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EFFECT OF IMMUNIZATION AGAINST CHOLECYSTOKININ (CCK) ON PERFORMANCE OF PRIMIPAROUS SOWS AND NURSERY PIGS

By

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ABSTRACT

EFFECTS OF IMMUNIZATION AGAINST CHOLECYSTOKININ (CCK) ON PERFORMANCE OF PRIMAPAROUS SOWS AND NURSERY PIGS

By

Josep Garcia-Sirera

Three experiments were designed to test the effect of immunization against CCK on parity-one sow productivity, plasma CCK concentrations during lactation and growth performance of nursery pigs. In Experiment 1, seventy-two gilts were either vaccinated against CCK or with a placebo during gestation. Sow performance was monitored for the parity-one lactation period (26 ± 1 d). Analysis by RIA showed a large range of anti-CCK titer for sows in the CCK group (log serum titer values ranged between 0 and 4.15). There were no differences (P>.05) between CCK and control sows, respectively, in ADFI (kg) in wk 1 (4.26 vs 4.25) wk 2 (5.00 vs 4.94), wk 3 (5.70 vs 5.46), and for the total duration of the experiment (4.99 vs 4.88). Likewise, there were no differences (P>.05) in piglet ADG (CCK vs Control, respectively) in wk 1 (.17 vs. .17), wk 2 (.23 vs .24), wk 3 (.21 vs .21) and for the duration of the experiment (.21 vs .21). Weaning-to-estrus interval (days) was similar for sows in CCK and control groups (4.84 vs 5.20, respectively). Active immunization against CCK did not improve the sow's lactation and reproductive performance in parity one. In Experiment 2 fourteen gilts were used. A similar protocol as in Experiment 1 was followed. In addition, gilts were fitted with an ear vein catheter and blood samples obtained at

15 minutes interval during a six-hour collection period, to determine free and total CCK concentration in plasma. Feed intake pattern was also visually recorded during the six-hour period. Anti-CCK antibody titer, total and free CCK concentration were analyzed by RIA. Analysis of plasma free-CCK showed sows immunized against CCK had similar (P>.05) peak values as control sows (12 ± 3 pmol/L CCK vs 19 pmol/L ± 5 Control). Feed intake pattern was not altered by CCK vaccination.

In Experiment 3, forty-eight, early-weaned crossbred pigs (10 to 12 d of age) from sows vaccinated against CCK or with a placebo (Control), were used to test the benefits of passive immunization on performance of nursery pigs. Pigs were housed in pens of four. Individual pig weights were recorded on d 0, 7, 14, 21, 28, and 35. Feed disappearance was monitored daily. Blood samples for serum were collected from each pig on days of age 14, and 21 and anti-CCK titer was determined by RIA. There was a difference in ADG (P<.01) between immunized (CCK) and control pigs during week 1 (.23 kg vs .20 kg,respectively). Values for ADG for individual wk 2, 3, 4 and 5 were not different between treatments. Values for ADFI among treatments differed during wk 2 (.42 kg; vs .35 kg; P< .05). Values for ADFI for individual wk 1,3,4 and 5 were not different between treatments. For the entire experiment, ADFI (.44 vs .41) was greater (P<.05) and ADG (.67 vs .63) tended to be greater (P<.1) for pigs in the CCK treatment. There were no differences in Feed/Gain ratio (F/G) for any week or entire experiment. Passive immunization against CCK improved growth performance of early-weaned nursery pigs.

Als meus amics, tinc la sort de tenir els millors.

To my friends, I'm very lucky to have the best.

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INTRODUCTION

Modern swine production requires that sows wean large, heavy litters, and have short weaning-to-estrus intervals. Averages of 2.5 litters/sow/year, 25 pigs weaned/sow/year and litter weights of 65 to 75 kg are attainable. Maximum lactation feed intake is essential to achieve this level of production.

Concurrent with an increase in sow productivity has been an increase in consumer demand for lean pork, which has forced the swine industry to select for animals with reduced fat reserves. This selection has coincidentally led to a reduction in voluntary feed intake of sows (Riley, 1989). Most lactating sows do not consume enough feed to maintain body weight during a 3-week lactation period. Sows typically lose more weight during the last stages of lactation, when milk output is maximized. A problem associated with excessive weight loss and body condition is delayed return to estrus (>10 days). Extended weaning-to-estrus intervals results in fewer litters per year. This problem is especially present in the primiparous sow.

