ABSTRACT

SEABOLT, BRYNN SHEA. Palatability of Feed Ingredients in Nursery Pigs. (Under the direction of Eric van Heugten.)

The objectives of this research were: 1) To evaluate nursery pig preference for diets containing various inclusion levels of dried distillers grains with soluble (DDGS), high protein dried distillers grains (HPDDG) or corn gluten meal (CGM); 2) To evaluate the effect of different qualities of DDGS on nursery pig preference; and 3) To evaluate growth performance and feed preference for diets containing various inclusion levels of DDGS with or without flavor supplementation.

For the first objective, 3 double-choice preference experiments were performed using a 2 day assay. In experiment 1, preference for diets containing DDGS (0, 10, 20, and 30%) was examined. A linear decrease ($P<0.001$) in preference was found with increasing inclusion levels of DDGS on day 1, day 2 and overall. In experiment 2, preference for diets containing CGM (0, 5, 10, and 15%) was examined. On day 1 and overall, a linear decrease ($P<0.06$) in preference was found with increasing inclusion level of CGM. Preferences for all CGM containing diets were lower ($P<0.05$) than 50% on day 1, day 2 and overall, indicating preference of the control diet over CGM containing diets, as no preference would result in equal consumption of both feeds (50% of the control feed and 50% of the test feed). In experiment 3, preference for diets containing HPDDG (0, 10, 20, and 30%) was examined. A linear decrease ($P<0.001$) in preference was found with increasing inclusion levels of HPDDG on day 1, day 2

and overall, and preference for all HPDDG containing diets was less than 50% on day 1, day 2 and overall ($P<0.0001$).

For the second objective, 2 experiments were performed. In experiment 1, preference for diets containing 30% good or poor quality DDGS was examined. DDGS sources were obtained from mills with known good and poor quality DDGS. Color of the sources was observed to ensure poor versus good quality, with the darker source being poor and the lighter source being good quality. Preference for the control diet was not different from the 30% good or poor quality DDGS diets. However, the diet containing 30% poor quality DDGS was preferred ($P<0.05$) over the diet containing 30% good quality DDGS on day 1, day 2 and overall. In experiment 2, preference for diets containing good quality DDGS (0, 10, or 20%) or poor quality DDGS (0, 10, or 20%) was examined. Inclusion of good quality DDGS linearly decreased ($P<0.01$) preference on day 1, 2, and overall. For the poor quality DDGS, inclusion of 20% resulted in a preference lower ($P<0.05$) than 50%. The negative impact of good DDGS on preference was greater compared to the poor DDGS, indicating that poor DDGS may have a higher preference compared to good DDGS.

For the third objective, 2 experiments were performed. In experiment 1, growth performance of nursery pigs fed diets containing various inclusion levels of DDGS (0, 10, and 20%) in the presence or absence of flavor was examined. Average daily gain (ADG) and average daily feed intake (ADFI) in the Starter 1

phase were negatively affected ($P<0.06$) by DDGS inclusion. No other performance parameters, such as feed efficiency and body weight, were affected by DDGS inclusion. ADFI was increased ($P=0.02$) by flavor in the Starter 1 phase only. No other performance parameters were affected by flavor. In experiment 2, feed preference for diets containing various inclusion levels of DDGS in the presence or absence of flavor was examined. Preference for unflavored and flavored DDGS containing diets was less than preference for the control diet. Presence of flavor decreased preference regardless of DDGS inclusion.

Overall, these studies indicate that DDGS, CGM and HPDDG containing diets are not preferred over control diets with corn and soybean meal. However, poor quality DDGS may be preferred over good quality DDGS. Also, addition of the present flavor seems to exacerbate the negative effect of DDGS palatability. Evaluation of volatile components via gas chromatography and headspace analysis in each DDGS and HPDDG sample indicated that compounds associated with rancidity are negatively correlated with palatability, and that the smoky, burnt characteristic of furfural may be palatable to pigs.

Palatability of Feed Ingredients in Nursery Pigs

by
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Biography

Brynn Shea Seabolt was born on June 1, 1984 in Asheboro, NC. She graduated from Asheboro High School in 2002. She received her Bachelor of Science degree in Animal Science with a minor in Nutrition from North Carolina State University in 2006. In the spring of 2007, she re-enrolled at North Carolina State University to obtain a Master of Science degree in Animal Science/Nutrition.

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Chapter 1: Literature Review

Introduction

Increased production of biofuels has resulted in a growing supply of byproducts, such as dried distillers grains with soluble (DDGS) and high protein dried distillers grains (HPDDG). Use of these byproducts in domestic animal diets is becoming common, however an understanding of their palatability is lacking. Feed intake is imperative to production and performance of domestic animals, and palatability is known to play a significant role in feed intake. This literature review will focus on components of palatability; taste, smell and texture; methods for measurement; and importance of ingredient palatability for feed intake in pigs.

Taste

Little research has been performed on taste anatomy and physiology in the pig, but taste is expected to function in the same way it functions in other mammals. Taste provides a way to distinguish between substances that should be digested and those that are harmful (Breslin and Huang, 2006). For example, in humans, and most other mammalian species, sweet receptors signal recognition of high calorie foods, which are often desirable and enjoyable, whereas bitter receptors signal recognition of toxic chemicals, which are typically rejected (Hoon et al., 1999). These reflexes are present even in human infants, and thus are thought to be survival mechanisms (Breslin and Huang, 2006).

In mammals, taste buds contain between 60 and 120 cells (Breslin and Huang, 2006), including precursor, support and receptor cells (Hoon et al., 1999).

Taste buds have a short life span, and therefore turn over continually during the life of the bud, which is approximately 10 days (Breslin and Huang, 2006). These taste buds are found in three distinct papillae regions of the tongue epithelium. Circumvallate papillae are located at the back of the tongue and are most numerous in taste buds, containing hundreds to thousands, depending on the species (Hoon et al., 1999). These taste buds are particularly sensitive to bitter stimuli (Hoon et al., 1999) and convey taste information through the glossopharyngeal nerve (Hellekant and Danilova, 1999). Foliate papillae are located on the posterior edge of the tongue epithelium and contain dozens to hundreds of taste buds (Hoon et al., 1999). These are most sensitive to bitter and sour stimuli (Hoon et al., 1999) and also utilize the glossopharyngeal nerve to send taste information (Hellekant and Danilova, 1999). Finally, fungiform papillae are located at the front of the tongue and only contain one or two taste buds (Hoon et al., 1999). These papillae are most sensitive to salty and sweet stimuli (Hoon et al., 1999) and transmit taste information via the chorda tympani (Hellekant and Danilova, 1999). The majority of papillae found on the tongue are called filiform, which do not contain taste buds (Breslin and Huang, 2006). They are present on the tongue to make the surface rough in order to facilitate food and beverage manipulation and possibly to enhance somatosensory function on the tongue (Breslin and Huang, 2006). Taste buds are also present in areas other than the

tongue, such as the uvula, epiglottis, pharynx, larynx and esophagus (Gilbertson et al., 2000).

The chorda tympani and glossopharyngeal nerves contain various taste fibers (Danilova et al., 1998). Hellenkant and Danilova (1999) identified 4 different types of fiber clusters and called them M, H, Q and S clusters based on the primary taste quality that stimulated the cluster. In the chorda tympani and glossopharyngeal nerves, M, H, Q and S clusters were most sensitive to umami, acid, bitter and sweet compounds, respectively (Hellekant and Danilova, 1999). However, in the chorda tympani, salty compounds also elicited responses in M clusters, and umami compounds elicited responses in S clusters (Hellekant and Danilova, 1999).

Although there are opposing views as to the method of encoding of taste qualities, the most likely model is called the labeled-line model (Chandrashekar et al., 2006). In this model, receptor cells respond to a specific taste stimulus; sweet, salty, sour, bitter, umami; and are innervated at their base by an individually tuned nerve fiber (Chandrashekar et al., 2006), that communicates information to taste centers in the cortex of the brain via synaptic firings (Hoon et al., 1999). In the two opposing models, called across fiber patterns, taste receptor cells are either tuned to more than one taste quality or are innervated by fibers that carry more than one taste quality (Chandrashekar et al., 2006). These are more complex and many

recent molecular studies have shown that the labeled line model is most likely the correct mode of encoding (Chandrashekar et al., 2006).

Although in many ways, taste receptor cells act like neurons, they are actually modified epithelial cells (Gilbertson et al., 2000). Taste information is received by neural fibers within the taste bud (Breslin and Huang, 2006). These fibers are connected with the central nervous system via the brain stem (Breslin and Huang, 2006). They are in contact with solutions of the oral cavity via microvilli at the end of the cells (Lindemann, 2001). These microvilli are mounted by taste receptor proteins (Lindemann, 2001) and extend into an opening in the epithelium of the tongue called the taste pore, located at the tip of each taste bud (Breslin and Huang, 2006). Four types of receptor cells exist in a taste bud. They include dark (type I), light (type II), intermediate (type III) and basal cells, named as such because of their appearances, shapes and positions in an image of a taste bud viewed through electron microscopy (Breslin and Huang, 2006). The basal cells are small, round and found at the base of the taste bud, whereas the other 3 types are elongated cells that stretch from basal to apical ends of the taste bud (Breslin and Huang, 2006). Cell types I and II have microvilli. Those associated with type II cells are shorter than those found on type I cells (Breslin and Huang, 2006). Type II cells are those responsible for the majority of taste transduction. Only they contain the components necessary for gustatory response, such as receptors and

effector enzymes (Breslin and Huang, 2006). Type III cells play a crucial role in that they contain most synapses with afferent axons (Breslin and Huang, 2006).

Sweet and umami tastes are controlled by a family of taste receptors called T1Rs. There are 3 identified receptors in this family; T1R1, T1R2 and T1R3. These receptors are all members of the class C G-protein coupled receptor (GPCR) family (Breslin and Huang, 2006). Some cells express T1R1 and T1R3, some express T1R2 and T1R3, and some express T1R3 alone. Nelson et al. (2001) examined expression of T1R3 in all papillae of the rat tongue and found that its pattern of expression is similar to the combination of T1R1 and T1R2, suggesting coexpression of T1R3 with both T1R1 and T1R2. Using in situ hybridization, Nelson et al. (2001) confirmed that T1R3 is coexpressed with T1R2 in all tongue papillae types, and with T1R1 in fungiform and palate papillae. Some fungiform and palate taste buds also contain T1R3 not in combination with T1R1 or T1R2.

In humans, receptor cells containing T1R2 and T1R3 (T1R2+3) are activated by all sweet-tasting compounds such as sugars, some amino acids, sweet proteins such as monellin and thaumatin, and synthetic sweeteners (Breslin and Huang, 2006). Those cells with only T1R3 respond only to some of these compounds, and at much higher concentrations (Breslin and Huang, 2006). Some studies have suggested the potential expression of multiple sweet receptors. Zhao et al. (2003) sought to define the role of T1R2+3 in sweet taste reception. To

accomplish this, they examined responses of knockout mice lacking T1R2 and T1R3. They found that mice lacking T1R2 or T1R3 experienced dramatic impairment of sweet stimulus response, indicating that T1R2+3 is the main sweet taste receptor (Zhao et al., 2003). However they also found that high concentrations of natural sugars, but not artificial sweeteners, caused slightly elevated responses in both T1R2 and T1R3 knockout mice, indicating either that there are other sweet receptors or that T1R2 and T1R3 function alone (Zhao et al., 2003). To determine which of these scenarios was true, they engineered knockout mice lacking both T1R2 and T1R3. This resulted in a complete loss of response to high sugar concentrations, indicating that sweet taste reception is completely dependent upon T1R2 and T1R3 (Zhao et al., 2003). T1R2 is invariably expressed along with T1R3, however T1R3 is frequently found alone on the tongue epithelium (Zhao et al., 2003). In an attempt to determine whether this receptor could provide animals with another means of detecting calorie-dense foods, Zhao et al. (2003) found that T1R3 alone does respond to high concentrations of natural sugars, supporting the idea that T1R3 functions alone as a sweet receptor. T1R3 did not respond to low concentrations or to artificial sweeteners, helping to explain why artificial sweeteners are not able to elicit the same level of sweetness response of high concentrations of natural sugars (Zhao et al., 2003).

Further evidence that T1R2+3 is the principle sweet taste receptor is found by observing the cat. Li et al. (2005) sought to understand the indifference of cats

to sweet stimuli. They reasoned that this defect in the cat could be caused by DNA amino acid substitution or an unexpressed pseudogene. They first identified the DNA sequence and studied the structures of T1R2 and T1R3 in the cat and compared them to the same genes in other mammals, such as dogs, humans, mice and rats, with functioning sweet taste modality. Results showed that T1R3 is expressed in cat taste buds in the same way as in other mammals and is likely functional (Li et al., 2005). However, T1R2 was found to be an unexpressed pseudogene in the cat, possibly by never being transcribed or by being rapidly degraded upon completion of transcription (Li et al., 2005). This leads to inability of formation of the T1R2+3 heteromer (Li et al., 2005).

Umami taste (taste of monosodium glutamate) is thought to be mediated by receptor cells with T1R1 and T1R3 receptors because they respond to many L-amino acids (Breslin and Huang, 2006). In 2002, Nelson and coworkers sought to determine whether a T1R protein was involved in taste reception of amino acids. They first expressed candidate receptors in human embryonic kidney (HEK) cells and assayed for changes in intracellular calcium. They began with T1R2+3 cells and found that they were not activated by L-amino acid stimuli. T1R1 and T1R3 were tested alone and in combination. No response was shown for the individual receptors; however most amino acids that are perceived to be sweet did activate the combination (Nelson et al., 2002). D-amino acids and other natural and artifical sweeteners did not yield the same response, indicating that the T1R1+3 receptor

is selective for L-amino acids (Nelson et al., 2002). This activation method can be further potentiated by ribonucleotides, such as inosine monophosphate (IMP), and guanosine monophosphate (GMP), which are distinct features of umami taste (Breslin and Huang, 2006). To determine the effect of IMP, Nelson et al. (2002) engineered HEK cells expressing the T1R1+3 receptor stimulated with L-amino acids in presence or absence of IMP. This resulted in drastic enhancement of taste of nearly all amino acids by even low concentrations of IMP (Nelson et al., 2002). However, IMP did not stimulate T1R1 or T1R3 alone or the T1R2+3 receptor (Nelson et al., 2002). Another receptor, mGluR4, has also shown to play a receptor role in savory taste reception (Breslin and Huang, 2006). In 2003, Zhao and coworkers sought to determine whether T1R1+3 was the true umami taste receptor by examining T1R1 and T1R3 knockout mice. Because monosodium glutamate contains sodium, they isolated salty taste from glutamate and found that T1R3 knockout mice experience a drastic loss of attraction to all umami stimuli (Zhao et al., 2003). These same results were also true in the T1R1 knockout mice, but not in T1R2 or control animals, indicating that the heteromeric T1R1+3 is the true mammalian umami receptor (Zhao et al., 2003).

Bitter taste receptors are also part of the G-protein-coupled family and are known as the T2Rs (Breslin and Huang, 2006). Like other G-protein-coupled receptors (GPCR), T2Rs contain a 7-transmembrane domain (Breslin and Huang, 2006). Unlike T1Rs, they have relatively short N and C terminal ends (Breslin and

Huang, 2006). These receptors are highly sensitive to bitter, and often toxic, compounds (Breslin and Huang, 2006). Because gustducin, a G protein alpha subunit, is found in a fraction of taste receptors in all taste buds, but is not coexpressed with T1Rs, Adler et al. (2000) deduced that another GPCR taste receptor much be present. To identify other taste receptors in gustducin-containing cells, they looked for a link between GPCRs and bitter taste perception by searching DNA databases for genes that could encode transmembrane proteins at a specific locus associated with bitter substance response (Adler et al., 2000). They were able to identify 20 total T2R receptor candidates. By examining various genomic resources, they were able to conclude that there may be 40 to 80 functional human T2Rs. To determine if these T2Rs function as taste receptors, Adler and coworkers performed in situ hybridizations of the different taste papillae allowing a closer look at patterns of T2R expression. They found that T2Rs are expressed in subsets of receptor cells on the tongue and palate, yielding more evidence that they are taste receptors themselves (Adler et al., 2000). Other results from Adler et al. (2000) indicate that each receptor cell expresses multiple T2Rs, indicating that mammals can recognize the bitterness of many compounds, but cannot distinguish between them. They also found that T2Rs are expressed only in gustducin-containing cells, providing further evidence that T2Rs are gustducin-linked (Adler et al., 2000).

