and IL- 23; these cytokines promote differentiation of Th17 cells increasing IL-22 secretion to alleviate intestinal inflammation (Sun et al., 2019).

We believe that the increase in consumed Trp increased the Trp-AhR activation pathway subsequently improving innate and adaptive immune responses following a vaccine challenge. The result from this study supports this conclusion. We found that increased Trp at 2X NRC (2012) recommended concentrations led to heavier pigs at the end of the nursery period regardless of vaccine-challenge. Notably, Trp supplemented pigs undergoing a vaccine-indued immune challenge were consistently heavier than vaccinated pigs provided 1X Trp. We also saw an overall improved average daily gain in supplemented pigs. However, we did not observe a change in feed intake or efficiency between treatments.

In conclusion, feeding 2X Trp to pigs undergoing transportation and undergoing a secondary stress event, in the form of an immune challenge, has a positive effect on growth performance of pigs during the nursery period regardless of immune challenge. Similar results on Trp supplementation on growth performance (Shen et al., 2015) and immune tolerance (Mándi and Vécsei, 2012) have been reported. Following 2X Trp supplemented pigs to market showed increased Trp during the nursery period improved hot carcass weights.

Literature Cited

Adeola, O., and R. O. Ball. 1992. Hypothalamic neurotransmitter concentrations and meat quality in stressed pigs offered excess dietary tryptophan and tyrosine. J Anim Sci. 70:1888–1894. doi:10.2527/1992.7061888x. Available from: https://academic.oup.com/jas/article/70/6/1888-1894/4705289

Bamberger, C. M., H. M. Schulte, and G. P. Chrousos. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev. 17:245–261. doi:10.1210/EDRV-17-3-245. Available from: https://pubmed.ncbi.nlm.nih.gov/8771358/

Bao, E., K. R. Sultan, B. Nowak, and J. Hartung. 2008. Expression and distribution of heat shock proteins in the heart of transported pigs. Cell Stress Chaperones. 13:459–466. doi:10.1007/s12192-008-0042-4.

Bayir, H. 2005. Reactive oxygen species. Crit Care Med. 33. doi:10.1097/01.CCM.0000186787.64500.12. Available from: https://journals.lww.com/ccmjournal/Fulltext/2005/12001/Reactive_oxygen_species.31.aspx

Blanchard, E. B., L. Keefer, J. M. Lackner, T. E. Galovski, S. Krasner, and M. A. Sykes. 2004. The role of childhood abuse in Axis I and Axis II psychiatric disorders and medical disorders of unknown origin among irritable bowel syndrome patients. J Psychosom Res. 56:431–436. doi:10.1016/S0022-3999(03)00627-5. Available from: https://pubmed.ncbi.nlm.nih.gov/15094028/

Blikslager, A. T., A. J. Moeser, J. L. Gookin, S. L. Jones, and J. Odle. 2007. Restoration of Barrier Function in Injured Intestinal Mucosa. https://doi.org/10.1152/physrev.00012.2006. 87:545–564. doi:10.1152/PHYSREV.00012.2006. Available from: https://pubmed.ncbi.nlm.nih.gov/17429041/

Brooks, P., C. Moran, J. Beal, V. Demechova, and A. Campbell. 2001. Liquid feeding for the young piglet. In: Proceedings of a British Society of Animal Science Occasional Meeting. CABI Publishing. p. 153–178. Available from: https://www.cabdirect.org/cabdirect/abstract/20013155806

CADENAS, E. 1985. Oxidative Stress and Formation of Excited Species. PERGAMON PRESS INC. Available from: http://dx.doi.org/10.1016/B978-0-12-642760-8.50017-X

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. J Anim Sci Biotechnol 2013 41. 4:1–4. doi:10.1186/2049-1891-4-19. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-4-19

Chen, J. Y., C. M. Wu, C. M. Liao, K. C. Chen, C. C. You, Y. W. Wang, C. Huang, and M. S. Chien. 2019. The impact of porcine circovirus associated diseases on live attenuated classical swine fever vaccine in field farm applications. Vaccine. 37:6535–6542. doi:10.1016/J.VACCINE.2019.08.039.