The limitation that voluntary feed intake puts on maximizing productivity has been studied with the lactating sow and with growing pigs (Pekas, 1985;Matzat, 1990). Lactating sows, which were superalimented via a gastric fistula and provided with 20% more feed than those sows allowed ad libitum access to feed, were able to preserve a greater amount of body mass and were also able to synthesize greater quantities of milk (Matzat, 1990). This study proved that appetite is a limiting factor in increasing sow productivity, and that increasing feed intake would result in better performance of sows.

One approach to increase sow feed intake has involved immunoneutralization of systemic cholecystokinin (CCK), a hormone involved in regulation of appetite and feed intake. In a series of studies (Pekas and Trout, 1990; Pekas, 1993; Pekas, 1996) with growing pigs, active immunization against CCK stimulated feed intake and enhanced growth, in proportion to the anti-CCK antibody (CCK-Ab) titer elicited in the animal. The same technique was used to increase the feed intake of lactating sows (Nelson, 1996). The studies with CCK immunization show promising results but leave their approach to increasing feed intake in sows in need of future elucidation.

The positive correlation between feed intake and anti-CCK titer in serum of immunized sows shown by Nelson(1996), indicates a potential benefit of immunizing sows against the hormone, but a true comparison of feed intake between immunized and control animals needs to be examined. Furthermore, the potential effects of CCK immunization on subsequent reproduction performance of the sows needs to be addressed. Lastly, a possible improvement in growth performance of the sow's offspring via passive immunization has been postulated (Nelson,1996) but not investigated.

Chapter 1

REVIEW OF LITERATURE

Introduction

Eating behavior can be influenced by several factors such as environmental conditions, sensory cues (sight, smell, taste), nutrients in the diet, gastrointestinal factors, hormones, neurotransmitters and metabolites. Pen density, feeder design, water intake, alternative feedstuffs (fiber, fat), genotype, photoperiod and temperature are all examples of environmental factors which can influence feed intake. Alternatively, distention of the stomach due to feeding, activates pressure sensitive receptors that signal the brain to induce satiety. Metabolites, hormones and neurotransmitters are released when food enters the gastrointestinal tract (GI), and also influence appetite. The primary site responsible for the integrated control of feed intake and energy balance is the central nervous system (CNS).

It is generally accepted that swine eat to meet their energy requirements. This is not followed in a period of great biological demand such as lactation, where the animal does not eat enough to obtain the energy necessary for maintenance and lactation. Also, when fed diets with higher nutrient density, swine tend to eat more total nutrients, indicating that there are other factors that influence appetite.

One of the hormones involved in control of feed intake is CCK. A review on CCK structure, mechanism(s) and site(s) of action and possible ways to increase feed intake by means of altering CCK action follows.

Cholecystokinin – Introduction

The first studies involving what later would be known as Cholecystokinin were undertaken by Ivy and Oldberg (1927,1928). The authors reported that infusion of lipid into the duodenum of experimental animals stimulated the release of a chemical substance that caused contractions of the gall bladder. They proposed the substance be termed *cholecystokinin* as "it excites or moves the gall bladder". In 1943, Harper and Raper isolated a substance from extracts of the mucosa of the upper intestine which, upon intravenous injection, stimulated the release of pancreatic hormones. They called it *pancreozymin*. Over two decades later, researchers (Jorpes and Mutt, 1966; Mutt and Jorpes, 1971) purified cholecystokinin extracts from the mucosal cells of the porcine small intestine and characterized it as a 33 amino acid peptide. They demonstrated that, in addition to producing contraction of the gallbladder, cholecystokinin also caused secretion of pancreatic hormones (Mutt and Jorpes, 1968). Thus, it became apparent that cholecystokinin and pancreazymin were one and the same substance. For many years thereafter, the gut peptide was referred to as *cholecystokinin-pancreozymin*, but more recently it has been referred to as *cholecystokinin* (CCK). Subsequent work has revealed the

existence of several other molecular forms of CCK besides CCK-33. Larger molecular forms of the peptide with 39 and 58 amino acids, and smaller forms with 4, 5 and 8 amino acids have been found in the periphery and brain of several species, including rat, pig, dog and man (Calam et al., 1982; Walsh et al.,1982; Eberlien et al., 1988).