In bitter taste transduction, bitter compounds such as denatonium and PROP (6-n-propyl-2-thiouracil) activate the T2R receptor, stimulating the G-protein to dissociate into its alpha (α-gustducin) and beta-gamma ($G\beta_3\gamma_{13}$) subunits (Breslin and Huang, 2006). α-Gustducin goes on to activate phosphodiesterase (PDE) (Gilbertson et al., 2000) and possibly guanylyl cyclase (GC) (Breslin and Huang, 2006), hydrolyzing cAMP and leading to activation of protein kinase A (PKA) and nitric oxide synthase (Breslin and Huang, 2006). PKA modulates several ion channels by changing membrane potentials. $G\beta_3\gamma_{13}$ released from activated gustducin activates phospholipase $C\beta_2$, hydrolyzing phosphatidylinositol-4,5-bisphosphate, which produces the second messengers diacylglycerol (DAG) and IP3 (Breslin and Huang, 2006). DAG activates protein kinase C, leading to phosphorylation of many intracellular proteins, such as ion channels (Breslin and Huang, 2006). IP3 binds to its receptor, and intracellular calcium is released, opening the monovalent cation channel TRPM5 (Breslin and Huang, 2006). Other bitter compounds, such as quinine and divalent cations seem to be transduced in a different way. These are able to bypass the receptor and block K^+ channels, thus causing receptor cell depolarization (Gilbertson et al., 2000).

In sweet and umami taste transduction, the process is similar to the gustducin mediated bitter pathway, however in response to stimulation, the α-subunit is thought to regulate activity of adenylyl cyclase (AC), instead of PDE and GC, and cAMP levels, stimulating PKA (Breslin and Huang, 2006). It is also

believed that leptin receptors may play a role in sweet taste transduction and that truncated metatropic glutamate receptors tmGluR1 and tmGluR4 contribute to umami sensation (Breslin and Huang, 2006). Regardless of these differences, bitter, sweet and umami taste transduction results in activation of TRPM5 (Breslin and Huang, 2006). It is not completely understood how opening of this channel leads to action potential generation on afferent nerves (Breslin and Huang, 2006). However, the theory is that influx of monovalent cations through the channel leads to depolarization of the taste bud cell and, ultimately, neurotransmitter release (Breslin and Huang, 2006).

Salty taste is thought to enable animals and humans to detect food rich in minerals but to avoid overly salty foods in order to maintain ion-water homeostasis (Breslin and Huang, 2006). Salty taste is caused by Na^+ and other cations and believed to be received through ion channels sensitive to amiloride (Lindemann, 2001). In rodents, this amiloride-sensitive channel is the epithelial-type sodium channel (ENaC) (Gilbertson et al., 2000). It provides a direct pathway for sodium current into taste cells when the concentration of sodium is high enough (Lindemann, 2001). This current leads to action potential and synaptic firing (Lindemann, 2001). Amiloride sensitivity is less pronounced in humans than in rats, indicating the potential function of other ion channels (Lindemann, 2001). The exact mechanism for salty taste is still unknown, because a large portion of the

molecular components of this and the sour pathways are still unidentified (Hoon et al., 1999).

Presence of acids produces the sour taste (Breslin and Huang, 2006). Sometimes this taste is attractive, as is the case with certain fruits, such as oranges and grapefruits. However, sour tastes of spoiled foods and unripe fruits lead to rejection (Breslin and Huang, 2006). Studies have shown that perceived sourness depends on pH in strong acids such as HCl (Breslin and Huang, 2006). Like saltiness, sourness is thought to be received by ion channels (Breslin and Huang, 2006). Members of the ENaC family are also predicted to function in sour taste by resulting in receptor cell depolarization (Gilbertson et al., 2000). In addition, acid-sensing channels, such as MDEG1 (mammalian degenerin-1 channel), ASIC (acid-sensing ion channel) and DRASIC (dorsal root acid-sensing ion channel) (Gilbertson et al., 2000) are thought to be involved in sour transduction. Many possible mechanisms have been suggested for the complex sour taste, however, the exact mechanism is still unknown (Breslin and Huang, 2006).

The close proximity of taste bud cells to one another allows for essential cell-to-cell communication (Breslin and Huang, 2006). Many bioactive agents, such as serotonin, norepinephrine, cholecystokinin, somatostatin and their receptors have been located on mammalian taste cells (Breslin and Huang, 2006). When taste receptor cells are stimulated, these agents are released and have the

ability to act as autocrine or paracrine signals, modulating producing cells or adjacent cells (Breslin and Huang, 2006). They may also trigger output of neurotransmitters onto the cranial nerve (Breslin and Huang, 2006).

In addition to electrical responses, taste stimuli evoke release of bioactive agents from taste cells and changes in intracellular calcium concentration (Breslin and Huang, 2006). Increase or decrease in calcium concentration is experienced under stimulus of any of the 5 tastes, and this is not dependent on extracellular calcium changes (Breslin and Huang, 2006). However, in the presence of extracellular calcium, magnitude and length of response are increased (Breslin and Huang, 2006).

Taste stimuli are recognized and filtered during transduction via sensory coding (Breslin and Huang, 2006). The peripheral receptor molecule is the first level of filtering and integration of chemical information (Breslin and Huang, 2006). Chemical structure of the ligand for a specific receptor can determine the first binding event, which affects all other processes thereafter (Breslin and Huang, 2006). This is the case when a receptor is highly specific and leads to molecular identification of the ligand (Breslin and Huang, 2006). However, when several ligands are capable of activating the same receptor, this identification specificity is lost and the receptor only recognizes that a single member of a set of chemical stimuli is present (Breslin and Huang, 2006). This is referred to as the principle of univariance (Breslin and Huang, 2006). Only location and degree of activation can

be determined, but not the specific chemical compound (Breslin and Huang, 2006). This is the more common form of recognition, filtering and integration, as specific ligand recognition is quite rare (Breslin and Huang, 2006). Also, most ligands are capable of activating more than one receptor. Kuhn et al. (2004) demonstrated this idea by studying the bitter aftertaste of low-caloric sweeteners, saccharine and acesulfame K. Tastants were applied to transfected cells functionally expressing T2Rs and psychophysical studies were performed to determine the effect of the tastants on the transfected cells. Results from this study showed that T2R43 and T2R44 were activated by saccharine and acesulfame K at concentrations known to induce bitter aftertastes (Kuhn et al., 2004). To ensure that these receptors functioned only in the bitter aftertaste, they also determined that these specific receptors do not contribute to the sweet taste sensation of the sweeteners. They found that sweet compounds sucrose and D-tryptophan did not activate these receptors, indicating they only play a role in the bitter aftertaste of the sweeteners (Kuhn et al., 2004). Not only does this study unveil 2 new bitter receptors, but it indicates that a single tastant is able to activate multiple receptors.

The receptor cell is another location for integration (Breslin and Huang, 2006). Each taste receptor has its distinct receptive field, but all receptor activations lead to cellular excitation (Breslin and Huang, 2006). To date, it is not believed that sweet and savory, and bitter receptor genes are expressed in the

same receptor cells. However, as previously shown by Adler at al. (2000), members within a class are coexpressed. Multiple T2Rs are often found together within a certain type of cell. However T1R1 (necessary for umami taste) and T1R2 (necessary for sweet taste) are not coexpressed, and T1R3 is not always coexpressed with T1R1, indicating that receptor cells in a given taste bud specialize for certain receptor types (Breslin and Huang, 2006). For example, in 2003, Damak and coworkers studied the effects of removing T1R3. Using a two-bottle preference test, they performed behavioral tests on knockout mice lacking the T1R3 receptor to determine the responses of these knockout mice to all five taste qualities. Results from this study indicate that T1R3 is possibly the only receptor responsible for taste of artificial sweeteners, and that it plays no role in sour, salty or bitter taste reception (Damak et al., 2003), further indicating that taste qualities are encoded by specialized receptor cell types.

Smell

At one point, olfaction was believed to be the least understood of the special senses (Allison, 1953). However much has been discovered in the last 15 years relating to olfaction. Behavioral reactions associated with feeding are greatly dependent upon olfactory function in most vertebrate animals (Allison, 1953), as well as behaviors not associated with feeding, such as detection of predators or the opposite sex, and navigation. In fishes and amphibians, the olfactory region

makes up almost 1/6 of the brain, and in mammals it comprises 25% of the surface area of the cerebral cortex (Allison, 1953).

The nose is divided into two halves by a bony structure called the nasal septum (Hornung, 2006). The olfactory cells lining the interior of the nose have a rich blood supply (Hornung, 2006). These cells are covered by mucus that continuously flows into the back of the throat (Hornung, 2006). The olfactory epithelium contains a lamina wherein lies a layer of basal cells, which ultimately divide into mature neurons, called olfactory receptor neurons (Rawson and Yee, 2006). These neurons contain dendrites that extend into the lumen and end in an olfactory knob. From this knob, there are cilia that project into the mucus of the nasal cavity for the purpose of interaction with odorants (Rawson and Yee, 2006). Proteins contained on these cilia interact with the odorants that get through the mucus, leading to excitation via a second messenger cascade event (Rawson and Yee, 2006). Other types of cells, such as supporting and microvillar cells, are also present in the olfactory epithelium, however their functions are not known (Rawson and Yee, 2006).

Upon inhalation through the nose, odorant molecules enter through the nasal valve area before arriving at the headspace above the olfactory receptors, which are located high in the nose in the superior turbinate (Hornung, 2006). In this headspace, odorants bind to cilia located on the ends of the olfactory receptor cells (Hornung, 2006). Upon binding, membrane-bound proteins change in

structure in order to allow extracellular calcium to enter, which leads to a change in membrane potential at the tip of the receptor cell (Hornung, 2006). This creates an electronic signal that travels along axons of olfactory neurons ultimately to the olfactory bulb (Hornung, 2006).

In the olfactory bulb are many types of cells. However, axons from receptor cells first communicate with glomerular cells (Hornung, 2006). Those receptors which respond to the same chemical compound send signals to certain glomerular cells (Hornung, 2006). The majority of odorant compounds stimulate multiple types of receptors. Therefore, each odorant produces a unique response pattern across the glomerular cell, reflecting chemical and physical properties of the odorants themselves (Hornung, 2006). The odorant molecule is disassembled into a pattern of its functional groups by the olfactory receptor sheet. This pattern is later sent to the primary olfactory cortex, where it is reassembled for further processing (Hornung, 2006). These patterns ("inherent" patterns) are one potential way the central nervous system is able to identify particular smells (Moulton, 1976). Receptor cells that are similar in sensitivity are located together on the olfactory receptor sheet (Hornung, 2006). Therefore, different smells are able to produce different electrical patterns in the mucosa and olfactory bulb (Hornung, 2006). The brain is able to distinguish a smell based on these patterns (Hornung, 2006). Molecules from highly mucus-soluble chemicals produce uneven distribution of odorants along the mucosal sheet (Hornung, 2006). Conversely, odorants from

only slightly mucus-soluble chemicals produce a more even distribution along the axis (Hornung, 2006). The distribution pattern ("imposed" pattern) of a chemical based on its solubility in mucus is another potential way the central nervous system identifies a particular smell (Moulton, 1976), although it is not unlikely that the two mechanisms are able to work together to allow animals to identify a broader range of smells (Hornung, 2006). The actual role these inherent and imposed patterns play in olfaction is still unclear (Hornung, 2006). However, based on animal studies, it is believed that these patterns do mirror olfactory quality perception (Hornung, 2006).

Although olfaction consists of sniffing (inhalation of air) and smelling (lack of inhalation of air) (Mainland and Sobel, 2006), examination of the primary olfactory cortex has shown that its activity is higher when air is moving through the nasal cavity than in the absence of air flow (Mainland and Sobel, 2006). Sniffing appears to have great influence upon odorant intensity and identity (Mainland and Sobel, 2006), and because airflow through the two nostrils of the nose is different, it is believed that odors should smell differently to the two (Hornung, 2006).

In mammals, when an odorant binds to the olfactory receptor, a confirmation change of the protein occurs, resulting in dissociation of the G-protein (G_{olf}) (Rawson and Yee, 2006). The G-protein activates adenylate cyclase III, converting ATP to the second messenger, cAMP (Rawson and Yee, 2006). This second messenger is then able to bind to the cyclic nucleotide gated ion channels

(cNcs), causing them to open (Rawson and Yee, 2006). Positive ions such as Na^+ and Ca^+ enter the cell leading to depolarization and excitation (Rawson and Yee, 2006). The involvement of Ca^+ was alluded to in the study done by Miyamoto et al. (1992). By isolating olfactory receptor neurons from catfish, they were able to study the effect of IP3 and cAMP application on these neurons. They found that repolarization after cAMP-induced depolarization depended on influx of Ca^{2+}, and that removal of extracellular Ca^{2+} sustained cAMP-induced depolarization (Miyamoto et al., 1992). The same results occurred with application of IP3. These results indicate that Ca^{2+} cations passing through IP3 and cAMP-gated channels are involved in termination of the second-messenger response (Miyamoto et al., 1992). Further evidence of the involvement of Ca^+ comes came in 1993 when Restrepo and coworkers isolated human olfactory neurons and studied the response to olfactory stimuli. They found that neurons respond to stimulation with odorant molecules with an increase in intracellular Ca^{2+} most likely via influx through the plasma membrane (Restrepo et al., 1993).

Transduction through adenylate cyclase is thought to be the main mechanism of olfaction. However, other pathways are possible. Boekhoff et al. (1990) studied formation of different second messengers by different odorants. They applied citralva (compound characterized by a fruity, citrus odor) and pyrizine (parent compound of many potent compounds, such as the bell pepper) to rat cilia and studied the effects on cAMP and IP3 (Boekhoff et al., 1990). Application of

citralva resulted in rapid increase of cAMP, but had no effect on IP3 formation (Boekhoff et al., 1990). Conversely, application of pyrazine increased levels of IP3 and had no effect on cAMP, indicating activation of different second messenger systems by different odorant compounds (Boekhoff et al., 1990). A later study by Breer and Boekhoff (1991) showed that even odors within the same category (floral, fruity, putrid) are able to activate different second messengers, but always cAMP or IP3 (Breer and Boekhoff, 1991).

Because of controversies surrounding the validity of IP3 serving as a second messenger to some odorants and cAMP a second messenger to others, Brunet et al. (1996) set out to study the role of the cyclic nucleotide-gated ion channel associated with activation through the second messenger cAMP. They engineered a knockout mouse with a mutation in the gene for the cyclic nucleotide-gated channel, and studied the effects when stimulated with various odorants. Results from this study showed an absence of olfactory response to nine odorant compounds of varying chemical structures and odor qualities and to 4 complex mixtures, including mineral oil, mouse urine, coyote urine and peanut butter (Brunet et al., 1996). Some of these compounds have shown in the past to utilize a cAMP second messenger system, while others employ the IP3 system. However, these results indicate that cAMP is required for most, if not all olfactory response to odorant stimulation (Brunet et al., 1996).

While olfaction transduction is more thoroughly studied and familiar than taste transduction, there are still many aspects of it that continue to be a mystery.

Somatosensing

Studies have shown that palatability is related to non-taste/non-odorous sensations experienced as food enters the mouth. According to Szczesniak (2002), there are 11 chemical or mechanical sensations often experienced when food enters the mouth and immediately before swallowing. They are 1) viscosity (thin, thick and viscous); 2) feel on soft tissue surfaces (smooth, pulpy and creamy); 3) carbonation-related terms (bubbly, tingly and foamy); 4) body-related terms (heavy, watery and light); 5) chemical effect (astringent, burning, sharp); 6) coating of oral cavity (mouth clinging, coating, fatty, oily); 7) resistance to tongue movement (slimy, syrupy, pasty and sticky); 8) afterfeel mouth (clean, drying and lingering); 9) afterfeel physiological (refreshing, warming, thirst-quenching and filling); 10) temperature-related (cold or hot); and 11) wetness-related (wet or dry).

Okabe (1979) evaluated factors that affect the palatability of cooked rice using a trained human taste panel. He found that hardness and stickiness were the most influential factors, with hardness being the most important. Blossfeld et al. (2007) studied the effect of pureed versus chopped cooked carrots on preference in infants. They found that pureed carrots were significantly preferred over chopped carrots. Sola-Oriol (2008) determined the correlation between feed preference in nursery pigs and texture analysis. He found that hardness and

chewing work of the feed was significantly and negatively correlated with preference with correlation coefficients of 0.20, and 0.32, respectively, while variables related to particle size were only marginally correlated, with correlation coefficients of 0.07, 0.05, and 0.05 for mean particle size, number of particles per gram, and % of fine particles, respectively.

Importance of palatability in pigs

The pig contains more than 1.5 times more circumvallate and foliate taste buds, and more than 3 times more fungiform taste buds than the human (Hellekant and Danilova, 1999). Because taste buds contain receptor cells involved in transduction of taste mechanisms, it is evident that the pig's ability to taste is even more pronounced than that in the human. Research shows that, while the basic mechanisms of taste and olfaction are true for most mammals, there are marked differences between species. In 1996, Hellekant and Danilova studied species differences in preference for sweeteners. Using two-bottle preference tests, they applied various sweeteners to chimpanzees, rhesus monkeys, hamsters, and pigs. They showed that only 5 of 13 sweeteners used actually tasted sweet to all animals (Hellekant and Danilova, 1996). Then in 1999, Hellekant and Danilova studied various compounds with differing taste qualities in the pig. They stimulated the chorda tympani and glossopharengeal nerves with compounds understood to taste sweet, sour, bitter, salty, or umami and measured electrophysiological response. They found that citric acid and ascorbic acid elicit the most response for

the chorda tympani nerve, indicating that acids give a distinct taste to the pig (Hellekant and Danilova, 1999). Also, many natural and artificial sweeteners that elicit large responses in humans were either weak or non-existent in the pig, such as acesulfame-K, alitame, aspartame, cyclamate, and saccharine (Hellekant and Danilova, 1999). These results indicate that marked taste differences do exist between species.