Correa, J. A., S. Torrey, N. Devillers, J. P. Laforest, H. W. Gonyou, and L. Faucitano. 2010. Effects of different moving devices at loading on stress response and meat quality in pigs. J Anim Sci. 88:4086–4093. doi:10.2527/jas.2010-2833.

dalla Costa, O. A., L. Faucitano, A. Coldebella, J. V. Ludke, J. V. Peloso, D. dalla Roza, and M. J. R. Paranhos da Costa. 2007. Effects of the season of the year, truck type and location on truck on skin bruises and meat quality in pigs. Livest Sci. 107:29–36. doi:10.1016/j.livsci.2006.08.015.

Dallman, M., and D. Hellhammer. 2017. Regulation of the Hypothalamo-Pituitary-Adrenal Axis, Chronic Stress and Energy: The Role of Brain Networks. In: The Handbook of Stress Science: Biology, Psychology, and Health . Springer. p. 11–36.

De Groot, J., M. A. W. Ruis, J. W. Scholten, J. M. Koolhaas, and W. J. A. Boersma. 2001. Long-term effects of social stress on antiviral immunity in pigs. 73:145–158. doi:10.1016/S0031-9384(01)00472-3.

De Oliveira, D., M. J. R. Paranhos Da Costa, M. Zupan, T. Rehn, and L. J. Keeling. 2015. Early human handling in non-weaned piglets: Effects on behaviour and body weight. Appl Anim Behav Sci. 164:56–63. doi:10.1016/j.applanim.2015.01.002. Available from: http://dx.doi.org/10.1016/j.applanim.2015.01.002

de Ridder, K., C. L. Levesque, J. K. Htoo, and C. F. M. M. de Lange. 2012. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. J Anim Sci. 90:3485–3491. doi:10.2527/jas.2011-4830. Available from: https://academic.oup.com/jas/article/90/10/3485/4717878

Deakin, J. F., and F. G. Graeff. 1991. 5-HT and mechanisms of defence. J Psychopharmacol. 5:305–15. doi:10.1177/026988119100500414. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22282829

Djordjević, V. B. 2004. Free radicals in cell biology. Int Rev Cytol. 237:57–89. doi:10.1016/S0074-7696(04)37002-6.

Dröge, W. 2002. Free radicals in the physiological control of cell function. Physiol Rev. 82:47–95. doi:10.1152/PHYSREV.00018.2001. Available from: https://pubmed.ncbi.nlm.nih.gov/11773609/

Edwards, L. N., T. Grandin, T. E. Engle, S. P. Porter, M. J. Ritter, A. A. Sosnicki, and D. B. Anderson. Use of exsanguination blood lactate to assess the quality of pre-slaughter pig handling. doi:10.1016/j.meatsci.2010.05.022.

Ekkel, E. D., B. Savenije, W. G. P. Schouten, V. M. Wiegant, and M. J. M. Tielen. 1997. The effects of mixing on behavior and circadian parameters of salivary cortisol in pigs. Physiol Behav. 62:181–184. doi:10.1016/S0031-9384(97)00037-1. Available from: https://www.sciencedirect.com/science/article/pii/S0031938497000371?casa_token=aDLmvoBNpjcAAAAA:VtX-Vs5zv8U7mxOaKuqabfR3wAu5IeccqYkix_SnPbVaautuBlS3Ke3NFjmHqcHvjH6hUFWP_w

Gao, J., K. Xu, H. Liu, G. Liu, M. Bai, C. Peng, T. Li, and Y. Yin. 2018. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front Cell Infect Microbiol. 8. doi:10.3389/fcimb.2018.00013.

Geor, R. J., M. Coenen, and P. A. Harris. 2013. Equine Applied and Clinical Nutrition : Health, Welfare and Performance. Saunders [Imprint]. Available from: https://books.google.com/books?hl=en&lr=&id=rlBfYgLiqtwC&oi=fnd&pg=PA304&dq=NRC+2006+donkey+nutrition&ots=ShgLOCLP0l&sig=5AsR5p8vvz4S-L2Z45_i_gmtz84#v=onepage&q=NRC 2006 donkey nutrition&f=false

Guide for the Care and Use of Agricultural Animals in Research and Teaching. 2020. 4th ed. Available from: www.adsa.org

Harris, D. L. (“Hank”). 2000. Multi-Site Pig Production. John Wiley and Sons. Available from: https://books.google.com/books?hl=en&lr=&id=xEXw23repU4C&oi=fnd&pg=PR7&ots=O2Rl5hrle6&sig=oX-oSvEGif2xI-8jAtlAZDg445I#v=onepage&q&f=false

Henry, Y., B. Sè ve, A. Mounier, and P. Ganier. 1996. Growth Performance and Brain Neurotransmitters in Pigs as Affected by Tryptophan, Protein, and Sex 1. J Anim Sci. 74:2700–2710. Available from: https://academic.oup.com/jas/article/74/11/2700/4624826

Indiana Packers Corporation. 2015. Delphi.