The peripheral biological activity of CCK appears to be contained within the C-terminal octapeptide with an O-sulphated tyrosine residue (CCK-8S) as reported by Ondetti and coworkers (1970), and is well conserved across mammalian species. More recently, a cDNA fragment encoding a 115 amino acid precursor to CCK has been reported (Deschenes et al., 1984). Various molecular forms of CCK are obtained from this precursor by post-translational processing (Gubler et al., 1984). With regard to degradation, a number of peptidases have been shown to degrade the various forms of CCK into inactive fragments by cleavage, including membrane-bound aminopeptidases (Deschodt-Lanckman et al., 1981) and an enkephalinase enzyme (Zuzel et al., 1985).

Anatomical Distribution of CCK

Cholecystokinin is widely produced and distributed, both in the periphery and in the central nervous system. Sjolund and co-workers (1983) revealed the presence of CCK-like immunoreactivity (CCK-LI) in high concentrations in the upper small intestine. The main site for CCK-LI localization is within mucosal I-cells of the duodenum and jejunum. Depending on the species, the forms of

CCK present in the small intestine can vary. Other forms of CCK such as CCK-58, CCK-39, CCK-33 and CCK-8 are abundant in dogs, cats, and humans, while in rats and pigs, smaller forms such as CCK-33, CCK-22, CCK12 and CCK-8 predominate (Eberlien et al., 1988). The different forms of CCK can also be found in enteric nerves, mucosa and smooth muscle of the lower gastrointestinal tract (Larsson and Rehfeld, 1979), in ascending afferent fibers of the vagus nerve (Dockray et al., 1981), and in the testis (Persson et al., 1988; Pelto-Huikko et al., 1989). In addition, CCK-LI is also found in blood plasma, and it is generally believed that CCK-8S is the most abundant circulating form, although other forms, such as CCK-33, CCK-39 and CCK-58 are also present (Calam et al., 1982; Walsh et al., 1982).

Vanderhaeghen and co-workers (1975) first demonstrated the possible existence of CCK-LI in the CNS, using antibodies against gastrin. A year later Dockray (1976) showed that most of this immunoreactivity corresponded to the carboxyl terminal octapeptide of CCK. Subsequent work revealed that CCK-8S is the main molecular form of the peptide in the CNS (Docray, 1978; Robberecht et al., 1978). Other forms of CCK also present in significant amounts in the CNS include CCK-8 unsulfated (US), CCK-5 and CCK-4 (Rehfeld, 1978, 1986; Shively et al., 1987). Larger forms of CCK are almost absent in the CNS except CCK-58 which has been isolated both from dog and pig brains (Eysselein et al., 1984; Tatemoto et al., 1984). Techniques such as immunohistochemistry, radioimmunoassay and *in situ* hybridization have helped to provide strong evidence that CCK-8S is released from nerve terminals and acts as a

neurotransmitter within the CNS (Schick et al., 1994). There is also evidence that CCK may co-exist with other neurotransmitters within the CNS, such as dopamine (Hokfelt et al., 1980) and substance P (Skirboll et al., 1982). Cholecystokinin is widely distributed within the CNS and studies in rats have demonstrated the existence of the peptide in neurons of cerebral cortex, hippocampus, septum, amygdala, olfactory bulb, hypothalamus, thalamus, parbrachial nucleus, raphe nucleus, substancia nigra, ventral mesencephalon, nucleus tractus solatarious, ventral medulla, and spinal cord (Vanderhaeghen et al., 1975; Hokfelt et al., 1988; Vanderhaeghen and Schiffman, 1992).

CCK Receptors: types and anatomical distribution

Different studies have been performed to determine the location and structure of CCK receptors. Innis and Snyder (1980) carried out radioligand binding studies with different fragments of CCK in tissue homogenates from brain and pancreas. They found that the unsulphated form of CCK-8 (CCK-8US), pentagastrin (CCK-5), and CCK-4 inhibited the binding of [^{125}I] Bolton-Hunter CCK-33 to brain homogenates with much greater efficiency than to pancreatic homogenates. These results suggested the possibility of two classes of CCK receptors and led to the use of the terms "peripheral type" receptors and " brain type" receptors. Subsequent studies by Moran and co-workers (1986) provided evidence for the presence of both subtypes of receptors in the brain. They reclassified the two subtypes of CCK receptors as CCK_A and CCK_B, respectively.

Type A receptors are found mainly in the periphery, but also in some areas of the CNS. They have a high affinity for CCK-8S. Type B receptors are found mainly in the CNS and have a high affinity for CCK-8S, CCK-8US, CCK-5 and CCK-4.