Ettle and Roth (2004) studied dietary selection of tryptophan by piglets. They found that piglets were able to detect tryptophan-deficient diets and select against them when offered a choice. Pigs were given a choice between 0.11% (deficient) and 0.20% (adequate) tryptophan and chose the diet adequate in tryptophan 87% of the time. Similarly, Kirchgessner et al. (1999) found that piglets were able to select for lysine-adequate diets when offered a choice between lysine-adequate (1.25%) and lysine-inadequate (0.70%) diets. Over a 6 week trial, pigs consumed a total of 22.5 kg of the lysine adequate diet as opposed to only 8.67 kg of the lysine inadequate diet. In addition, Sola-Oriol (2008) found that young pigs prefer a diet with broken rice over diets containing sorghum, corn, or rye.

Decrease in feed intake can be observed in pigs when they experience stressful situations such as weaning, location change or encounter of disease (Hellekant and Danilova, 1999). Leibbrandt et al. (1975) determined the effect of weaning and weaning age on growth performance in nursery pigs. Results showed

that weight gain and feed intake resumed only after 1 week post-weaning, indicating that weaning has a profound effect on pig performance (Leibbrant et al., 1975). They also found that growth performance was highest for pigs weaned at 4 week of age as opposed to those weaned at 2 and 3 week of age (Liebbrandt et al., 1975). McGlone et al. (1993) studied the effects of pig shipping on performance parameters. They tested 19 growing pigs randomly assigned to travel 4 hours to another facility, or to remain in their resident facility. Blood samples were taken at both locations before shipping, immediately after the 4-hour trip, and 72 hours after arrival at the new location. Results from this study showed that shipped pigs performed significantly worse after 4 and 72 hours. Weight gain and feed intake were both significantly reduced by shipping (McGlone et al., 1993). Finally, Pijpers et al. (1991) studied the feed and water consumption of pigs faced with a disease challenge. Six healthy pigs were inoculated with A. pleuropneumoniae toxin, while 1 served as a control inoculated with saline. Pigs were fed and disappearance of feed and water was recorded twice daily. Before disease challenge, all 7 pigs consumed 750 g of feed twice daily and 3 to 7 liters of water daily (Pijpers et al., 1991). After the disease challenge, experimental pigs showed a significantly decreased feed and water intake as compared to the control (Pijpers et al., 1991). These studies show the need for more palatable diets during stressful situations that typically lead to disease, reduced feed intake and reduced weight gain.

Measuring palatability in pigs

According to Parfet and Gonyou (1991), newborn piglets are able to discriminate olfactory stimuli immediately after birth. Olfactory stimuli included birth fluids, sow's milk and water absorbed onto clean gauze. Piglets were allowed a 1-minute orientation phase, followed by 5 minutes for testing of odor cues, including birthing fluids, sows milk and water as a control, all applied to sterile gauze. They found that piglets spend more time with maternal odors than with water, indicating their ability to process olfactory stimuli immediately after birth.

Double choice models for testing diet preference in pigs have been widely utilized. McLaughlin et al. (1983) used a double choice method for testing feed preference in pigs called the T-maze. 10-week old pigs were trained to run in this T-maze by first being held individually in the holding pen for 2 minutes. The pig was then released and allowed to enter into a chamber with 2 arms, each containing a different diet. Here, a choice between feeds could be made. Once the choice was made, the door to that feeder section was closed, and the pig was able to sample feed for 20 seconds. The pig was then brought back into the holding pen. In the next run, the pig was forced to sample feed from the other side of the T-maze for 20 seconds. This concluded a training period and diets compared were an unpalatable diet containing corn meal, and a commercial piglet diet that piglets typically prefer (McLaughlin et al., 1983). In runs 3-7, the pig was allowed to choose the feed it preferred, and in runs 8-12, the feeders were switched. Preference was determined by dividing number of times the test diet was chosen

by the 10 test runs, and percentage feed intake was determined by dividing intake of the test diet by total intake of the test diet plus cornmeal diet (McLaughlin et al., 1983). They also did a study to determine if 5 runs of the experiment would be as effective as 10 runs, and concluded that 5 runs was as sufficient as 10 (McLaughlin et al., 1983). Finally they conducted an experiment to determine if the diets that were preferred in the T-maze would be preferred during an experiment with longer exposure, lasting 5 days. The pigs were able to choose between 2 commercial pig diets or the same diet plus 3 different inclusion levels of flavors previously preferred in the T-maze experiment. Of the five flavors preferred in the T-maze experiment, 3 of them were again preferred in the longer-term double choice test (McLaughlin et al., 1983). Sola-Oriol (2008) measured preference of various feed ingredients in nursery pig diets by utilizing a double-choice preference test lasting 8 to 11 days, depending on the experiment. He found that pigs showed significant preferences after 1 or 2 days, and that they did not significantly change that preference throughout the remainder of the experiment. Measuring preference for a short period of time lessens the chances for the animal to choose a diet based on nutrient composition or adaptation (Sola-Oriol, 2008).

Use of byproducts in animal diets

Several byproduct ingredients are currently being used in animal diets. Because of the increasing demand for biofuels, there has been an increase in

supply for associated byproducts, such as dried distiller's grains with solubles (DDGS), and high protein dried distiller's grains.

Dried distiller's grains with solubles are a byproduct of the ethanol industry. Corn is the most common grain used to produce ethanol; however sorghum, wheat and barley are sometimes used, producing DDGS that are much different in nutrient composition than corn DDGS (Shurson et al., 2003). During fermentation, starch from the grain is converted into ethanol, yielding DDGS that are 2 to 3 times more concentrated in the remaining nutrient fractions (protein, oil and fiber) (Shurson et al., 2003). Nutrient content of DDGS varies between ethanol plants. New generation, modern ethanol plants produce DDGS that are higher in digestible and metabolizable energy, digestible amino acids, and available phosphorus compared to DDGS produced in old generation ethanol plants. New generation DDGS contain approximately 34.6% neutral detergent fiber (NDF) and 16.3% acid detergent fiber (ADF), whereas corn grain only contains 9.6% NDF and 2.8% ADF (NRC, 1998). However, new generation DDGS also contains approximately 8.4% crude fat compared to corn grain's 3.9% (NRC, 1998), resulting in energy values that are similar to corn (Shurson et al., 2003). For this reason, as well as high available phosphorus content, DDGS are being included in swine diets more frequently as supply increases. Performance of pigs fed DDGS containing diets is variable. Whitney et al. (2006) reported including 10% DDGS in grow-finish diets without drastically affecting performance. However, a diet

consisting of 20% DDGS and formulated on a total amino acids basis resulted in reduced average daily gain and final body weight (Whitney et al., 2006). Thong et al. (1978) found that DDGS inclusion at 44.2% had no negative effect on gilt reproductive performance. Whitney and Shurson (2004) found no significant effects of diets containing up to 25% DDGS on performance parameters. Variation in performance of pigs fed DDGS could be attributed to DDGS quality, which can be variable among new generation plants and within plants from batch to batch. Quality of DDGS is often determined by color and odor. DDGS color can be affected by initial grain color, amount of solubles added, and drying time and temperature (US Grains Council, 2008). DDGS dark in color exhibit a burned, smoky odor, likely caused by overheating in the drying process of DDGS production (Cromwell et al., 1993). DDGS are especially affected by overheating because of the high concentration of reducing sugars present in the solubles fraction (Amezcua and Parsons, 2007). This overheating causes the Maillard reaction, resulting in a decrease in available lysine and explaining the lower analyzed lysine concentrations found in dark colored DDGS (Cromwell et al., 1993). Analyzed lysine concentrations vary among samples of DDGS because of the variation in drying time and temperature among DDGS producers (US Grains Council, 2008). Cromwell et al. (1993) found a significant correlation between subjective color score and lightness/darkness, and growth rate and feed to gain in chicks. They found that chicks fed diets containing dark, smoky DDGS

demonstrated lower growth rates than those fed golden colored DDGS (Cromwell et al., 1993). Color is measured using the Hunter and Minolta colorimeters. Color is defined by three factors, L*, a* and b*, and each is measured on a scale from 0 to 100. The L* score determines darkness to lightness (0 to 100). The a* score determines yellowness to redness (0 to 100). The b* score determines blueness to greenness (0 to 100) (US Grains Council, 2008). Ergul et al. (2005) found that L* and b* scores were significantly correlated with lysine, cystine and threonine digestibilities, but a* scores were not, indicating that lighter colored, more yellow DDGS were higher in amino acid digestibility than darker, redder DDGS.

High protein dried distiller's grains are produced in the same way as DDGS; however they do not contain solubles, which consist mainly of fat and vitamins. If solubles are the main cause of overheating during the drying process of DDGS, HPDDG are less likely to be overheated, leading to higher lysine digestibility (Stein, 2007). However, this has not been studied. HPDDG contains approximately 40% crude protein (Stein, 2007), is similar in amino acid and phosphorus digestibility to DDGS, and contains higher digestible and metabolizable energy values than DDGS (Widmer et al., 2007). Widmer et al. (2008) reported including up to 20% HPDDG in growing pig diets before seeing effects on performance. Corn gluten meal is a byproduct of the corn syrup and corn starch industry. It is a popular natural herbicide and is more commonly used in dairy rations. This ingredient contains approximately 60.2% crude protein (NRC, 1998), and its sulfur

amino acid are highly digestible and available to growing chicks and pigs (Sasse and Baker, 1973; Knabe et al., 1989). Mahan (1993) studied the effect of replacing corn and dried whey with corn gluten meal and lactose in nursery pig diets, and found an improvement in daily gain and in feed intake when fed diets with CGM and lactose.

Summary

As shown in this review, taste and olfaction are complicated processes. Combined with somatosensing, these processes make up the main components of palatability. While it is clear that the discussed byproduct feeds may be capable of replacing more traditional, higher cost ingredients, research on their palatability is still lacking. The purpose of this research was to address the issue of byproduct palatability in order to determine the potential for inclusion in pig diets, resulting in economic benefits.

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Chapter 2: Feed preferences by nursery pigs fed diets containing various inclusion levels of dried distillers grains with solubles (DDGS), high protein dried distillers grains (HPDDG), or corn gluten meal (CGM).

Abstract

Three experiments were conducted to evaluate feed preference of nursery pigs for diets containing various inclusion levels of dried distillers grains with solubles (DDGS), corn gluten meal (CGM) or high protein dried distillers grains (HPDDG), without solubles. Pigs were weaned at 21 days of age and adjusted to a commercial diet (without DDGS) for at least 10 d and subsequently housed individually. Each pen contained two identical feeders positioned side by side and preference was measured for two days. In Exp. 1, 60 pigs (11.6 ± 0.27 kg BW) were given a choice between a control diet (0% DDGS) and diets containing 0, 10, 20, or 30% DDGS. In Exp. 2, 80 pigs (9.6 ± 0.16 kg BW) were given a choice between a control diet (0% CGM) and diets containing 0, 5, 10, or 15% CGM. In Exp. 3, 80 pigs (10.8 ± 0.13 kg BW) were given a choice between a control diet (0% HPDDG) and diets containing 10, 20, or 30% HPDDG. Preference was calculated as intake of the test diet as a percentage of total intake. In Exp. 1, a linear decrease ($P<0.01$) in preference for DDGS containing diets was observed, where preferences were 50.0, 34.8, 26.4, and 16.3% for the 0, 10, 20, and 30% DDGS inclusions, respectively. In Exp. 2, decreased preference for CGM containing diets was observed, where preferences were 29.7, 29.6, 33.4, and

29.8% for the 0, 5, 10, and 15% CGM inclusions, respectively. All comparisons were different from 50% ($P<0.05$), indicating dietary preference for one feed over the other. In Exp. 3, a linear decrease ($P<0.01$) in preference for HPDDG containing diets was observed, where preferences were 56.0, 22.4, 19.5, and 13.2% for the 0, 10, 20, and 30% HPDDG inclusions, respectively. Nursery pigs prefer a diet without DDGS, CGM, or HPDDG over a diet containing either of these ingredients, even at low inclusion levels.

Introduction

Feed intake is a major driving factor in pork production. Feed intake can be affected by nutrient composition of the diet, environmental temperature, disease, gender, genetics, and palatability of feed ingredients (Frederick and van Heugten, 2002). One method to increase feed consumption, even during times of stress, is to choose ingredients that are highly palatable. Research has shown that pigs are able to clearly distinguish palatability of different diets. Ermer et al. (1994) found that weanling pigs preferred diets with spray dried porcine plasma over diets containing dried skim milk. Yang et al. (1997) found that inclusion of a milk chocolate product even at 5% of the diet is strongly preferred by nursery pigs over a traditional control diet containing milk whey. Finally, Sola-Oriol (2008) found that grains uncommonly used in pig diets, such as rice and naked oats, were preferred over traditional grains, such as corn.

Traditional feed ingredients, such as corn, wheat, and milk whey are increasing in price, forcing producers to consider alternative feed ingredients. According to Leibtag (2008), corn prices have increased from $2 per bushel in 2005 to $3.40 per bushel in 2007, and prices for soybeans have increased from approximately $5.60 per bushel in 2005 to nearly $8 per bushel in 2007. While, economically, byproduct ingredients may improve profitability of pork production by replacing high cost ingredients, little is known about their palatability.

Dried distillers grains with solubles (DDGS) is a byproduct of the ethanol industry. Production of DDGS is increasing rapidly due to the growing demand for biofuels and, specifically, ethanol. Studies have shown that DDGS contain similar energy values and significantly higher phosphorus and available phosphorus compared to corn grain (Shurson et al., 2004), and are a reasonable source of protein for pigs, containing approximately 27.7% crude protein (NRC, 1998). Whitney et al. (2006) reported including 10% DDGS in grow-finish diets without seeing detrimental effects on performance, or carcass and pork quality. A diet consisting of 20% DDGS and formulated on a total amino acids basis resulted in reduced average daily gain and final body weight (Whitney et al., 2006). Thong et al. (1978) studied the effects of feeding DDGS to gestating sows at increasing inclusion levels. They found that DDGS inclusion at 44.2% had no negative effect on gilt reproductive performance. Whitney and Shurson (2004) studied the effects of increasing DDGS inclusion on performance of nursery pigs. They found no

significant effects of diets containing up to 25% DDGS on performance parameters. Widmer et al. (2007) studied energy, amino acid, and phosphorus digestibility in growing pigs fed diets containing high protein dried distillers grains (HPDDG), which are produced in ethanol production, but lack solubles, which are rich in vitamins and high in fat. HPDDG contains approximately 40% crude protein (Stein, 2007), is similar in amino acid and phosphorus digestibility to DDGS, and contains higher digestible and metabolizable energy values than DDGS (Widmer et al., 2007). Widmer et al. (2008) reported including up to 20% HPDDG in growing pig diets before seeing effects on performance. Corn gluten meal is another corn byproduct, more commonly used in dairy rations, that contains approximately 60.2% crude protein (NRC, 1998). Mahan (1993) studied the effect of replacing corn and dried whey with corn gluten meal and lactose in nursery pig diets, and found an improvement in daily gain and in feed intake when fed diets with CGM and lactose. While these byproduct ingredients may be nutritionally adequate to replace other protein sources, palatability has scarcely been considered. In one study, Hastad et al. (2005) found that adding 30% DDGS to pig diets resulted in a significant decrease in feed intake. Widmer et al. (2008) found that inclusion levels of 20% and 40% HPDDG negatively affected feed intake. Little work has been done to determine the preference of diets containing corn gluten meal.

The aim of this study was to evaluate preference of nursery pigs for diets containing increasing inclusion levels of either DDGS, HPDDG or CGM. It was hypothesized that increasing levels of the tested byproduct feeds would result in decreased preference.

Materials and Methods

Experiment 1

Pigs were weaned at 21 days of age and housed approximately eight pigs per pen in a nursery room with 12 pens. After a 2-week nursery period (to ensure adequate feed consumption of a complex starter diet containing corn and soybean meal), 20 pigs were selected and moved to a nursery room with 20 pens (1.73 m × 0.83 m). Each pen contained two identical feeders (side-by-side) and housed one pig. One feeder contained a diet with 0% DDGS as a control and the other feeder contained a diet with either 10, 20, or 30% DDGS, resulting in 4 possible comparisons. The position of the feeders was alternated from each group of 4 comparisons to the next to minimize side preferences (see Figure 1). Thus, 10 of the feeders containing the control diet were positioned on the left side of the pen and 10 feeders containing the control diet were positioned on the right side of the pens. In addition, treatment comparisons were assigned to pens such that each comparison occurred in each of the 20 pens at least one time during the experiment. Pigs were allowed to consume feed freely from either feeder for 48 hours and feed disappearance was measured after 24 hours and again at the end

of the 48 hour trial. Pigs were weighed at the onset of the trial and again at the end of 48 hours. Pigs were returned to the original nursery room at the end of the experiment. This process was repeated 3 times using a total of 60 pigs (11.6 ± 0.27 kg BW), 15 pigs per treatment comparison.