Jan Koopmans, S., M. Ruis, R. Dekker, H. van Diepen, M. Korte, and Z. Mroz. 2005. Surplus dietary tryptophan reduces plasma cortisol and noradrenaline concentrations and enhances recovery after social stress in pigs. Physiol Behav. 469–478. doi:10.1016/j.physbeh.2005.05.010.

Jensen, P., and J. Yngvesson. 1998. Aggression between unacquainted pigs - Sequential assessment and effects of familiarity and weight. Appl Anim Behav Sci. 58:49–61. doi:10.1016/S0168-1591(97)00097-X. Available from:

https://www.sciencedirect.com/science/article/pii/S016815919700097X?casa_token=ZbUn0_qY8_YAAAAA:Mg8vxdEq8wQbzBGgmxRFylQhrEME9jrD_oBEmx8aJWVfnbuxXwDsSQQaTspnKReXUjLWHmNI8A

Keszthelyi, D., F. J. Troost, and A. A. M. Masclee. 2009. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. Neurogastroenterol Motil. 21:1239–1249. doi:10.1111/J.1365-2982.2009.01370.X. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2982.2009.01370.x

Kitikoon, P., D. Nilubol, B. J. Erickson, B. H. Janke, T. C. Hoover, S. A. Sornsen, and E. L. Thacker. 2006. The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination. Vet Immunol Immunopathol. 112:117–128. doi:10.1016/J.VETIMM.2006.02.008.

Koopman, N., D. Katsavelis, A. S. Ten Hove, S. Brul, W. J. De Jonge, J. Seppen, and K. Nonogaki. 2021. The Multifaceted Role of Serotonin in Intestinal Homeostasis. Mol Sci. 22. doi:10.3390/ijms22179487. Available from: https://doi.org/10.3390/ijms22179487

Koopmans, S. J., A. C. Guzik, J. Van Der Meulen, R. Dekker, J. Kogut, B. J. Kerr, and L. L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets 1,2. Available from: https://academic.oup.com/jas/article/84/4/963/4804233

Koopmans, S. J., F. J. van der Staay, N. Le Floc, R. Dekker, J. M. Th van Diepen, and A. J. M Jansman. 2012. Effects of surplus dietary l-tryptophan on stress, immunology, behavior, and nitrogen retention in endotoxemic pigs 1. J Anim Sci. 90:241–251. doi:10.2527/jas.2010-3372. Available from: https://academic.oup.com/jas/article/90/1/241/4764560

Lauridsen, C., and S. K. Jensen. 2005. Nonruminant Nutrition: Oxidative Stress and the Use of Antioxidants for Nonruminant Animals.

Lewis, N. J., and R. J. Berry. 2006. Effects of season on the behaviour of early-weaned piglets during and immediately following transport. Appl Anim Behav Sci. 100:182–192. doi:10.1016/j.applanim.2005.12.006. Available from: https://www.sciencedirect.com/science/article/pii/S0168159105004223?casa_token=5ZiOx_eRykgAAAAA:Hy4I6Ytd7OxvC2Qy7mqpVLJfhbr4MNfvIhv0XZSoilXAzrUOO_-m1avik_4NDJoQNhlYSUvHdQ

Liang, H., Z. Dai, J. Kou, K. Sun, J. Chen, Y. Yang, G. Wu, and Z. Wu. 2018. Dietary l-Tryptophan Supplementation Enhances the Intestinal Mucosal Barrier Function in Weaned

Piglets: Implication of Tryptophan-Metabolizing Microbiota. Int J Mol Sci. 20:20. doi:10.3390/ijms20010020. Available from: https://www.mdpi.com/1422-0067/20/1/20/htm

Liu, J. B., H. L. Yan, S. C. Cao, J. Liu, Z. X. Li, and H. F. Zhang. 2019. The response of performance in grower and finisher pigs to diets formulated to different tryptophan to lysine ratios. Livest Sci. 222:25–30. doi:10.1016/j.livsci.2019.01.016.