Wank and co-workers (1992a,b) have sequenced and cloned the genes of the two subtypes of CCK receptors from rat pancreas and rat brain. Similarly, the genes for the two CCK receptors have also been cloned from canine and human stomach and human brain (Pisegna et al., 1992). Sequence analysis of rat pancreatic and rat brain CCK receptor genes has revealed a 48% homology between the two receptors. The cloning of the receptors has also confirmed that both peripheral and CNS CCK_A receptors are identical and that the CCK_B receptor has high homology to gastrin receptors (Wank et al., 1994).

Autoradiography binding studies have been used to determine the distribution of CCK_A and CCK_B receptors in peripheral tissue and in the CNS. As expected, studies in rats have indicated high populations of CCK_A receptors in peripheral tissue, and high populations of CCK_B receptors in the CNS. Type A receptors have been found in the pancreatic acinar cells, gall bladder, smooth muscle of the pylorus and vagal afferents. Local populations of CCK_A receptors have been found in the CNS in the area postrema (AP), the dorsal medial hypothalamus, the interpenduncular nucleus and the havenulum (Moran et al., 1986; Hill et al., 1987, 1990, 1992; Mercer and Lawrence, 1992). Type B receptors are found on vagal afferents and in the CNS regions, such as the cerebral cortex, olfactory bulb, nucleus accumbens, caudate nucleus, hippocampus, hypothalamus, amygdala, substantia nigra, dorsal raphe nucleus

and the dorsal horn of the spinal cord (Gaudreau et al., 1983; Hill et al., 1992; Corp et al.,1993). There are also some species differences in the distribution of CCK receptors subtypes. For example, CCK_A receptors are the predominant subtype in rat pancreatic acinar cells, CCK_B receptors predominate in the pig pancreatic acinar cells (Morisset et al., 1996).

CCK as a satiety factor

The role of Cholecystokinin as a satiety factor has been studied extensively. CCK participates in rapid, preabsorptive satiety in pigs (Anika et al., 1981). Intraperitoneal injections of partially-purified CCK produces a dose related suppression of feed intake (Gibbs et al., 1973; Stallone et al., 1989). Lateral cerebral ventricle injections of CCK also depresses feed intake in rats (Tsai et al., 1984). Systemic infusions of CCK-8 reduce meal size in pigs (Houpt, 1983).

Evidence from Kow and Pfaff (1986) suggest that the role of CCK-8 in satiety induction is two fold: CCK-8 serves as a satiety agent in the periphery (non CNS), mediated through vagal afferent nerves to the brain, and as a neurotransmitter in the brain to convey the information originating in the periphery. The results of Linden (1989) concur with these findings, indicating that peripheral CCK receptor mechanisms induce a release of CCK in the brain. In the brain and in the periphery CCK may serve as a neurotransmitter (Kow and Pfaff, 1986; Linden, 1989). Ultimately, the satiety center of the hypothalamus is

stimulated by these endogenous compounds, and the animal terminates the meal.

Artificial synthesis of the specific CCK_A receptor antagonist devazepide, has made possible the testing of the CCK's role in control of satiety. Studies with different species indicate that the inhibitory effects of exogenous peripheral CCK on food intake could be completely abolished by intraperitoneal pretreatment with devazepide (Hewson et al., 1988; Ebenezer et al., 1990a; Ebenezer et al., 1993). These results suggest that a peripheral CCK_A receptor mechanism is involved in the suppression of feeding produced by the peptide. Furthermore, it was found that devazepide, on its own, increased the size of a test meal when administered systemically to several species, including the rat, pig, mouse, monkey, dog, cat and chicken. These tests have been conducted under a number of different feeding schedules and dietary conditions (Hewson et al., 1988; Silver et al., 1989; Ebenezer et al., 1990a; Bado et al. 1991; Cheng et al., 1993; Moran et al., 1993; Weatherford et al., 1993; Covasa and Forbes, 1994; Ebenezer and Baldwin, 1995). These experiments have provided another clear indication that endogenous CCK, acting via CCK_A receptors, plays an important role in the control of food intake.

Some researchers question whether CCK "satiety" action is due to creating discomfort to the animal such as vomiting and nausea, and not directly by controlling food intake. Activation of both, CCK_A and CCK_B, receptors have been described as being responsible for interruption of food intake. In addition, activation of CCK_A receptors seems to be responsible for release of vasopresin

shortly after activation, and release of cortisol after a longer period of time after receptor activation (Parrot et al., 1991). Recent research questions the involvement of CCK_A in cortisol release (Parrot and Forsling, 1992). The authors used a CCK_A receptor antagonist to block the receptor action, and still observed release of cortisol after infusion of CCK in the circulation. CCK_B receptors have been associated with stress-related disorders. In general, these studies promote the idea that the animal stops eating due to the discomfort produced by CCK action. The first indications in favor of CCK as a true satiation agent are that CCK decreases food intake but not water intake, suggesting that the decrease in intake is not secondary to general malaise (Gibbs et al., 1973). Other research also shows that CCK's action on satiety are more focused on fat intake than other ingredients which would rule out the "discomfort" theory, since discomfort would generate a rejection of any food.