Experiment 2

Experimental procedures were identical to those described in the first experiment, except diets included 0, 5, 10, and 15% CGM. The process was repeated 4 times using a total of 80 pigs (9.6 ± 0.16 kg BW), 20 pigs per treatment comparison.

Experiment 3

Experimental procedures were identical to those described in the first experiment, except diets included 0, 10, 20, and 30% HPDDG. The process was repeated 4 times using a total of 80 pigs (10.8 ± 0.13 kg BW), 20 pigs per treatment comparison.

Diets

All diets were mixed at the NC State University Grinnells laboratory using a common basal diet, formulated for lysine, and presented in mash form. Diets were analyzed at DairyOne Forage Laboratory Services in Ithaca, NY. Their ingredient and chemical compositions can be found in Tables 1 through 3. Representative samples of the test ingredients used in this study were obtained and analyzed for crude protein, acid detergent insoluble crude protein (ADICP), ADF, NDF, calcium,

phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, and molybdenum. Additionally, DDGS and HPDDG were analyzed for aflatoxin, vomitoxin (DON), zearalenone, and T-2 toxin (Table 4). Additionally, sensorial testing of volatile compounds via gas chromatography and headspace analysis was performed on the test ingredients. A 2-gram sample of each DDGS or HPDDG sample was placed into a 20 ml vial and extracted by solid phase microextraction (SPME) fibers. After 30 minutes of extraction of the volatile compounds from the headspace of the sample onto the fiber, the fiber was automatically transferred into a gas chromatograph (GC) for 10 minutes of desorption of the compounds followed by separation into a capillary chromatographic column, resulting in identification and quantification by mass spectrometry. Finally, Hunter Minolta color scores were determined for the DDGS source.

Statistical Analysis

Data were analyzed using the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC). Preference was calculated as:

$$\text{Preference} = \frac{\text{Intake of Test Diet}}{\text{Total Intake}}(100)$$

Therefore, preference values ranged from 0 to 100% and a value of 50% indicated no preference. The model included block and diet as main effects. Preference values were compared to the 50% no-effect level by t-test and differences from

50% were interpreted as a preference over the comparison diet. Significance was declared at P<0.05.

Results

Experiment 1

Two-day performance and preference results are shown in Table 5. Performance parameters were not affected by inclusion level of DDGS. On day 1, preference for diets containing 20 and 30% DDGS were found to be lower (P < 0.05) than 50%, with a preference for the 20% DDGS diet of 26.9% and a preference for the 30% DDGS diet of 16.5%. Preference for the control diet and 10% DDGS diet were not different from 50%. There was a decreasing linear response in preference to increasing inclusion level of DDGS (P<0.001). On day 2, preference for all 3 DDGS containing diets was lower (P < 0.05) than 50% at 29.4% for the 10% diet, 28.0% for the 20% diet, and 18.8% for the 30% diet. The control diet preference was not different from 50%. There was a decreasing linear response in preference to increasing inclusion of DDGS (P<0.001). Overall, preference for all 3 DDGS containing diets was lower (P < 0.05) than 50% at 34.8% for the 10% diet, 26.4% for the 20% diet, and 16.3% for the 30% diet and the response was linear (P < 0.001). The control diet preference was not different from 50%.

Experiment 2

Two-day performance and preference results are shown in Table 6. Performance parameters were not affected by inclusion level of CGM. On day 1, all preferences except the control versus control comparison were found to be different from 50%. Preferences were 35.3%, 32.6%, 32.1% and 27.2% for 0%, 5%, 10% and 15% CGM, respectively. There was a decreasing linear response to increasing levels of CGM in the diet on day 1 (P=0.02). On day 2, all preferences were found to be significantly different from 50%. Preferences were 29.7, 27.2, 34.8 and 31.9% for 0, 5, 10, and 15% CGM, respectively. No linear or quadratic responses were found. Overall, preferences of 29.7, 29.6, 33.4, and 29.8% for 0, 5, 10, and 15% levels of CGM, respectively, were observed. All preferences were significantly different from 50%. Overall, there tended to be a decreasing linear response to increasing inclusion level of CGM (P=0.06).

Experiment 3

Two-day performance and preference results are shown in Table 7. Performance parameters were unaffected by inclusion level of HPDDG. On day 1, all HPDDG containing diets were significantly different from 50%. Preferences were 49.9, 23.3, 22.9, and 15.3% for 0, 10, 20, and 30% HPDDG, respectively. On day 2, all preferences except control versus control were different (P<0.0001) from 50%. Preferences were 60.8, 23.4, 18.1, and 13.3% for 0, 10, 20, and 30% HPDDG, respectively. Overall, only HPDDG containing diets were different

(P<0.0001) from 50% with preferences of 56.0, 22.4, 19.5, and 13.2% for 0, 10, 20, and 30% HPDDG, respectively. The response to HPDDG inclusion was linear, indicating decreased preference with increasing levels of HPDDG in the diet, on day 1, day 2, and overall (P < 0.001).

Discussion and Conclusions

We have previously demonstrated in a double choice preference test comparing corn and rice based diets that preferences could be clearly measured and were well-established after 2 days (van Heugten et al., 2006). Similarly, Sola-Oriol (2008) demonstrated clear differences in palatability could be detected after one to two days and these differences remained unaffected when measurements were conducted during a longer time period. Thus, this preference model provides a quick, efficient method to determine ingredient and diet preference in pigs, and the use of a single pig may eliminate errors associated with feeding competition. Further, we determined preferences in pigs that had been weaned for 2 weeks to ensure adequate feed intake and improve the sensitivity of the experiments. Indeed, Sola-Oriol (2008) demonstrated similar responses between newly weaned pigs and post-weanling pigs in their preferences for feed ingredients; however, preferences in post-weanling pigs were much more pronounced. Studies of preference and palatability are vital to understanding how to increase or maintain feed intake in pigs, ultimately maximizing production. In the current study, only corn and soybean meal were altered when replacing them with the test

ingredients, to avoid confounding preference measures with changes in diet composition other than the ingredient of interest. Each experiment contained control versus control comparisons within each replicate to determine the validity of the study. To accomplish this, right feeder feed intake was divided by left feeder feed intake for an entire replication of all 4 comparisons. For the next 4 comparisons, the side of control feeder was switched, and left feeder feed intake was divided by right feeder feed intake. For validation, control versus control preferences were also calculated in this way. Thus, the control versus control comparison should not be different from 50% preference to validate that factors in the study were properly controlled and not confounded by placement of feeders, temperature and ventilation differences within the room, or other factors. Further, the control versus control comparison allowed us to determine potential preferences for the location of the feeder (right or left). On day 2 of the DDGS inclusion trial, preference for the right feeder over the left feeder was found. This was accounted for in the experimental design of all experiments by assigning equal numbers of treatment and control diets to feeders on each side. Also, each pen in each experiment received each comparison at least once.

All 3 experiments clearly show that the sources of DDGS, CGM and HPDDG used in the present study led to reductions in preference, even at relatively low levels of inclusion. As inclusion levels of DDGS increased, preference was significantly reduced. These results agree with those obtained by

Hastad et al. (2005) who found that preference decreased linearly as DDGS inclusion increased from 0% to 30% in the diet. Analysis of the DDGS source for experiment 1 showed that it contained 4 ppm vomitoxin (DON). Feeding DDGS at 30% would constitute nearly 1.2 ppm in the as-fed diet. Swine are particularly sensitive to vomitoxin and reduced feed intake and weight gain can be observed after feeding 2 to 3 ppm (van Heugten, 2001); however, Smith et al. (1997) found that increasing inclusion level of vomitoxin from 0 to 1.9 ppm created a significant decreasing linear feed intake response over a three week period. Finally, after performing sensorial analyses of the test ingredients, it was found that the DDGS source contained many volatile compounds characteristic of rancidity, such as valerianate (Figure 2) and aldehyde C-6 (Figure 3), which could have caused decreased preference for this diet. Greenberg et al. (1953) fed rats diets containing rancid fat and found that feed intake was lower than feed intake by rats fed diets without rancid fat. Also, Kimura et al. (2004) found that rats given a choice between fresh oil and oxidized (rancid) oil significantly preferred fresh oil, indicating an unpalatable characteristic of rancid fat. The current DDGS source contained 27.5% neutral detergent fiber and 11.5% acid detergent fiber, as compared to corn grain which contains 9.6% NDF and 2.8% ADF (NRC, 1998). In the diets for this study, DDGS replaced mostly corn and some soybean meal. As DDGS increased in the diet, neutral detergent fiber also increased. Sola-Oriol (2008) found a negative correlation between preference and crude fiber, possibly

caused by low energy density of high fiber diets. This could be another factor affecting palatability of DDGS containing diets.

Results from this study also show that as HPDDG increased, preference was reduced. This agrees with results from Widmer et al. (2008) who found that inclusion levels of 20% and 40% HPDDG reduced feed intake. In the current study, preference was significantly reduced even at inclusion levels of 10%. Neither mycotoxins nor rancidity seemed to play a role in the negative preference of HPDDG containing diets, based on mycotoxin screening and sensorial analysis. However, similar to the DDGS, HPDDG are also higher in fiber than corn grain. The current source of HPDDG contained 35.1% NDF and 15.0% ADF. Increasing HPDDG in the diet resulted in increased neutral detergent fiber in the diet, which, as mentioned previously, could explain lower palatability due to decreased energy density, according to Sola-Oriol (2008).

The same decrease in preference was found for CGM containing diets, even at 5% of the diet. However, the comparison between 0% CGM and 0% CGM showed a preference different from 50% ($P<0.05$), indicating the presence of confounding factors that may have affected preference besides inclusion of CGM. Fiber content of this ingredient is similar to that of corn grain. However, crude protein content is much higher. CGM contains 60.2% crude protein compared to 8.3% found in corn grain. In the current CGM diets, crude protein content was 19.1%, 23.1%, 25.6% and 27% of the 0%, 5%, 10% and 15% CGM diets,

respectively. Although few studies of pig preference for differing levels of crude protein are available, it has been shown that sheep are able to select for a diet with a level of protein that meets their requirements, while avoiding diets with excess protein (Kyriazakis and Oldham, 1993). The 15% CGM containing diet in the current study contained approximately 50% more methionine than the basal diet. Methionine takes on a bitter taste and sulfur smell (Edmonds et al., 1987). Including a 4% excess of DL-methionine in the diet of newly weaned pigs resulted in significant taste aversion (Edmonds et al, 1987). Whereas the diets in the current study had much lower methionine levels than the diets used by Edmonds et al. (1987), taste aversion to this amino acid could explain the significant preference for control over CGM containing diets. Mycotoxins and sensorial analyses were not performed on this ingredient, so volatile components present in the utilized CGM source are not known.

Conclusion

Based on the present studies, inclusion of DDGS, CGM or HPDDG even at relatively low inclusion levels leads to reductions in preference. Even though these ingredients have proven to be effective as nutrient sources in pig diets, their palatability may compromise feed consumption in situations where no choice is offered. During times of stress, such as weaning, palatability of the diet becomes more critical, as animals are adjusted from sow milk to a solid feed and feed intake tends to be very low. Feeding a diet with highly palatable ingredients may be able

to assist recovery of feed intake, leading to improvement of performance and gut health.

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Table 1: Composition of dried distillers grain with solubles (DDGS) containing diets (Experiment 1)[1]

	DDGS inclusion level, %			
	0	**10**	**20**	**30**
Ingredient, % as fed				
Corn	63.9	56.3	48.7	41.0
Soybean meal	30.0	27.6	25.3	22.9
Corn oil	2.5	2.5	2.5	2.5
Corn DDGS	0	10	20	30
Dicalcium phosphate	1.59	1.59	1.59	1.59
Calcium carbonate	0.88	0.88	0.88	0.88
Salt	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
L-Lys	0.24	0.24	0.24	0.24
Copper sulfate	0.08	0.08	0.08	0.08
DL-Met	0.04	0.04	0.04	0.04
L-Thr	0.002	0.002	0.002	0.002
Analyzed Nutrient Composition				
Dry matter, % as fed	90.2	90.3	90.6	90.4
Crude protein, % as fed	20.1	21.3	21.2	21.6
ADF, % as fed	5.1	4.5	5.6	6.0
NDF, % as fed	8.1	9.6	12.2	13.5
Calcium, % as fed	0.81	0.93	1.00	0.97
Phosphorus, % as fed	0.68	0.73	0.79	0.78
Magnesium, % as fed	0.18	0.22	0.25	0.27
Potassium, % as fed	0.86	0.88	1.01	0.98
Sodium, % as fed	0.21	0.22	0.32	0.33
Iron, ppm	302	306	338	354
Zinc, ppm	149	216	322	365
Copper, ppm	192	218	295	227
Manganese, ppm	43	113	199	266
Molybdenum, ppm	2.1	1.0	1.1	1.1

[1]Diets were formulated to contain 1.25% lysine

[2]Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine·HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B-12 , 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO, 21 mg Fe as $FeSO_4$, 9 mg Cu as $CuSO_4$, 21 mg Mn as $MnSO_4$, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na_2SeO_3.

Table 2: Composition of corn gluten meal (CGM) containing diets (Experiment 2)[1]

	CGM inclusion level, %			
	0	**5**	**10**	**15**
Ingredient, %				
Corn	63.9	60.3	56.7	53.0
Soybean meal	30.0	28.6	27.3	25.9
Corn oil	2.5	2.5	2.5	2.5
Corn gluten meal	0	5	10	15
Dicalcium phosphate	1.59	1.59	1.59	1.59
Calcium carbonate	0.88	0.88	0.88	0.88
Salt	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
L-Lys	0.24	0.24	0.24	0.24
Copper Sulfate	0.08	0.08	0.08	0.08
DL-Met	0.04	0.04	0.04	0.04
L-Thr	0.002	0.002	0.002	0.002
Analyzed Nutrient Composition				
Dry Matter, % as fed	89.4	90.4	89.8	89.7
Crude Protein, % as fed	19.1	23.1	25.6	27.0
ADF, % as fed	5.6	4.9	4.4	4.1
NDF, % as fed	8.1	7.1	7.6	6.3
Calcium, % as fed	0.85	0.98	0.87	0.84
Phosphorous, % as fed	0.70	0.74	0.71	0.65
Magnesium, % as fed	0.18	0.17	0.15	0.15
Potassium, % as fed	0.83	0.88	0.82	0.78
Sodium, % as fed	0.23	0.32	0.22	0.25
Iron, ppm	307	353	319	291
Zinc, ppm	172	189	192	185
Copper, ppm	177	286	217	212
Manganese, ppm	49	51	46	52
Molybdenum, ppm	0.5	1.6	3.0	1.5

[1]Diets were formulated to contain 1.25% lysine

[2]Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine·HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B-12 , 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO , 21 mg Fe as $FeSO_4$, 9 mg Cu as $CuSO_4$, 21 mg Mn as $MnSO_4$, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na_2SeO_3.

Table 3: Composition of the high protein distillers grains (HPDDG) containing diets (Experiment 3)[1]

	HPDDG Inclusion level, %			
	0	**10**	**20**	**30**
Ingredient, %				
Corn	63.9	56.3	48.7	41.0
Soybean meal	30.0	27.6	25.3	22.9
Corn oil	2.5	2.5	2.5	2.5
Corn HPDDG	0	10	20	30
Dicalcium phosphate	1.59	1.59	1.59	1.59
Calcium carbonate	0.88	0.88	0.88	0.88
Salt	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.25	0.25	0.25	0.25
L-Lys	0.24	0.24	0.24	0.24
Copper Sulfate	0.08	0.08	0.08	0.08
DL-Met	0.04	0.04	0.04	0.04
L-Thr	0.002	0.002	0.002	0.002
Analyzed Nutrient Composition				
Dry Matter, % as fed	88.3	89.2	89.5	89.3
Crude Protein, % as fed	18.4	19.7	21.5	22.1
ADF, % as fed	5.1	4.2	4.2	4.5
NDF, % as fed	6.7	8.8	12.6	16.2
Calcium, % as fed	0.85	0.69	0.80	0.60
Phosphorous, % as fed	0.65	0.65	0.67	0.60
Magnesium, % as fed	0.15	0.17	0.17	0.16
Potassium, % as fed	0.70	0.81	0.78	0.76
Sodium, % as fed	0.24	0.22	0.21	0.23
Iron, ppm	272	236	269	207
Zinc, ppm	173	171	211	151
Copper, ppm	252	155	168	134
Manganese, ppm	44	43	48	39
Molybdenum, ppm	1.7	1.0	2.0	2.1

[1]Diets were formulated to contain 1.25% lysine

[2]Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine·HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B-12 , 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO , 21 mg Fe as $FeSO_4$, 9 mg Cu as $CuSO_4$, 21 mg Mn as $MnSO_4$, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na_2SeO_3.