Ma, D., J. M. Guedes, A. W. Duttlinger, J. S. Johnson, S. M. Zuelly, D. C. Lay, B. T. Richert, and Y. H. B. Kim. 2021. Impact of L-glutamine as replacement of dietary antibiotics during post weaning and transport recovery on carcass and meat quality attributes in pigs. Livest Sci. 244:104350. doi:10.1016/j.livsci.2020.104350.

Mándi, Y., and L. Vécsei. 2012. The kynurenine system and immunoregulation. J Neural Transm. 119:197–209. doi:10.1007/S00702-011-0681-Y. Available from: https://pubmed.ncbi.nlm.nih.gov/21744051/

Mao, X., M. Lv, B. Yu, J. He, P. Zheng, J. Yu, Q. Wang, and D. Chen. 2014. The effect of dietary tryptophan levels on oxidative stress of liver induced by diquat in weaned piglets. J Anim Sci Biotechnol. 5:49. doi:10.1186/2049-1891-5-49. Available from: http://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-5-49

Mao, X., M. Lv, B. Yu, J. He, P. Zheng, J. Yu, Q. Wang, and D. Chen. 2014. The effect of dietary tryptophan levels on oxidative stress of liver induced by diquat in weaned piglets. J Anim Sci Biotechnol. 5:49. doi:10.1186/2049-1891-5-49. Available from: http://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-5-49

Marshall, K. A., R. J. Reiter, B. Poeggeler, O. I. Aruoma, and B. Halliwell. 1996. Evaluation of the antioxidant activity of melatonin in vitro. Free Radic Biol Med. 21:307–315. doi:10.1016/0891-5849(96)00046-9.

Martínez-Miró, S., F. Tecles, M. Ramón, D. Escribano, F. Hernández, J. Madrid, J. Orengo, S. Martínez-Subiela, X. Manteca, and J. J. Cerón. 2016. Causes, consequences and biomarkers of stress in swine: An update. BMC Vet Res. 12. doi:10.1186/s12917-016-0791-8.

Matlock, T. 2022. United States hog inventory down 2%. United States Dep Agric. Available from: https://www.nass.usda.gov/Newsroom/2022/03-30-2022.php

McLamb, B. L., A. J. Gibson, E. L. Overman, C. Stahl, and A. J. Moeser. 2013. Early Weaning Stress in Pigs Impairs Innate Mucosal Immune Responses to Enterotoxigenic E. coli Challenge and Exacerbates Intestinal Injury and Clinical Disease. PLoS One. 8. doi:10.1371/journal.pone.0059838.

Meerts, P., G. Misinzo, D. Lefebvre, J. Nielsen, A. Bøtner, C. S. Kristensen, and H. J. Nauwynck. 2006. Correlation between the presence of neutralizing antibodies against porcine circovirus 2 (PCV2) and protection against replication of the virus and development of PCV2-associated disease. BMC Vet Res. 2. doi:10.1186/1746-6148-2-6.

Melchior, D., B. Sève, and N. Le Floc'h. 2004. Chronic lung inflammation affects plasma amino acid concentrations in pigs. J Anim Sci. 82:1091–1099. doi:10.2527/2004.8241091x. Available from: https://academic.oup.com/jas/article-abstract/82/4/1091/4835319

Moeser, A. J., C. S. Pohl, and M. Rajput. 2017. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim Nutr. 3:313–321. doi:10.1016/J.ANINU.2017.06.003.

Munn, D. H., and A. L. Mellor. 2013. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol. 34:137–143. doi:10.1016/j.it.2012.10.001. Available from: http://dx.doi.org/10.1016/j.it.2012.10.001

Nayak, B. N., R. B. Singh, H. S. Buttar, and Corresponding. 2019. Role of Tryptophan in Health and Disease: Systematic Review of the Anti-Oxidant, Anti-Inflammation, and Nutritional Aspects of Tryptophan and Its Metabolites.

Olsen, R. E., K. Sundell, T. M. Mayhew, R. Myklebust, and E. Ringø. 2005. Acute stress alters intestinal function of rainbow trout, Oncorhynchus mykiss (Walbaum). Aquaculture. 250:480–495. doi:10.1016/j.aquaculture.2005.03.014. Available from: https://www.sciencedirect.com/science/article/pii/S004484860500178X?casa_token=_bIA6-BeuuQAAAAA:6bNye9isVFFP3YHydm9844xr9objNyIMmNP29UTjqm_K_IWNCRps3Dsn8UEKZdnlX637soWg7Q

Packer, L., and A. N. Glazer. 1984. Oxygen radicals in biological systems. 600.