Peripheral CCK and Food Intake

Cholecystokinin cannot penetrate the blood brain barrier (Passaro et al., 1982). Thus it is likely that systemic CCK acts at a peripheral site to inhibit feeding (Weller et al., 1990). The target site for the hypophagic actions of the peptide is not clear. Recent studies have suggested that peripheral exogenous CCK-8S may activate vagal afferent fibers that relay the signal to brain areas concerned with feeding where it is translated into behavior consistent with satiety

(Smith et al., 1981, 1985; Crawley et al., 1984; Crawley and Kiss, 1985; Smith and Gibbs, 1992).

It has also been proposed that peripheral CCK may act at certain brain sites where the blood brain barrier is interrupted, for example AP and NST. Indeed peripheral CCK-A receptors have been identified in AP as mentioned before in this review. Van der Kooy (1984) showed that lesions of the AP specifically blocked the satiety effect induced by CCK.

CCK action in the CNS

Cholecystokinin has been shown to act in the CNS affecting behaviour of pigs trained to make operant responses for food and water (Parrot and Baldwing, 1981). Injections of bolus doses of CCK-8S into the lateral cerebral ventricles of pigs decreased operant responding for food in a dose-related manner. The study was done with several doses of CCK-8S, and none of them inhibited water intake in thirsty animal indicating the specificity of the peptide for food. Later studies using specific CCK receptor agonist has shown that the hypophagic effect of CCK in pigs in the CNS is mediated by CCK_A receptors (Parrot, 1994; Ebenezer et al., 1996).

CCK and other hormones and neuropeptides

In addition to CCK direct action on feed intake, there are different hormones and peptides related to feed intake control that appear to be related to CCK.

Bombensin, a hormone that has been isolated in the GI tract, induces a satiety effect in the pig. Injection of bombensin both, peripherally and directly in the brain has been shown to induce satiety. Bombensin also induces a release of peripheral CCK, raising the question whether bombensin action is mediated by CCK. The use of antagonists for CCK receptors has shown that bombensin has a satiety effect by itself, independent of its action through CCK (Parrot and Baldwin, 1982).

Neuropeptide Y (NPY) has been isolated in the pig's brain (Tatemoto et al.,1982). In the brain, CCK induces a reduction of NPY levels, suggesting that NPY could be in the sequential chain of events brought on by CCK (Gourch et al., 1990). Because the majority of studies on feeding behavior have been conducted in satiated rats, the question emerged whether NPY actually stimulates feeding behavior or, rather, attenuates satiety signals. Schick and coworkers (1991) showed that intracerebral NPY did not augment food intake initially, but rather delayed the reduction in feeding that occurs normally with satiation, suggesting that NPY does not act to stimulate feeding behavior per se, but rather to suppress satiety signals.

Role of CCK in lactation

In lactating rats and sows, daily ad libitum feed intake generally increases as lactation progresses. It has been suggested that the increase in energy spent associated with increasing amounts of milk synthesis is responsible for a large proportion of hyperphagia observed in rats and sows (Flemming, 1976). However, there could be other mechanisms associated with this hyperphagia.

Daily feed intake is the sum of the number of meals eaten within a day and the size of each meal. Meal size is the combination of the duration of time spent eating, and the rate of ingestion. Cholecystokinin mediates its effects on feed intake by reducing meal size (Leibowitz, 1986). In lactating rats, the gradual increase in feed intake occurs primarely because of an increase in meal size (Strubbe and Gorissen, 1980; McLaughlin et al., 1983), rather that an increase in meal frequency. Meal size becomes larger in lactating rats in spite of the fact that plasma CCK concentrations are elevated (McLaughlin et al., 1983; Linden, 1989). A possible explanations for the elevated CCK concentrations in lactating rats could be that CCK is released in lactating rats (and dogs) in response to suckling stimulus (Linden et al., 1990). This release of CCK is immediate and short lasting, and in rats, of smaller magnitude that the release produced after feeding (Linden et al., 1990). Even with elevated CCK concentrations as lactation progresses, feed intake gradually increases also during lactation. For this reason, several studies (McLaughlin et al., 1983; Wager-Srdar et al., 1986, Linden, 1989) speculate with the idea that marked hyperphagia of lactating rats reflects an insensitivity of the animals to the inhibitory effects of CCK on food