Table 4: Chemical analysis of test ingredients

	Test Ingredient		
	DDGS	**60% CGM**	**HPDDG**
Nutrient, %			
Dry Matter	85.4	92.1	89.8
Crude Protein	24.9	64.5	26.1
ADICP	1.9	NA[1]	1.8
ADF	11.5	3.9	15.0
NDF	27.5	6.8	35.1
Calcium	0.39	0.05	0.05
Phosphorous	0.73	0.46	0.39
Magnesium	0.48	0.06	0.14
Potassium	1.04	0.21	0.41
Sodium	0.44	0.03	0.08
Iron, ppm	272	108	66
Zinc, ppm	552	36	41
Copper, ppm	40	3	8
Manganese, ppm	534	8	10
Molybdenum, ppm	1.1	0.8	0.9
Aflatoxin, ppb	0.0	NA[1]	0.6
Vomitoxin, ppb	4000	NA[1]	0
Zearolenone, ppb	138	NA[1]	0
T-2 Toxin, ppb	0	NA[1]	0

[1]ADICP and mycotoxin content was not analyzed for CGM.

Table 5: Effect of DDGS inclusion on performance and diet preference

	DDGS comparison (% included-0 Control)					Contrast	
Item	**0-0**	**10-0**	**20-0**	**30-0**	**SEM**	**Linear**	**Quadratic**
Initial wt., kg	11.58	11.62	11.32	12.01	0.21	0.314	0.137
Final wt., kg	12.66	12.85	12.42	12.99	0.28	0.651	0.503
ADG, kg/d	0.54	0.62	0.55	0.49	0.06	0.478	0.290
ADFI, kg/d	0.75	0.80	0.75	0.78	0.03	0.857	0.696
G/F	0.74	0.77	0.71	0.67	0.07	0.398	0.593
Preference, %[a]							
Day 1	47.99	41.40	26.94[†]	16.53[†]	6.71	<0.001	0.857
Day 2	52.04	29.43[†]	27.98[†]	18.78[†]	7.22	<0.001	0.047
Overall	50.02	34.84[†]	26.42[†]	16.33[†]	6.36	<0.001	0.242

[a]Preference is expressed as the intake of the test diet as a percent of total intake

[†]Denotes significant preference different from 50% at P<0.01

*Denotes significant preference different from 50% at P<0.05

Table 6: Effect of CGM inclusion on performance and diet preference

	CGM comparison (% included-0 Control)					Contrast	
Item	**0-0**	**5-0**	**10-0**	**15-0**	**SEM**	**Linear**	**Quadratic**
Initial wt., kg	9.62	9.44	9.65	9.64	0.30	0.836	0.774
Final wt., kg	10.57	10.41	10.71	10.76	0.34	0.576	0.759
ADG, kg/d	0.48	0.49	0.53	0.56	0.05	0.231	0.847
ADFI, kg/d	0.64	0.61	0.66	0.69	0.04	0.265	0.374
G/F	0.73	0.78	0.78	0.76	0.07	0.791	0.586
Preference, %[a]							
Day 1	35.29[b]	32.64[†]	32.12[†]	27.18[†]	6.20	0.020	0.344
Day 2	29.70[b]	27.17[†]	34.75*	31.91*	7.18	0.157	0.178
Overall	29.66[b]	29.56[†]	33.40*	29.83[†]	6.20	0.056	0.200

[a]Preference is expressed as the intake of the test diet as a percent of total intake

*Denotes significant preference different from 50% at P<0.05

[†]Denotes significant preference different from 50% at P<0.01

[b]Control versus control shows significant difference from 50% at P<0.05

Table 7: Effect of HPDDG inclusion on performance and diet preference

	HPDDG comparison (% included-0 Control)					Contrast	
Item	**0-0**	**10-0**	**20-0**	**30-0**	**SEM**	**Linear**	**Quadratic**
Initial wt., kg	10.91	10.86	10.76	10.82	0.27	0.752	0.854
Final wt., kg	11.61	11.48	11.62	11.59	0.32	0.965	0.874
ADG, kg/d	0.35	0.31	0.43	0.39	0.05	0.289	0.989
ADFI, kg/d	0.62	0.59	0.62	0.63	0.04	0.616	0.586
G/F	0.54	0.45	0.67	0.61	0.07	0.193	0.859
Preference, %[a]							
Day 1	49.88	23.29[†]	22.85[†]	15.31[†]	5.36	<0.001	0.027
Day 2	60.84[a]	23.43[†]	18.06[†]	13.26[†]	5.24	<0.001	0.039
Overall	55.96	22.43[†]	19.48[†]	13.24[†]	4.29	<0.001	0.006

[a]Preference is expressed as the intake of the test diet as a percent of total intake

[†]Denotes preference different from 50% at P<0.0001

[a]Control versus control shows significant difference from 50% at P<0.05

-		-		-		-
		-		-		
10	20	exp		c	21	11
	19	c		exp	22	
9	18	c		c	23	12
	17	c		exp	24	
8	16	c		c	25	13
	15	exp		c	26	
7	14	c		exp	27	14
	13	exp		c	28	
6	12	c		exp	29	15
	11	exp		c	30	
5	10	c		exp	31	16
	9	c		c	32	
4	8	exp		c	33	17
	7	c		c	34	
3	6	exp		c	35	18
	5	c		exp	36	
2	4	exp		c	37	19
	3	c		exp	38	
1	2	c		c	39	20
	1	c		exp	40	
-		-		-		-
		-		-		

Door

Figure 1: Basic room setup for Chapter 2 experiments

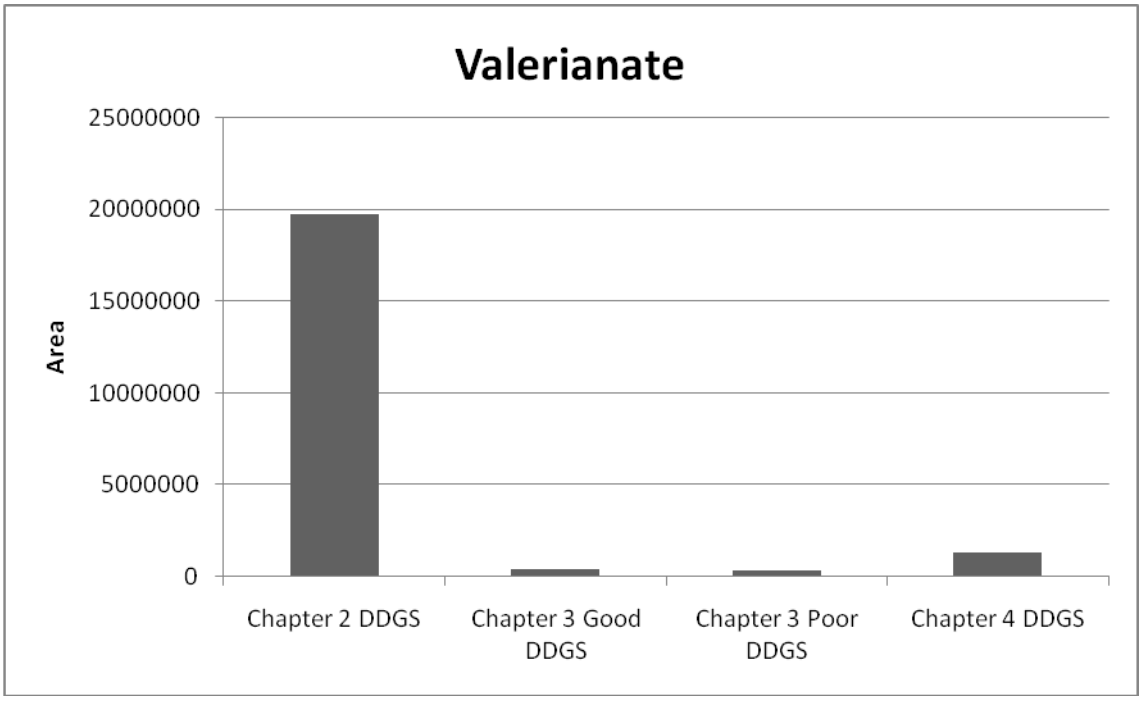

Figure 2: Valerianic Aldehyde concentration in test ingredients

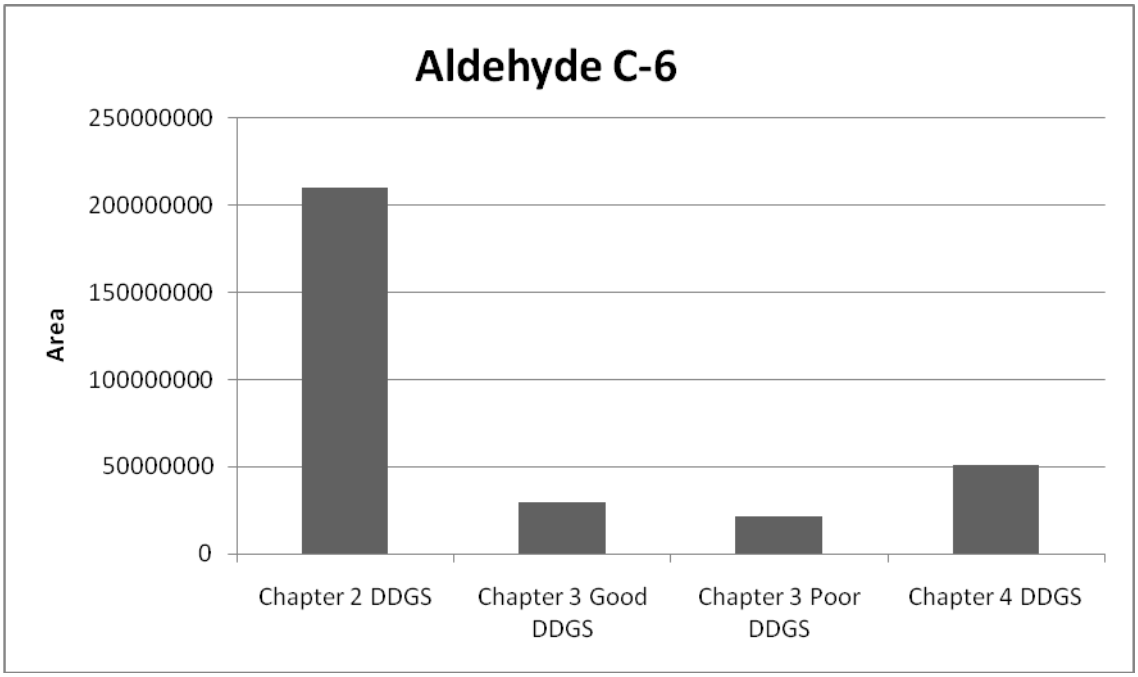

Figure 3: Aldehyde C-6 concentration in test ingredients

Chapter 3: Feed preferences in nursery pigs fed diets containing different qualities of dried distillers grains with solubles.

Abstract

Two experiments were conducted to evaluate nursery pig preference of diets containing dried distillers grains with solubles (DDGS) of different quality and levels of inclusion. At weaning, pigs were adjusted to a commercial diet (without DDGS) for at least 10 d and subsequently housed individually. Each pen contained two identical feeders positioned side by side and preference was measured for two days. In Exp. 1, 80 pigs (10.3 ± 0.20 kg BW) were given a choice between a control diet (0% DDGS) and a diet containing either 0% DDGS, 30% good quality DDGS or 30% poor quality DDGS. Quality was determined by odor and color of the DDGS**.** In Exp. 2, 80 pigs (11.2 ± 0.18 kg BW) were given a choice between a control diet without DDGS and a diet containing either 10% or 20% good quality, or 10% or 20% poor quality. Preference was calculated as intake of the test diet as a percentage of total intake. In Exp. 1, only preference of poor quality DDGS over good quality DDGS each at 30% of the diet was different from 50% at day 1, day 2 and overall, indicating a higher preference for the poor quality DDGS diet over the high quality DDGS diet ($P<0.05$). Preferences of the control diet over good and poor quality DDGS containing diets were not significant. In Exp. 2, preferences were less than 50% for the 10% ($P<0.05$) and 20% ($P<0.01$) good quality DDGS over the control diet on day 1. Preferences were less

than 50% for the 20% good quality DDGS (P<0.05) and the 20% poor quality DDGS (P<0.01) on day 2 and overall. Overall, control preference over 10% good and poor quality DDGS was not different from 50%. Quality of DDGS may be a preference-influencing factor, along with inclusion level, with poor quality DDGS being more palatable than good quality DDGS.

Introduction

The nursery phase is a stressful time during which a piglet leaves its mother, experiences a diet and location change, and is mixed with pigs from other litters often resulting in fighting and establishment of hierarchy. During this process, feed intake is typically drastically reduced (Liebrandt et al., 1975), often resulting in wasting pig syndrome (Pluske et al., 1997). Increasing feed intake is a way for pigs to recover from this decrease in performance.

In practice, quality of DDGS is often determined by color and odor. DDGS color can be affected by initial grain color, amount of solubles added, and drying time and temperature (US Grains Council, 2008). Typically, DDGS dark in color also exhibit a burned, smoky odor, likely caused by overheating in the drying process of DDGS production (Cromwell et al., 1993). As with other protein sources, this overheating causes formation of Maillard reaction products, yielding a decrease in available lysine and explaining the lower analyzed lysine concentrations found in dark colored DDGS (Cromwell et al., 1993). Analyzed lysine concentrations vary among samples of DDGS because of the variation in

drying time and temperature among DDGS producers (US Grains Council, 2008). Several amino acids, such as arginine and cysteine, also seem to be related to DDGS color (Cromwell et al., 1993). Cromwell et al. (1993) found a significant correlation between subjective color score and lightness/darkness, and growth rate and feed to gain in chicks. They found that chicks fed diets containing dark, smoky DDGS demonstrated lower growth rates than those fed “normal” colored DDGS (Cromwell et al., 1993). This indicates that color analysis is an appropriate way to distinguish between good and poor quality DDGS, and agrees with results obtained by Ergul et al. (2003) who found color to be a quick, efficient method of determining DDGS quality. Color is measured using the Hunter and Minolta colorimeters. Color is defined by three factors, L*, a* and b*, and each is measured on a scale from 0 to 100. The L* score determines darkness to lightness (0 to 100). The a* score determines yellow to redness (0 to 100). The b* score determines blueness to greenness (0 to 100) (US Grains Council, 2008). Ergul et al. (2005) found that L* and b* scores were significantly correlated with lysine, cystine and threonine digestibilities, but a* scores were not.

The purpose of this study was to determine preferences of pigs for good or poor quality DDGS compared to diets without DDGS. It was hypothesized that preference would be lower in pigs fed DDGS, with poor quality DDGS being the least preferred.

Materials and Methods

Experiment 1

Pigs were weaned at 21 days of age and housed approximately eight pigs per pen in a nursery room with 12 pens. After a 2-week nursery period (to ensure adequate feed consumption of a complex starter diet containing corn and soybean meal), 20 healthy pigs were selected and moved to a nursery room with 20 pens (1.73 m × 0.83 m). Each pen contained two identical feeders (side-by-side) and housed one pig. Comparisons included control versus control, control versus 30% good quality DDGS, control versus 30% poor quality DDGS and the good quality versus poor quality DDGS diets, resulting in 4 possible comparisons. The position of the feeders was alternated from each group of 4 comparisons to the next group to minimize side preferences (see Figure 1). Thus, 10 of the feeders containing the control diet were positioned on the left side of the pen and 10 feeders containing the control diet were positioned on the right side of the pens. In addition, comparisons were assigned to the pens such that each comparison occurred in each pen at least once during the experiment. Pigs were allowed to consume feed freely from either feeder for 48 hours and feed disappearance was measured after 24 hours and again at the end of the 48 hour trial. Pigs were weighed at the onset of the trial and again at the end of 48 hours. Pigs were returned to the original nursery room at the end of the experiment. This process was repeated 4 times using a total of 80 pigs (10.3 ± 0.20 kg BW), resulting in 20 pigs per comparison.

Experiment 2

Pigs were weaned at 21 days of age and housed approximately eight pigs per pen in a nursery room with 12 pens. After a 2-week nursery period (to ensure adequate feed consumption of a complex starter diet containing corn and soybean meal), 20 pigs were selected and moved to a nursery room with 20 pens (1.73 m × 0.83 m). Each pen contained two identical feeders (side-by-side) and housed one pig. One feeder contained a diet with 0% DDGS as a control and the other feeder contained a diet with either 0%, 10% good quality DDGS, 10% poor quality DDGS, 20% good quality DDGS or 20% poor quality DDGS, resulting in 5 possible comparisons. Good quality DDGS were obtained from a new technology DDGS producer known to produce high quality DDGS, while poor quality DDGS were obtained from an old technology producer known for poor quality DDGS. Quality was also assessed by Hunter Minolta L, a, and b scores. The position of the feeders was alternated from each group of 5 comparisons to the next group to minimize side preferences (see Figure 4). Thus, 8 of the feeders containing the control diet were positioned on the left side of the pen and 8 feeders containing the control diet were positioned on the right side of the pens. Procedures were the same as in experiment 1. This process was repeated 4 times using a total of 80 pigs (11.2 ± 0.18 kg BW), resulting in 16 pigs per comparison.