Page, T. G., L. L. Southern, T. L. Ward, S. M. Neal, T. R. Cline, and R. H. Simms. 1993. Effect of Dietary Tryptophan in Excess of the Requirement on Growth and Carcass Characteristics of Finishing Pigs. Prof Anim Sci. 9:86–88. doi:10.15232/S1080-7446(15)32056-8.

Papadimitriou, A., and K. N. Priftis. 2009. Regulation of the hypothalamic-pituitary-adrenal axis. Neuroimmunomodulation. 16:265–271. doi:10.1159/000216184. Available from: https://pubmed.ncbi.nlm.nih.gov/19571587/

Perlman, R. L., and M. Chalfie. 1977. Catecholamine release from the adrenal medulla. Clin Endocrinol Metab. 6:551–576. doi:10.1016/S0300-595X(77)80071-6. Available from: http://dx.doi.org/10.1016/S0300-595X(77)80071-6

Phaniendra, A., D. B. Jestadi, and L. Periyasamy. 2015. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 30:11. doi:10.1007/S12291-014-0446-0. Available from: /pmc/articles/PMC4310837/

Poletto, R., R. L. Meisel, B. T. Richert, H. W. Cheng, and J. N. Marchant-Forde. 2010. Aggression in replacement grower and finisher gilts fed a short-term high-tryptophan diet and the effect of long-term human-animal interaction. Appl Anim Behav Sci. 122:98–110. doi:10.1016/j.applanim.2009.11.015.

Pomorska-Mól, M., K. Kwit, and I. Markowska-Daniel. 2012. Major acute phase proteins in pig serum from birth to slaughter. Bull Vet Inst Pulawy. 56:553–557. doi:10.2478/V10213-012-0097-Y.

Qiu, J., X. Guo, Z. ming E. Chen, L. He, G. F. Sonnenberg, D. Artis, Y. X. Fu, and L. Zhou. 2013. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. Immunity. 39:386–399. doi:10.1016/J.IMMUNI.2013.08.002. Available from: https://pubmed.ncbi.nlm.nih.gov/23954130/

Rao, R. 2008. Oxidative stress-induced disruption of epithelial and endothelial tight junctions. Front Biosci. 13:7210–7226. doi:10.2741/3223.

Rivier, C., and W. Vale. 1983. Modulation of stress-induced ACTH release by corticotropin-releasing factor, catecholamines and vasopressin. Nature. 305:325–327. doi:10.1038/305325A0. Available from: https://pubmed.ncbi.nlm.nih.gov/6312319/

Rodrigues, L. A., J. C. Panisson, L. A. Kpogo, J. C. González-Vega, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2022. Functional amino acid supplementation postweaning mitigates the response of normal birth weight more than for low birth weight pigs to a subsequent Salmonella challenge. Animal. 16. doi:10.1016/J.ANIMAL.2022.100566.

Romero, L. M. 2019. Fight or Flight Responses. Encycl Anim Behav. 547–552. doi:10.1016/B978-0-12-809633-8.20760-7.

Sailaja Rao, P., S. Kalva, A. Yerramilli, and S. Mamidi. 2011. Free Radicals and Tissue Damage: Role of Antioxidants. Free Radicals Antioxidants. 1:2–7. doi:10.5530/AX.2011.4.2.

Salt, J. S., P. V. Barnett, P. Dani, and L. Williams. 1998. Emergency vaccination of pigs against foot-and-mouth disease: Protection against disease and reduction in contact transmission. Vaccine. 16:746–754. doi:10.1016/S0264-410X(97)86180-4.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How Do Glucocorticoids Influence Stress Responses? IntegratingPermissive, Suppressive, Stimulatory, and Preparative Actions. Endocr Rev. 21:55–89. doi:10.1210/EDRV.21.1.0389. Available from: https://academic.oup.com/edrv/article/21/1/55/2423840

Shastri, A., R. Srivastava, B. Jyoti, and M. Gupta. 2016. The Antioxidants-Scavengers of Free Radicals for Immunity Boosting and Human Health/ Overall Well Being. Int J Contemp Med Res. 3:2454–7379. Available from: www.ijcmr.com

Shen, Y. B., G. Voilqué, J. D. Kim, J. Odle, and S. W. Kim. 2012. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs1. J Anim Sci. 90:2264–2275. doi:10.2527/jas.2011-4203. Available from: https://academic.oup.com/jas/article/90/7/2264/4702013

Shen, Y. B., M. T. Coffey, and S. W. Kim. 2015. Effects of short term supplementation of L-tryptophan and reducing large neutral amino acid along with L-tryptophan supplementation on growth and stress response in pigs. Anim Feed Sci Technol. 207:245–252. doi:10.1016/j.anifeedsci.2015.06.020.