Diets

All diets were mixed at the NC State University Grinnells laboratory using a common basal, formulated for lysine, and presented in mash form. Diets were analyzed at DairyOne Forage Laboratory Services in Ithaca, NY. Their ingredient and chemical compositions can be found in tables 8 and 9. Representative samples of the test ingredients used in this study were obtained and analyzed for crude protein, acid detergent insoluble crude protein (ADICP), ADF, NDF, calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, and molybdenum. Additionally, samples of DDGS were analyzed for aflatoxin, vomitoxin (DON), zearolenone and T-2 toxin (Table 10). Sensorial testing of volatile compounds via gas chromatography and headspace analysis was performed on the DDGS samples. A 2-gram sample of each DDGS or HP DDG sample was placed into a 20 ml vial and extracted by solid phase microextraction (SPME) fibers. After 30 minutes of extraction of the volatile compounds from the headspace of the sample onto the fiber, the fiber was automatically transferred into a gas chromatograph (GC) for 10 minutes of desorption of the compounds followed by separation into a Supelcowax capillary chromatographic column in an Agilent 6890 chromatograph, resulting in identification and quantification by mass spectrometry. Finally, Hunter Minolta color scores were assigned to each DDGS source for comparison.

Statistical Analysis

Data were analyzed using the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC). Preference was calculated as:

$$\text{Preference} = \frac{\text{Intake of Test Diet}}{\text{Total Intake}}(100)$$

Therefore, preference values ranged from 0 to 100% and a value of 50% indicated no preference. The model included block and diet as main effects. Preference values were compared to the 50% no-effect level by t-test and differences from 50% were interpreted as preference over the comparison diet. Significance was declared at $P<0.05$.

Results

Experiment 1

Two-day performance and preference results are shown in Table 11. Performance parameters were not significantly affected by DDGS quality. On days 1, 2 and overall, only preference for 30% poor quality DDGS over 30% good quality DDGS was different from 50% (all $P<0.05$), with poor quality DDGS being more highly preferred. Comparisons between the control diet and 30% DDGS containing diets were not significantly different from 50%.

Experiment 2

Two-day performance and preference results are shown in Table 12. Performance parameters were not significantly affected by inclusion level or quality of DDGS, except there was a quadratic relationship between ADG ($P=0.05$)

and ADFI (P=0.03), and increasing inclusion of poor quality DDGS. On day 1, preferences for diets containing 10% and 20% good quality DDGS, and 20% poor quality DDGS, were significantly lower than 50%. On day 2 and overall, only preference for the 20% good and poor quality DDGS diets was lower than 50%. Preference values for 20% good DDGS on day 1, day 2 and overall were 21.83%, 28%, and 25.3%, respectively, whereas values for 20% poor quality DDGS preference on day 1, day 2 and, overall were 37.36%, 36.78%, and 36.28%, respectively. On day 1, 2 and overall, there was a linear decrease (P<0.01) in preference for 0%, 10% and 20% good quality DDGS. A tendency for a linear decrease (P<0.07) was also found on day 2 and overall for preference of 0%, 10% and 20% poor quality DDGS diets. The control versus control comparison for day 1 and overall showed significant differences from 50%.

Discussion and Conclusions

It has been shown that pigs are often able to select diets that contain nutrients they need. Ettle and Roth (2004) found that nursery pigs were able to distinguish between a diet deficient in tryptophan and one with adequate tryptophan and choose the latter. In experiment 1, nursery pigs favored a poor quality DDGS containing diet over a diet containing high quality DDGS. The poor quality DDGS was darker in color, which typically is caused by overheating during the drying process. As overheating causes Maillard reactions, it could be assumed that this source of DDGS would be lower in digestible lysine. Ergul et al. (2003)

found that DDGS low in quality and dark in color contained approximately 0.38% digestible lysine, while lighter colored high quality DDGS contained 0.65% digestible lysine in poultry. Cromwell et al. (1993) found that subjective color score and Hunterlab L (lightness/darkness) scores were highly correlated with growth rate and feed efficiency in chicks, and that chicks can serve as models for nutritional value of DDGS in pig diets. The poor quality DDGS source had Hunterlab scores of 31.98, 13.08, and 35.69 for L, a, and b, respectively, whereas scores for the good quality DDGS source were 51.59, 11.23, and 48.69 for L, a, and b, respectively, indicating that the poor source was the darker, redder source. Results from experiment 1 show that pigs preferred the darker DDGS assumed to be lower in digestible lysine. The reason for this is unclear. Stein et al. (2005) found correlation coefficients for the relationship between ileal digestibility of lysine in the pig and color of DDGS, determined by the Hunter and Minolta L.a.b. score, to be only around 0.50, indicating that color may not be an effective way to characterize DDGS quality. DDGS replaced only corn and soybean meal in the DDGS containing diets to avoid dietary differences in other ingredients which could affect preference, such as specific amino acid, mineral and fat content.

After performing gas chromatography and headspace analysis of the 2 sources of DDGS, differences between the good and poor quality samples are represented in Figure 5, along with results from the DDGS source described in Chapter 2 for comparison. It is clear that the main difference between the good

and poor quality DDGS is the concentration of furfural, an aromatic compound often identified by its burned, smoky, almond aroma. In the current study pigs preferred the diet high in furfural (poor quality DDGS) over the diet much lower in this volatile compound. The reason for this is unclear.

Unlike the previous study (Chapter 2), experiment 1 shows that there was no difference in preference between the control diet and diets containing 30% DDGS, regardless of quality. In Chapter 2, inclusion of DDGS at a level as low as 10% yielded a significant preference for the control diet without DDGS. Figure 5 shows a partial comparison of volatile components between the good and poor quality DDGS used in the current study, and the DDGS from Chapter 2, which was highly unpalatable. Major differences between these sources include valerianate, aldehyde C-6, amyl alcohol, and furfural. In the DDGS sample from Chapter 2, flavor characterizing volatile compounds seemed to be valerianate, aldehyde C-6 and amyl alcohol. Valerianate and aldehyde C-6 contribute a rancid aroma to the sample. In rats, rancid oil is less preferred than fresh oil (Kimura et al., 2004). Amyl alcohol contributes a fermented, yeasty note to this sample that is not as evident, quantitatively, in the other DDGS samples. Lawlor et al. (2002) compared weanling pig feed intake of a control dry diet to an acidified liquid diet and a fermented liquid diet. They found that dry matter intake was higher for the fermented liquid diet than the control diet. If this greater intake is related to palatability, it would indicate that fermented volatile compounds may increase

preference, which would disagree with results found in the current study. However, preference could also have been a result of diet texture. Furfural was much higher in the poor quality DDGS sample than in the DDGS source used in Chapter 2 or the good quality DDGS source used in the present study. This compound contributes a burned, smoky aroma to the sample. Based on sensorial analysis in the current study, it seems that rancid, yeasty volatile compounds present in pig feed may decrease palatability, while burnt, smoky notes may increase palatability.

In experiment 2, inclusion of good quality DDGS linearly decreased ($P < 0.01$) preference on day 1, 2, and overall (Table 12). For the poor quality DDGS, inclusion of 20% resulted in a reduction of preference compared to the 50% no effect level. These results disagree with results from the first experiment, where inclusion of 30% DDGS had no effect on preference. The sources of DDGS were the same in both experiments. The impact of good DDGS on preference was greater compared to the poor DDGS, indicating that poor DDGS may have a higher preference compared to good DDGS. This is in agreement with experiment 1, in which poor DDGS was preferred over good DDGS when the direct comparison was made. Also, a validation test was performed on these experimental data to check for potential confounding. To accomplish this, right feeder feed intake was divided by left feeder feed intake for an entire replication of all 5 comparisons. For the next 5 comparisons, the side of control feeder was switched, and left feeder feed intake was divided by right feeder feed intake. For

validation, control versus control preferences were also calculated in this way. In this particular experiment, the validation test showed significant differences from 50% on day 1 and overall, indicating a problem with the design of the experiment. This could potentially be attributed to social interaction of the pigs in the study. Because they were housed in pens adjacent to other pigs, they may have preferred feeders closer to or furthest away from other pigs, regardless of diet preference. It may have been beneficial to have an empty pen between each animal, so that close contact with other pigs would not have been an option.

Conclusion

Results from this study indicate that quality of DDGS may be a preference-influencing factor, in addition to inclusion level. However, poor quality DDGS may be preferred over good quality DDGS when included at 30% of the diet. Further research on the effect of different inclusion levels of good and poor quality DDGS compared to one another is needed. Also, factors that may help predict palatability should be studied and defined.

It is important to understand palatability of certain ingredients in diets fed to livestock animals. Deeper understanding of such concepts will allow further research on chemicals or ingredients that may be successful in anorexia recovery during the weaning phase, when stress levels are heightened and feed intake is depressed. More palatable ingredients and additives can make a sizable difference in pork production.

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Table 8: Composition of good and poor quality dried distillers grain with solubles (DDGS) containing diets (Experiment 1)

	DDGS quality		
	0	**30% "Good"**	**30% "Poor"**
Ingredient, % as fed			
Corn	63.9	41.0	41.0
Soybean meal	30.0	22.9	22.9
Corn oil	2.5	2.5	2.5
Corn DDGS	0	30	30
Dicalcium phosphate	1.59	1.59	1.59
Calcium carbonate	0.88	0.88	0.88
Salt	0.50	0.50	0.50
Vitamin-mineral premix	0.25	0.25	0.25
L-Lys	0.24	0.24	0.24
Copper sulfate	0.08	0.08	0.08
DL-Met	0.04	0.04	0.04
L-Thr	0.002	0.002	0.002
Analyzed Nutrient Composition			
Dry Matter, % as fed	91.6	89.0	88.1
Crude Protein, % as fed	18.8	21.9	21.5
ADICP, % as fed	ND*	0.6	0.4
ADF, % as fed	4.6	7.1	7.0
NDF, % as fed	8.4	14.0	14.0
Calcium, % as fed	0.87	0.78	0.73
Phosphorus, % as fed	0.71	0.79	0.74
Magnesium, % as fed	0.17	0.22	0.20
Potassium, % as fed	0.81	0.82	0.79
Sodium, % as fed	0.21	0.21	0.22
Iron, ppm	321	271	246
Zinc, ppm	230	185	182
Copper, ppm	209	218	192
Manganese, ppm	50	45	44
Molybdenum, ppm	0.9	1.3	1.3

*ND-ADICP not determined for diets containing no DDGS.
[1]Diets were formulated to contain 1.25% lysine
[2]Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine·HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B-12 , 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO , 21 mg Fe as $FeSO_4$, 9 mg Cu as $CuSO_4$, 21 mg Mn as $MnSO_4$, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na_2SeO_3.

Table 9: Composition of good and poor quality dried distillers grain with solubles (DDGS) containing diets at different inclusion levels (Experiment 2)

	DDGS quality and inclusion level, %				
	0	**10 Good**	**10 Poor**	**20 Good**	**20 Poor**
Ingredient, % as fed					
Corn	63.9	56.3	56.3	48.7	48.7
Soybean meal	30.0	27.6	27.63	25.3	25.3
Vegetable fat	2.5	2.5	2.5	2.5	2.5
Corn DDGS	0	10	10	20	20
Dicalcium phosphate	1.59	1.59	1.59	1.59	1.59
Calcium carbonate	0.88	0.88	0.88	0.88	0.88
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix	0.25	0.25	0.25	0.25	0.25
L-Lys	0.24	0.24	0.24	0.24	0.24
Copper sulfate	0.08	0.08	0.08	0.08	0.08
DL-Met	0.04	0.04	0.04	0.04	0.04
L-Thr	0.002	0.002	0.002	0.002	0.002
Analyzed Nutrient Composition					
Dry Matter, % as fed	91.1	90.2	90.4	91.0	90.4
Crude Protein, % as fed	20.3	21.7	20.5	22.7	20.9
ADF, % as fed	4.0	4.7	4.1	5.6	6.4
NDF, % as fed	9.4	9.9	9.1	11.6	11.9
Calcium, % as fed	0.77	0.58	0.72	0.82	0.81
Phosphorus, % as fed	0.68	0.53	0.68	0.75	0.71
Magnesium, % as fed	0.17	0.15	0.19	0.21	0.20
Potassium, % as fed	0.84	0.76	0.81	0.97	0.81
Sodium, % as fed	0.19	0.21	0.20	0.26	0.21
Iron, ppm	273	188	255	271	279
Zinc, ppm	187	170	256	253	270
Copper, ppm	152	150	185	201	205
Manganese, ppm	54	37	63	53	56
Molybdenum, ppm	1.5	0.8	1.3	1.2	0.3

[1]Diets were formulated to contain 1.25% lysine

[2]Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine·HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B-12 , 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO , 21 mg Fe as $FeSO_4$, 9 mg Cu as $CuSO_4$, 21 mg Mn as $MnSO_4$, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na_2SeO_3.

Table 10: Chemical analysis of test ingredients

	Test Ingredient	
	"Good" DDGS	**"Poor" DDGS**
Nutrient, %		
Dry Matter	89.4	88.4
Crude Protein	29.6	24.9
ADICP	1.1	2.1
ADF	13.3	13.0
NDF	27.7	27.1
Calcium	0.03	0.07
Phosphorous	0.76	0.72
Magnesium	0.34	0.31
Potassium	0.90	0.78
Sodium	0.13	0.15
Iron, ppm	79	85
Zinc, ppm	91	82
Copper, ppm	5	4
Manganese, ppm	16	16
Molybdenum, ppm	1.1	1.1
Aflatoxin, ppb	6	5
Vomitoxin, ppb	0	0
Zearolenone, ppb	0	0
T-2 Toxin, ppb	0	0

Table 11: Effect of DDGS quality on performance and diet preference

Item	Distillers dried grains with solubles Quality Comparisons at 30% Inclusion			
	Control vs. Control	"Good" vs. Control	"Poor" vs. Control	"Poor" vs. "Good"
Initial wt., kg	10.18	10.24	10.29	10.28
Final wt., kg	11.10	11.37	11.26	11.08
ADG, kg/d	0.46	0.57	0.49	0.40
ADFI, kg/d	0.65	0.67	0.67	0.59
G/F	0.73	0.89	0.76	0.74
Preference, %[a]				
Day 1	43.78	51.85	52.10	65.28*
Day 2	43.04	48.75	48.46	67.96[†]
Overall	43.30	50.44	49.45	67.59[†]

[a]Preference is expressed as the intake of the test diet as a percent of total intake.

*Denotes significant difference (P<0.05) from 50% preference.

[†]D enotes significant difference (P<0.01) from 50% preference.

Table 12: Effect of DDGS quality and inclusion rate on performance and diet preference

	Good quality DDGS inclusion rate, %					
Item	**0**	**10**	**20**	**SEM**	**Linear**	**Quadratic**
Initial wt., kg	11.07	11.16	11.08	0.31	0.983	0.833
Final wt., kg	11.98	11.86	11.92	0.35	0.900	0.827
ADG, kg/d	0.45	0.35	0.42	0.04	0.519	0.075
ADFI, kg/d	0.66	0.57	0.58	0.03	0.097	0.186
G/F	0.80	0.59	0.71	0.08	0.406	0.111
Preference, %[a]						
Day 1	61.47[b]	38.18*	21.83[†]	5.24	0.009	0.323
Day 2	61.20	43.96	28.00[†]	6.22	0.003	0.746
Overall	62.47[b]	40.32	25.30[†]	5.58	0.001	0.798
	Poor quality DDGS inclusion rate, %					
Item	**0**	**10**	**20**	**SEM**	**Linear**	**Quadratic**
Initial wt., kg	11.07	11.27	11.15	0.29	0.849	0.664
Final wt., kg	11.98	12.00	12.09	0.32	0.810	0.927
ADG, kg/d	0.45	0.37	0.47	0.04	0.774	0.046
ADFI, kg/d	0.66	0.53	0.62	0.04	0.503	0.026
G/F	0.80	0.65	0.80	0.10	0.979	0.215
Preference, %[a]						
Day 1	61.47	51.25	37.36*	5.92	0.613	0.110
Day 2	61.20	53.27	36.78*	5.75	0.044	0.341
Overall	62.47[b]	51.99	36.28*	5.43	0.066	0.276

[a]Preference is expressed as the intake of the test diet as a percent of total intake

*Denotes significant preference different from 50% at P<0.05

[†]Denotes significant preference different from50% at P<0.01

[b]Control versus control shows significant difference from 50% at P<0.05

-		-		-		-
		-		-		
10	20	c		c	21	11
	19	exp		c	22	
9	18	c		c	23	12
	17	exp		exp	24	
8	16	c		c	25	13
	15	exp		exp	26	
7	14	c		c	27	14
	13	exp		exp	28	
6	12	c		c	29	15
	11	c		exp	30	
5	10	exp		c	31	16
	9	c		c	32	
4	8	exp		exp	33	17
	7	c		c	34	
3	6	exp		exp	35	18
	5	c		c	36	
2	4	exp		exp	37	19
	3	c		c	38	
1	2	c		exp	39	20
	1	c		c	40	
-		-		-		-
		-		-		

Door

Figure 4: Basic room setup for Chapter 3, Exp. 2

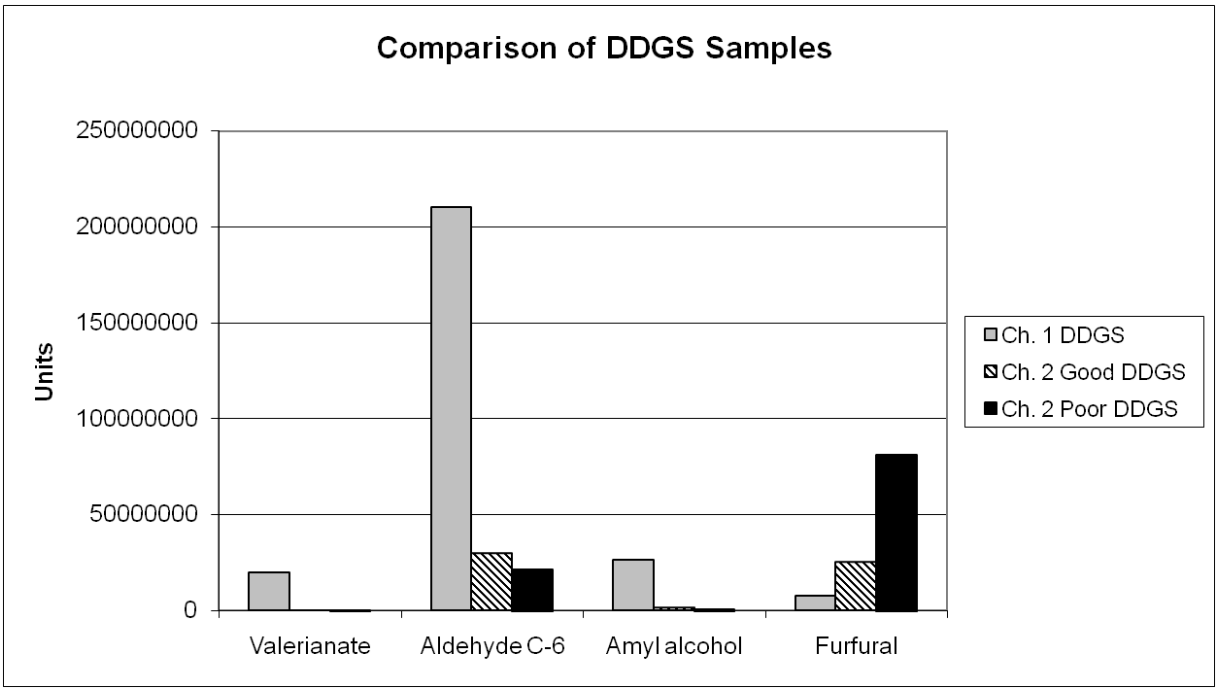

Figure 5: Comparison of DDGS from Chapters 2 and 3

Chapter 4: Effects of dried distillers grains with solubles and flavoring on performance and feed preferences in nursery pigs

Abstract

Two experiments were conducted to evaluate the effect of adding flavor to DDGS containing diets on nursery pig performance and diet preference. In Exp. 1, 192 pigs (6.7 ± 0.10 kg BW) were allocated to 48 pens with 4 pigs each, blocked by weight. Pens were randomly assigned 1 of 6 treatments in a 2x3 factorial design; 0% DDGS, 0% DDGS with flavor, 10% DDGS, 10% DDGS with flavor, 20% DDGS, 20% DDGS with flavor. Pens assigned treatments with flavor received a flavored Starter 1 diet lacking DDGS for 7 days. Pens assigned treatments without flavor received a non-flavored, complex Starter 1 diet lacking DDGS for 7 days. On day 7, a diet phase change occurred, and Starter 2 diets were presented. On day 21, another diet phase change occurred, and Starter 3 diets were presented until the end of the trial at day 35. Starter 2 and 3 diets included DDGS at either 0, 10, or 20% and were less complex than Starter 1, due to the increased ability for pigs to digest more economical diets. Pigs were weighed and feed intake was measured weekly. In Exp. 2, 108 pigs (9.0 ± 0.16 kg BW) were given a choice between a control diet (0% DDGS and no flavor) and a diet containing either 0% without flavor, 0% with flavor, 10% without flavor, 10% with flavor, 20% without flavor or 20% with flavor. Feed disappearance was measured for two days and preference was calculated as intake of the test diet as

a percentage of total intake. In Exp. 1, average daily gain (ADG) tended to decrease with DDGS inclusion during the Starter 2 phase (P=0.06). Average daily feed intake (ADFI) tended to decrease (P=0.10) with DDGS inclusion during week 3 and was significantly decreased (P=0.03) during the overall Starter 2 phase. Feed efficiency (G/F) was significantly increased (P=0.01) with DDGS during week 4, but tended to decrease during week 5 (P=0.08). ADFI was significantly increased with flavor supplementation only during the Starter 1 phase (P=0.02). In experiment 2, preference for the non-flavored diet containing 20% DDGS was lower (P<0.01) than 50% on day 1, day 2 and overall. Preference for flavored diets at all inclusion levels was lower (P<0.05) than 50% on day 1, day 2 and overall, regardless of inclusion level of DDGS. DDGS may have a negative effect on ADG and ADFI when first introduced into the diet. Flavor may improve ADFI immediately after weaning.

Introduction

Feed flavors have been used in nursery pig diets since the 1960s as palatability enhancers and feed attractants (Torrallardona et al., 2000). This can be especially important for piglets after weaning, when stress is heightened. During this phase, pigs often reduce feed intake and growth performance until they are able to adapt to their new surroundings. McLaughlin et al. (1983) found that pigs preferred 3 of 5 flavors tested. The flavors preferred were cheesy, meaty and sweet, and sweet molasses caramel. Tested flavors that were not preferred were very sweet and meaty buttery.

While it is clear that weanling pigs often show a preference for a diet when given a choice, results vary for growth performance of pigs fed a single diet containing flavors or sweeteners versus pigs fed diets without flavors. In a 5-week performance study applying preferred flavors, McLaughlin et al. (1983) found that cumulative daily feed intake and body weight gain were significantly increased only during the first week of the study. In another study with more pigs, the cheesy flavored diet improved feed intake in piglets during week 2.5 to 3.5 (McLaughlin et al., 1983). Kornegay et al. (1979) found that performance of pigs fed diets with preferred flavors and given no choice was similar to performance of pigs fed diets without flavors.

Studies have shown the DDGS are a reasonable source of protein for pigs. While this ingredient is somewhat high (27.7%) in crude protein (NRC, 2008), its

quality, like corn, is poor, relative to the pig's amino acid requirements (Spiehs et al., 2002). Whitney and Shurson (2004) studied the effects of DDGS inclusion on nursery pig performance and found that pigs fed up to 25% DDGS in the diet performed the same as pigs fed a control diet with no DDGS. Wahlstrom et al. (1970) found that average daily gain of growing pigs fed diets with DDGS at 0, 5, 10 and 20% were not different from each other, but that pigs fed the diet with 20% required significantly more feed per unit of gain.

The purpose of this study was to evaluate the effects of DDGS inclusion and presence of feed flavor on diet preference and growth performance of nursery pigs.

Materials and Methods

Experiment 1

A total of 192 pigs (6.7 ± 0.10 kg BW) were weaned at approximately 21 days of age. Pigs were weighed and assigned within weight block to one of 6 dietary treatments. Pigs were housed 4 pigs per pen using 48 pens (1.63 m x 0.91 m) and there were 8 replicates per treatment. Dietary treatments (Tables 13 through 15) were arranged in a 2 x 3 factorial randomized complete block design. Factors consisted of: 1) DDGS inclusion in Starter 2 and 3 diets (0, 10 or 20%), and 2) presence or absence of flavor in all 3 diet phases. Diets were formulated based on least cost. Feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good

Manufacturing Processes. A basal diet of only dry ingredients was manufactured within each level of DDGS for each diet phase. Each basal was split in half. One half received fat only (non-flavored diet) and one half received a flavor, characterized by creamy, milky cheese, sweet and vanilla volatile notes. Fat was then added to the flavored diet. All diets were presented in pelleted form.

Pigs were fed a three-phase dietary program. The first phase diet (Starter 1) was fed immediately following weaning for 7 days. Half of the pigs received a flavored Starter 1 diet, while the other half received the non-flavored basal Starter 1 diet, differing only in presence of flavor. The second and third phase diets (Starter 2 and Starter 3) were fed for 2 weeks each. Pigs that were fed the flavored Starter 1 were fed flavored Starter 2 and Starter 3 diets with either 0, 10, or 20% DDGS, while those fed non-flavored Starter feed were fed non-flavored Starter 2 and Starter 3 diets.

Pigs were weighed weekly on an individual basis throughout the 5 week period. Feed added to the feeders was recorded and feeders with remaining feed were weighed weekly to determine feed disappearance.

Experiment 2

Pigs were weaned at 21 days of age and housed approximately eight pigs per pen in a nursery room with 12 pens. After a 2-week nursery period (to ensure adequate feed consumption of the non-flavored Starter 1 diet), 18 pigs were selected and moved to a nursery room with 18 pens (1.73 m × 0.83 m). Each pen

contained two identical feeders (side-by-side) and housed one pig. One feeder contained a non flavored diet with 0% DDGS as a control and the other feeder contained a diet with either 0%, 10% or 20% DDGS, non-flavored or flavored, resulting in 6 possible comparisons. Pigs were allowed to consume feed freely from either feeder for 48 hours and feed disappearance was measured after 24 hours and again at the end of the 48 hour trial. Pigs were weighed at the onset of the trial and again at the end of 48 hours. Pigs were returned to the original nursery room at the end of the experiment. This process was repeated 6 times using a total of 108 pigs (9.0 ± 0.20 kg BW), resulting in 18 pigs per treatment comparison. The position of the feeders was alternated from each group of 6 comparisons to the next group to minimize possible side preferences (see Figure 6). Thus, half of the feeders containing the control diet were positioned on the left side of the pen and half of the feeders containing the control diet were positioned on the right side of the pens. In addition, each of the comparisons was assigned to pens such that each comparison occurred in each of the 18 pens at least one time. Experimental diets were the same as those used in experiment 1.

Chemical Analyses

Diets were analyzed at DairyOne Forage Laboratory Services in Ithaca, NY. Their ingredient and chemical compositions can be found in Tables 13 through 15. Representative samples of the test ingredients used in this study were obtained and analyzed for crude protein, acid detergent insoluble crude protein (ADICP),

ADF, NDF, calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, and molybdenum. Additionally, DDGS were analyzed for aflatoxin, vomitoxin (DON), zearalenone and T-2 toxin (Table 16). Also, sensorial testing of volatile compounds via gas chromatography and headspace analysis was performed on the test ingredients. A 2-gram sample of each DDGS sample was placed into a 20 mL vial and extracted by solid phase microextraction (SPME) fibers. After 30 minutes of extraction of the volatile compounds from the headspace of the sample onto the fiber, the fiber was automatically transferred into a gas chromatograph (GC) for 10 minutes of desorption of the compounds followed by separation into a Supelcowax capillary chromatographic column in an Agilent 6890 chromatograph, resulting in identification and quantification by mass spectrometry. Finally, Hunter Minolta color scores were assigned the DDGS source.

Statistical Analysis

Data were analyzed using the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC). The model for experiment 1 included the weight block, DDGS levels, flavor and the interaction between DDGS and flavor. In experiment 2, preference was calculated as:

$$\text{Preference} = \frac{\text{Intake of Test Diet}}{\text{Total Intake}}(100)$$

Therefore, preference values ranged from 0 to 100% and a value of 50% indicated no preference. The model included block and diet. Preference values were

compared to the 50% no-effect level by t-test and differences from 50% were interpreted as a preference over the comparison diet. Significance was declared at $P<0.05$.

Results

Experiment 1

Five-week performance results are shown in Table 17. DDGS inclusion had no effect ($P>0.123$) on body weight. Average daily gain (ADG) tended to decrease with DDGS inclusion during the Starter 2 phase ($P=0.056$). Average daily feed intake (ADFI) tended to decrease ($P=0.097$) with DDGS inclusion during week 3 and was decreased ($P=0.032$) during the overall Starter 2 phase. Feed efficiency (G/F) was increased ($P=0.013$) with DDGS during week 4, but tended to decrease during week 5 ($P=0.084$). Flavoring had no effect on body weight, ADG, or feed efficiency. ADFI was increased with flavoring only during the Starter 1 phase ($P=0.024$). There were no interactions between DDGS inclusion and flavor; however this interaction did tend to affect feed efficiency during week 3, week 4 and during the Starter 3 phase.

Experiment 2

Two-day performance and preference results are shown in Table 18. Performance parameters were not significantly affected by the treatments except for average daily feed intake of the non-flavored diets. A quadratic response ($P=0.010$) was found for this parameter with values of 0.45 kg/d, 0.32 kg/d and

0.41 kg/d for 0% DDGS, 10% DDGS and 20% DDGS within non-flavored diets, respectively. Preference for the non-flavored diet containing 20% DDGS was lower than 50% on day 1, day 2 and overall. Preference for flavored diets at all inclusion levels was lower than 50% on day 1, day 2 and overall and flavor decreased preference regardless of DDGS inclusion.

Discussion and Conclusions

Studies have shown that pigs fed isocaloric diets containing up to 25% DDGS perform the same as pigs fed control diets with no DDGS (Whitney and Shurson, 2004). In the current study, ADG and ADFI in the Starter 2 phase seem to be negatively affected by DDGS inclusion. DDGS inclusion affected feed efficiency during week 4. A similar tendency was found for week 5. However, feed efficiency for the overall starter-2 phase was unaffected. Overall, results from the present study agree with Whitney and Shurson (2004) as no significant DDGS effects were evident for any performance parameter, even though fiber content in the DDGS containing diets was higher. Studies have shown that high fiber diets negatively affect ADG, feed intake and feed efficiency of young pigs (Pond et al., 1988). These results partly agree with that, as ADG and ADFI decrease when DDGS are first added to the diet. However, as pigs grow, they are more capable of digesting these higher fiber diets, which may explain the lack of a DDGS effect during the Starter 3 phase.

ADFI was positively affected by the addition of a flavor to the diet only in the Starter 1 (week 1) phase. No other performance parameters were affected by flavor. These results partially agree with McLaughlin et al. (1983), who found that both weight gain and feed intake were only affected by the addition of flavor for the week immediately after weaning. These results indicate that flavor additives may be able to increase feed intake in pigs experiencing the new stress of weaning.

Results from experiment 2 show that preference decreased for diets with increasing inclusion of DDGS and no flavor added. This agrees with results from chapter 2 where preference linearly decreased with DDGS increasing from 0% to 30% of the diet, and with Hastad et al. (2005) who found the same. However, only preference for the control diet over the 20% DDGS unflavored diet was different from 50%, which agrees with results from chapter 2, which showed decreased preference for a diet with 10% DDGS when measured on day 1, but disagrees with overall results from chapter 2 where preference for the 10% DDGS diet was different from 50%. Analysis of the DDGS source in the present study showed that it contained 2.8 ppm vomitoxin (DON). Feeding DDGS at 30% would contribute nearly 0.56 ppm of vomitoxin to the diet. Swine are particularly sensitive to vomitoxin and reduced feed intake and weight gain can be observed after feeding 2 to 3 ppm (van Heugten, 2001); however, Smith et al. (1997) found that increasing inclusion level of vomitoxin from 0 to 1.9 ppm created a significant decreasing linear feed intake response over a three week period.

Figure 7 shows a partial comparison between volatile compounds in the DDGS source used in this study and those found in the DDGS used in chapter 2 and 3. Valerianate and aldehyde C-6 are volatile components associated with rancidity. Preference studies have shown that rancid oil is less preferred than fresh oil in rats (Greenberg et al., 1953, Kimura et al., 2004). Amyl alcohol contributes a fermented, yeasty note to the DDGS. According to Lawlor et al. (2002), fermented liquid diets increase feed intake when compared to dry diets fed to weanling pigs. Furfural contributes a burned, smoky aroma to the DDGS. The DDGS source used in the present study differs from the source used in chapter 2 in valerianate, aldehyde C-6 and amyl alcohol, which all seemed to negatively correlate with palatability. Thus, it would be expected that the current DDGS source would be more palatable than that used in chapter 2, based on these 3 components. However, the present source also differs with poor quality DDGS from chapter 3, which was preferred over good quality DDGS, in furfural content. In chapter 3, the DDGS source higher in furfural was preferred over the source lower in this component. Similar to the comparison between poor and good quality DDGS, the current DDGS source was lower in furfural, which would lead to the expectation that it would be less palatable than poor quality DDGS. Lower concentrations of valerianate, aldehyde C-6 and amyl alcohol, combined with a slightly higher concentration of furfural may explain why preference for the unflavored 10%

DDGS diet was not significantly different from 50%, while the 10% DDGS diet from chapter 2 was not preferred.