Shen, Y. Bin, G. Voilqué, J. Odle, and S. W. Kim. 2012. Dietary L-Tryptophan Supplementation with Reduced Large Neutral Amino Acids Enhances Feed Efficiency and Decreases Stress Hormone Secretion in Nursery Pigs under Social-Mixing Stress. J Nutr. 142:1540–1546. doi:10.3945/jn.112.163824. Available from: https://academic.oup.com/jn/article/142/8/1540/4630956

Sheng, J. A., N. J. Bales, S. A. Myers, A. I. Bautista, M. Roueinfar, T. M. Hale, and R. J. Handa. 2021. The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions. Front Behav Neurosci. 14:256. doi:10.3389/FNBEH.2020.601939/BIBTEX.

Sies, H. 1985. Oxidative Stress - 1st Edition. Acade. Available from: https://www.elsevier.com/books/oxidative-stress/sies/978-0-12-642760-8

Sies, H. 1991. Oxidative stress: from basic research to clinical application. Am J Med. 91. doi:10.1016/0002-9343(91)90281-2. Available from: https://pubmed.ncbi.nlm.nih.gov/1928209/

Smith, F., J. E. Clark, B. L. Overman, C. C. Tozel, J. H. Huang, J. E. F. Rivier, A. T. Blisklager, and A. J. Moeser. 2010. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. Am J Physiol - Gastrointest Liver Physiol. 298. doi:10.1152/ajpgi.00081.2009.

Stockinger, B., K. Hirota, J. Duarte, and M. Veldhoen. 2011. External influences on the immune system via activation of the aryl hydrocarbon receptor. Semin Immunol. 23:99–105. doi:10.1016/J.SMIM.2011.01.008. Available from: https://pubmed.ncbi.nlm.nih.gov/21288737/

Sun, M., N. Ma, T. He, L. J. Johnston, and X. Ma. 2019. Tryptophan (Trp) modulates gut homeostasis via aryl hydrocarbon receptor (AhR). https://doi.org/10.1080/10408398.2019.1598334. 60:1760–1768. doi:10.1080/10408398.2019.1598334. Available from: https://www.tandfonline.com/doi/abs/10.1080/10408398.2019.1598334

Sutherland, M., B. Backus, and J. McGlone. 2014. Effects of Transport at Weaning on the Behavior, Physiology and Performance of Pigs. Animals. 4:657–669. doi:10.3390/ani4040657. Available from: http://www.mdpi.com/2076-2615/4/4/657

Tank, A. W., and D. L. Wong. 2015. Peripheral and central effects of circulating catecholamines. Compr Physiol. 5:1–15. doi:10.1002/CPHY.C140007. Available from: https://pubmed.ncbi.nlm.nih.gov/25589262/

Tossou, M. C. B., Hongnan Liu, M. Bai, S. Chen, Y. Cai, V. Duraipandiyan, Hongbin Liu, T. O. Adebowale, N. A. Al-Dhabi, L. Long, H. Tarique, A. O. Oso, G. Liu, and Y. Yin. 2016. Effect of High Dietary Tryptophan on Intestinal Morphology and Tight Junction Protein of Weaned Pig. Biomed Res Int. 2016. doi:10.1155/2016/2912418.