The control diet was highly preferred over all flavored diets, regardless of DDGS inclusion, indicating that the flavor may have intensified the negative impact of DDGS. This disagrees with Duran et al. (2000) who found that depression of feed intake of diets containing various inclusion levels of rapeseed meal and canola meal was recovered by addition of flavor. These results also disagree with the performance results, which indicated that feed intake was increased during the Starter 1 phase with the addition of flavor. During the Starter 1 phase of experiment 1, pigs were around 21 days of age and had just been weaned and switched to a dry diet. The pigs used for the preference study were significantly older, ranging from 31 to 49 days of age and had been consuming a dry diet for at least 10 days before the beginning of the preference study. In addition, the ingredient composition of the Starter 1 diet was much different from the diet that was used to conduct the preference study. Interactions of flavor with diet components may explain differences observed between the two experiments.

Conclusions

DDGS negatively affected preference at 20% of the diet when no flavor was added. The specific flavor used in this study negatively affected preference regardless of inclusion of DDGS. When given no choice, DDGS containing diets seem to have a negative effect on performance only in the Starter 1 phase,

possibly because pigs are able to adapt to this ingredient as they grow and as they are more capable of digesting diets higher in fiber. Addition of flavoring seems to positively affect ADFI only in the Starter 1 phase, a critical time for gut health and development of the weanling pig.

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Table 13: Composition of flavored and non-flavored Starter I diets (Experiment 1)

	Non-flavored	Flavored
Ingredient, % as fed		
Corn	46.2	46.1
Whey dried	22.5	22.5
Soybean Meal	16.1	16.1
Fish Meal	5.0	5.0
Blood Plasma Meal	4.0	4.0
Poultry Fat	2.4	2.4
Blood Cells	1.5	1.5
Limestone	0.56	0.56
Trace Mineral Premix	0.50	0.50
Monocalcium Phosphate (21%)	0.42	0.42
Zinc Oxide	0.34	0.34
Salt	0.25	0.25
Luctarom Flavoring	0	0.20
Lysine	0.13	0.13
DL-methionine	0.08	0.08
L-threonine	0.05	0.05
Analyzed Nutrient Composition		
Dry Matter, % as fed	88.3	88.3
Crude Protein, % as fed	21.4	21.7
ADF, % as fed	1.2	1.1
NDF, % as fed	5.9	4.5
Calcium, % as fed	0.78	0.81
Phosphorous, % as fed	0.66	0.67
Magnesium, % as fed	0.15	0.16
Potassium, % as fed	0.97	0.97
Sodium, % as fed	0.35	0.37
Iron, ppm	434	385
Zinc, ppm	2240	2180
Copper, ppm	21	21
Manganese, ppm	70	67
Molybdenum, ppm	1.1	1.2

Table 14: Composition of flavored and non-flavored Starter II diets with different inclusion levels of DDGS (Experiment 1)

	DDGS inclusion level (%) and flavoring (NF/F)					
	0 NF	**0 F**	**10 NF**	**10 F**	**20 NF**	**20 F**
Ingredient, % as fed						
Corn	54.8	54.7	46.9	46.8	39.1	39.0
Soybean meal	25.0	25.0	23.1	23.1	21.2	21.2
DDGS	0	0	10	10	20	20
Whey dried	10	10	10	10	10	10
Poultry fat	3.3	3.3	3.2	3.2	3.0	3.0
Fish meal	2	2	2	2	2	2
Blood Cells	1.5	1.5	1.5	1.5	1.5	1.5
Monocalcium Phosphate (21%)	1.2	1.2	1.1	1.1	0.9	0.9
Limestone	0.67	0.67	0.72	0.72	0.77	0.77
Vitamin/Mineral Premix	0.5	0.5	0.5	0.5	0.50	0.50
Salt	0.4	0.4	0.4	0.4	0.40	0.40
Lysine	0.24	0.24	0.26	0.26	0.28	0.28
Zinc Oxide	0.20	0.20	0.20	0.20	0.20	0.20
Luctarom Flavoring	-	0.20	-	0.20	-	0.20
L-threonine	0.12	0.12	0.09	0.09	0.06	0.06
DL-methionine	0.05	0.05	0.02	0.02	0.003	0.003
Analyzed Nutrient Composition						
Dry Matter, % as fed	87.6	87.9	88.7	88.8	89.3	88.2
Crude Protein, % as fed	20.0	20.0	21.5	22.1	23.0	22.2
ADF, % as fed	3.9	4.4	4.5	5.3	5.4	6.2
NDF, % as fed	7.4	6.9	8.6	8.3	10.7	9.4
Calcium, % as fed	0.93	0.83	0.52	0.73	0.86	0.82
Phosphorus, % as fed	0.70	0.69	0.46	0.68	0.74	0.71
Magnesium, % as fed	0.17	0.16	0.14	0.19	0.27	0.25
Potassium, % as fed	0.87	0.85	0.70	0.99	0.98	0.96
Sodium, % as fed	0.24	0.23	0.24	0.33	0.36	0.34
Iron, ppm	384	375	245	335	383	362
Zinc, ppm	1210	1320	1030	1370	1510	1420
Copper, ppm	20	24	17	29	25	23
Manganese, ppm	88	84	56	73	88	88
Molybdenum, ppm	1.1	1.0	0.6	1.4	1.1	1.9

Table 15: Composition of flavored and non-flavored Starter-III diets with different inclusion levels of DDGS (Experiment 1)

	DDGS inclusion level (%) and flavoring (NF/F)					
	0 NF	**0 F**	**10 NF**	**10 F**	**20 NF**	**20 F**
Ingredient, % as fed						
Corn	64.3	64.2	56.5	56.4	48.6	48.6
Soybean Meal	28.4	28.4	26.6	26.5	24.7	24.6
DDGS	0	0	10	10	20	20
Poultry Fat	3.4	3.4	3.2	3.2	3.1	3.1
Monocalcium Phosphate (21%)	1.4	1.4	1.3	1.3	1.2	1.2
Limestone	0.9	0.9	0.9	0.9	1	1
Vitamin/Mineral Premix	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Luctarom Flavoring	-	0.20	-	0.20	-	0.20
Lysine	0.27	0.27	0.29	0.29	0.32	0.32
Copper Sulfate	0.08	0.08	0.08	0.08	0.08	0.08
L-threonine	0.10	0.10	0.07	0.07	0.04	0.04
DL-methionine	0.03	0.03	0.02	0.02	-	-
Analyzed Nutrient Composition						
Dry Matter, % as fed	90.6	88.2	88.7	89.4	89.7	90.5
Crude Protein, % as fed	20.5	19.6	20.1	20.7	21.0	22.1
ADF, % as fed	5.5	6.3	3.1	4.4	5.1	4.6
NDF, % as fed	6.9	7.1	8.7	9.2	8.8	9.7
Calcium, % as fed	0.75	0.76	0.69	0.74	0.85	0.77
Phosphorus, % as fed	0.65	0.65	0.64	0.68	0.70	0.72
Magnesium, % as fed	0.16	0.18	0.19	0.21	0.24	0.24
Potassium, % as fed	0.87	0.79	0.81	0.87	0.83	0.88
Sodium, % as fed	0.24	0.23	0.23	0.26	0.26	0.29
Iron, ppm	312	270	277	280	296	303
Zinc, ppm	160	168	163	171	183	319
Copper, ppm	185	195	179	209	230	182
Manganese, ppm	54	59	58	65	78	64
Molybdenum, ppm	2.1	1.6	2.2	3.2	0.8	1.2

Table 16: Chemical analysis of DDGS

	DDGS
Nutrient, %	
Dry Matter	87.5
Crude Protein	28.3
ADICP	4.7
ADF	13.9
NDF	23.7
Calcium	0.18
Phosphorous	0.81
Magnesium	0.53
Potassium	0.93
Sodium	0.39
Iron, ppm	121
Zinc, ppm	195
Copper, ppm	12
Manganese, ppm	80
Molybdenum, ppm	1.5
Aflatoxin, ppb	0
Vomitoxin, ppb	2846
Zearolenone, ppb	102
T-2 Toxin, ppb	0

Table 17. Effect of DDGS inclusion rate and flavoring on pig performance (Experiment 1)[1]

	Nonflavored Diets			Flavored Diets						
	DDGS inclusion in S-II and S-III, %			DDGS inclusion in S-II and S-III, %				P-Values[2]		
	0	10	20	0	10	20	SEM	D	F	F*D
Body weight, kg										
Initial	6.74	6.73	6.72	6.75	6.74	6.70	0.02	0.333	0.996	0.796
Week 1	7.93	7.87	7.89	8.01	8.00	7.95	0.11	0.904	0.300	0.958
Week 2	9.02	8.94	8.86	9.20	8.95	8.96	0.13	0.311	0.381	0.819
Week 3	11.31	10.60	10.85	11.18	10.88	10.88	0.24	0.124	0.756	0.688
Week 4	14.72	14.22	14.45	14.89	14.46	14.94	0.37	0.440	0.330	0.900
Week 5	17.51	16.65	16.70	17.65	16.98	17.47	0.42	0.219	0.238	0.748
Average daily gain, g/d										
Week 1	169	164	167	181	180	179	15	0.976	0.307	0.986
Week 2	157	152	139	170	136	144	13	0.235	0.953	0.504
Week 3	326	237	284	283	277	273	25	0.177	0.805	0.247
Week 4	488	517	514	529	511	581	30	0.379	0.167	0.451
Week 5	399	347	321	395	360	360	26	0.112	0.454	0.707
Starter 2	242	195	212	226	206	209	13	0.056	0.843	0.609
Starter 3	443	432	418	462	436	471	19	0.622	0.107	0.388
Overall	308	284	285	312	293	307	12	0.230	0.237	0.732
Average daily feed intake,g/d										
Week 1	211	195	214	228	234	227	12	0.878	0.024	0.514
Week 2	233	221	216	248	229	225	11	0.230	0.235	0.953
Week 3	483	426	434	469	436	458	20	0.097	0.681	0.625
Week 4	727	671	673	721	697	732	33	0.498	0.334	0.621
Week 5	697	662	657	690	678	673	31	0.646	0.756	0.922
Starter 2	354	320	325	359	333	342	11	0.032	0.207	0.850
Starter 3	712	667	665	706	688	702	28	0.515	0.455	0.738
Overall	465	430	439	471	455	463	15	0.263	0.143	0.803
Gain/feed, g/kg										
Week 1	803	834	775	788	776	780	46	0.831	0.551	0.781
Week 2	651	679	608	680	577	638	52	0.698	0.734	0.347
Week 3	685	538	650	610	646	594	41	0.414	0.810	0.057
Week 4	665	778	766	728	726	791	26	0.013	0.579	0.088
Week 5	571	521	482	573	536	533	28	0.084	0.316	0.643
Starter 2	679	604	641	634	624	611	30	0.364	0.448	0.510
Starter 3	619	651	629	655	632	671	15	0.673	0.110	0.088
Overall	661	658	650	662	646	664	12	0.735	0.907	0.549

[1] Each value represents the mean of 8 pens with 4 pigs per pen
[2] Probability values for the effects of DDGS (D), flavoring (F), and their interaction (F*D).

Table 18: Effect of DDGS inclusion rate and flavoring on diet preference

	DDGS inclusion rate in non-flavored diets, %			DDGS inclusion rate in flavored diets, %		
Item	**0**	**10**	**20**	**0**	**10**	**20**
Initial wt., kg	8.87	8.61	8.83	9.33	9.13	9.21
Final wt., kg	9.39	9.04	9.31	10.00	9.79	9.80
ADG, kg/d	0.26	0.21	0.24	0.34	0.33	0.30
ADFI, kg/d	0.45	0.32	0.41	0.46	0.43	0.47
G/F	0.51	0.23	0.59	0.75	0.72	0.43
Preference, %[a]						
Day 1	42.91	38.82	19.82†	22.61†	15.89†	13.88†
Day 2	45.78	37.77	21.91†	32.87*	30.91*	12.53†
Overall	44.44	40.36	20.16†	28.43†	23.04†	13.35†

[a]Preference is expressed as the intake of the test diet as a percent of total intake

*Denotes significant preference different from 50% at P<0.05

†Denotes significant preference different from50% at P<0.01

-		- -		- -		-
9	18 17	c exp		exp c	19 20	10
8	16 15	c exp		exp c	21 22	11
7	14 13	c c		exp c	23 24	12
6	12 11	exp c		c c	25 26	13
5	10 9	exp c		c exp	27 28	14
4	8 7	exp c		c exp	29 30	15
3	6 5	exp c		c exp	31 32	16
2	4 3	exp c		c exp	33 34	17
1	2 1	c c		c exp	35 36	18
-		- -		- -		-
-		- -		- -		-

Door

Figure 6: Basic room setup for Chapter 4 preference study

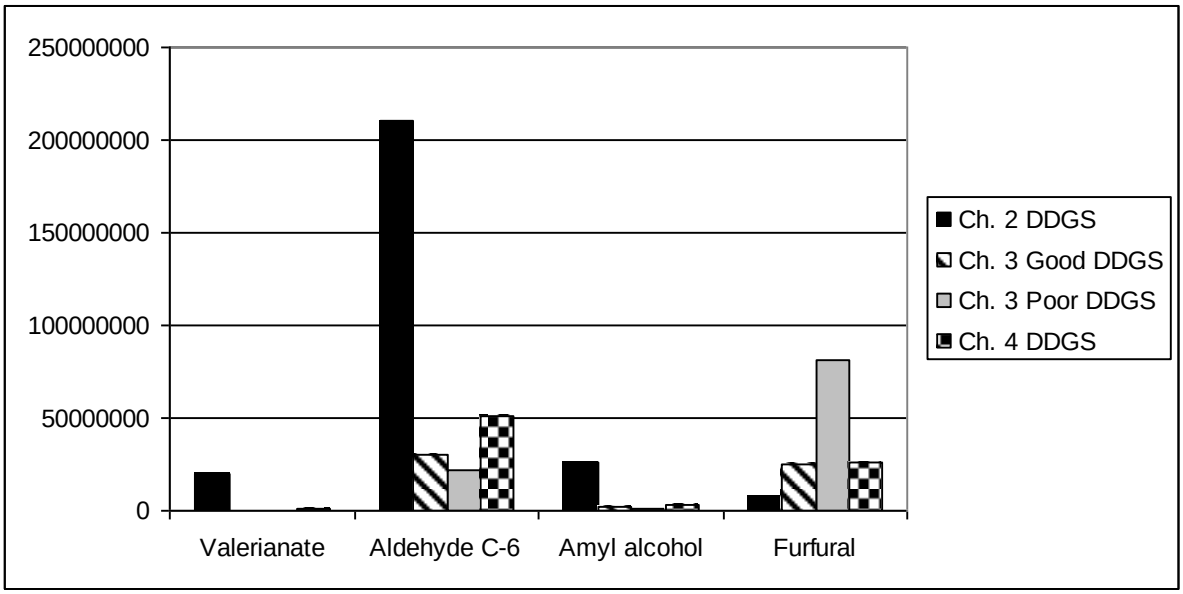

Figure 7: Comparison of DDGS from chapter 2, 3 and 4

Chapter 5: **General Summary**

The main objectives for this research were first, to determine nursery pig preference of byproduct feeds, specifically DDGS, HPDDG, and CGM. The second objective was to determine the effects of DDGS quality on preference. The third and final objective was to determine the effects of DDGS and flavor on preference and growth performance.

The first study shows evidence that DDGS and HPDDG are not highly palatable feed ingredients, as the control diet was highly preferred over diets containing these byproducts. Presence of rancid volatile components and vomitoxin may have played significant roles in decreased preference for DDGS. Preference for a control diet over a HPDDG containing diet was quite clear. Preference for CGM containing diets is less clear, due to possible confounding factors that may have affected preference.

The second study shows that DDGS quality may be a preference influencing factor. Pigs consumed more of this source of DDGS than they did in the first study, indicating that the source for the second study was more highly palatable. However, pigs seemed to prefer the darker poor quality DDGS over the good quality DDGS, even though the poor quality source is assumed to be lower in available lysine. A possible explanation for this preference is the presence of the volatile component, furfural, which is characterized by a burned, smoky volatile note.

The third study shows that DDGS can be included at 20% in nursery pig diets without affecting growth performance. A decrease in performance was shown during the first week after DDGS was included in the diet, but the animals recovered during the subsequent week and performed the same as those without DDGS. Flavor had no effect on performance except during the week immediately after weaning, when ADFI was increased. This is a critical time for gut health and development of weaned pigs. Addition of feed flavor to the diet at this stage may help to reduce the number of pig deaths that result from anorexia immediately after weaning. A diet without DDGS was preferred over the 20% DDGS diet during the preference study. However, a diet without DDGS and without flavor was highly preferred over all diets containing flavor, regardless of the level of DDGS, indicating that the specific flavor used in this study was not an effective palatability enhancer for nursery pigs.

Data from this research showed that poor quality DDGS may be more highly preferred than good quality DDGS. More research needs to be conducted to understand why that may be true. Furfural may be a component capable of enhancing palatability of unpalatable, but nutritional and economical ingredients. Also, this research showed that the specific flavor used was effective at increasing feed intake immediately after weaning in a growth performance study, even though it was not preferred with older pigs in a preference study. More research needs to be conducted to understand which components of this flavor may attract newly

weaned pigs to their feed. This could have a large economical impact on pork production in the future.