Upadhaya, S.-D., and I.-H. Kim. 2021. The Impact of Weaning Stress on Gut Health and the Mechanistic Aspects of Several Feed Additives Contributing to Improved Gut Health Function in Weanling Piglets—A Review. Anim 2021, Vol 11, Page 2418. 11:2418. doi:10.3390/ANI11082418. Available from: https://www.mdpi.com/2076-2615/11/8/2418/htm

Usatyuk, P. V., N. L. Parinandi, and V. Natarajan. 2006. Redox regulation of 4-hydroxy-2-nonenal-mediated endothelial barrier dysfunction by focal adhesion, adherens, and tight junction proteins. J Biol Chem. 281:35554–35566. doi:10.1074/JBC.M607305200. Available from: https://pubmed.ncbi.nlm.nih.gov/16982627/

Van Den Broeck, W., E. Cox, and B. M. Goddeeris. 1999. Receptor-dependent immune responses in pigs after oral immunization with F4 fimbriae. Infect Immun. 67:520–526. doi:10.1128/IAI.67.2.520-526.1999/ASSET/77608DBF-2262-49DE-90BC-DA38E3A3843B/ASSETS/GRAPHIC/II0290744007.JPEG. Available from: https://journals.asm.org/doi/10.1128/iai.67.2.520-526.1999

Vergauwen, H., B. Tambuyzer, K. Jennes, J. Degroote, W. Wang, S. De Smet, J. Michiels, and C. Van Ginneken. 2015. Trolox and Ascorbic Acid Reduce Direct and Indirect Oxidative Stress

in the IPEC-J2 Cells, an In Vitro Model for the Porcine Gastrointestinal Tract. PLoS One. 10:e0120485. doi:10.1371/JOURNAL.PONE.0120485. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0120485

Wang, H., Y. Ji, G. Wu, K. Sun, Y. Sun, W. Li, B. Wang, B. He, Q. Zhang, Z. Dai, and Z. Wu. 2015. L-Tryptophan Activates Mammalian Target of Rapamycin and Enhances Expression of Tight Junction Proteins in Intestinal Porcine Epithelial Cells. J Nutr. 145:1156–1162. doi:10.3945/JN.114.209817. Available from: https://academic.oup.com/jn/article/145/6/1156/4585840

Whittemore, C. T., and D. M. Green. 2001. Growth of the young weaned pig. In: The weaner pig: nutrition and management. Proceedings of a British Society of Animal Science Occasional Meeting, University of Nottingham, UK, September 2000. CABI. p. 1–15.

Williams, J. L., B. T. Richert, J. N. Marchant-Forde, and S. D. Eicher. 2012. Behavioral changes in neonatal swine after an 8-hour rest during prolonged transportation. J Anim Sci. 90:3213–3219. doi:10.2527/jas.2011-4597.

Wingfield, J. C., and L. M. Romero. 2015. Tempests, Poxes, Predators, and People. Tempests, Poxes, Predators, and People. doi:10.1093/ACPROF:OSO/9780195366693.001.0001.

Wu, G. 2013. Functional amino acids in nutrition and health. Amino Acids. 45:407–411. doi:10.1007/s00726-013-1500-6.

Young, J. F., K. Rosenvold, J. Stagsted, J. H. Nielsen, and H. J. Andersen. 2005. Significance of vitamin E supplementation, dietary content of polyunsaturated fatty acids, and preslaughter stress on oxidative status in pig as reflected in cell integrity and antioxidative enzyme activities in porcine muscle. J Agric Food Chem. 53:745–749. doi:10.1021/jf0490652. Available from: https://pubs.acs.org/sharingguidelines

Zhang, H., J. Yin, D. Li, X. Zhou, and X. Li. 2007. Tryptophan enhances ghrelin expression and secretion associated with increased food intake and weight gain in weanling pigs. Domest Anim Endocrinol. 33:47–61. doi:10.1016/J.DOMANIEND.2006.04.005. Available from: https://pubmed.ncbi.nlm.nih.gov/16707241/

Zhang, J. M., and J. An. 2007. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 45:27–37. doi:10.1097/AIA.0b013e318034194e. Available from: /pmc/articles/PMC2785020/

Zhang, M., Z. Yue, Z. Liu, A. Islam, B. Rehana, S. Tang, E. Bao, and J. Hartung. 2012. Hsp70 and HSF-1 expression is altered in the tissues of pigs transported for various periods of times. J Vet Sci. 13:253–259. doi:10.4142/jvs.2012.13.3.253. Available from:

https://synapse.koreamed.org/func/download.php?path=L2hvbWUvdmlydHVhbC9rYW1qZS9zeW5hcHNlL3VwbG9hZC9TeW5hcHNlRGF0YS9QREZEYXRhLzAxMThqdnMvanZzLTEzLTI1My5wZGY=&filename=anZzLTEzLTI1My5wZGY=