intake as lactation length progresses. However, pancreatic hypertrophy, a known bilogical response to elevated CCK concentrations still occurs in lactating rats (McLaughlin et al., 1983). This seems to indicate that CCK-insensitivity during lactation is restricted to satiety. In should be noted that some research did not observe a decrease in sensitivity to the anorexigenic effects of CCK as lactation progressed in rats (Helmereich et al., 1991).

Immunization against endogenous CCK

Several studies involving CCK antibodies (CCK-Ab) have been conducted in order to prove involvement of endogenous CCK in feeding behavior. Circulating CCK-Ab do not cross the blood-brain barrier, but are believed to sequester the peripheral, free circulating endogenous CCK, making it unavailable to the CCK receptor. This phenomenon is known as immunoneutralization.

Exogenous CCK-Ab, administered via continuous lateral cerebral ventricular injection, increases feed intake in whethers (Della-Fera et al., 1981). Feed intake of Zucker rats has also been increased by exogenous CCK-Ab administration, and also by endogenous CCK-Ab following active immunization against CCK (McLaughlin et al., 1985). Similarly, the feed intake and growth rate of growing pigs has been elevated (8.2 and 10.6% respectively) following immunization against desulfated CCK-9 conjugated to human serum globulin (Pekas and Trout, 1990). In another experiment, Pekas (1993) reported elevated feed intakes of growing pigs following immunization against desulfated CCK-8

conjugated to one of four different haptens; bovine serum albumin (BSA), human serum globulin (HSG), Keyhole limpet hemocyanin (KLH), or purified protein derivative (PPD). Nelson (1996) reported a correlation between serum titer and titer of colostrum of lactating sows and their average feed intake (ADFI) in week three of lactation (R^2=.43 to .67, P<.01 in all cases). Two other studies with sheep have reported no differences between immunized animals and controls (Trout et al., 1989; Spencer, 1992). In both studies feed intake was slightly depressed on the days following booster vaccinations. Pekas and Trout (1990) reported a similar observation in pigs. Spencer, when explaining the results of the study, hypothesized that potential reasons for immunized sheep failing to show a response were:

(1) Insufficient amount of CCK-Ab raised to effectively neutralize the endogenously produced CCK,

(2) CCK-Ab raised may have had too low of an affinity to effectively prevent the hormone from binding to the receptor,

(3) There is an elevated production and secretion rate of endogenous CCK due to decreased negative feedback as CCK-Ab sequestered CCK,

(4) CCK action is primarily autocrine or paracrine, and less affected by circulating CCK,

(5) The CCK-Ab may act like a plasma binding protein and protect the hormone from degradation thereby extending it's biological half-life,

(6) The Ab-bound hormone may be presented to the receptor in such a way as to enhance it's orientation at the receptor binding sites,

(7) The Ab-bound hormone may extend the hormone's transmembrane effectiveness by inhibiting internalization and clearance of the hormone.

The only report to date on immunized sows is that of Nelson (1996). His results appear to indicate an advantage of immunizing against CCK to obtain a better ADFI during lactation. The study though raises several questions. The statistical approach was that of a regression model. The author did not truly compare control against immunized animals. The regression (r) coefficients between values for ADFI and mean log titer of colostrum are low (ranging between .057 and .123) when comparing ADFI in weeks 1 and 2 of lactation with log titer of serum at d 7 and d 14. Values for r are higher when comparing ADFI in week 3 with log titer of serum in d 21 (.705). The highest r value reported was obtained by comparing ADFI on week 3 with a combination of serum log titer of samples taken during late gestation, d 7, 14, 21 of lactation and colostrum. While an r value of .705 appears to indicate a good correlation between immunization against CCK and feed intake, one could question the physiological significance of correlating feed intake during the third week of lactation with serum values obtained as far as 36 days earlier (day 92 of gestation, booster 2).

Summary

Cholecystokinin is involved in the control of feed intake. It's mechanism of action appears to involve both the periphery and the CNS. Active immunization against CCK has shown different results depending on the species and the phase of production, growing or lactating. The report on active immunization against CCK on lactating sows (Nelson, 1996) showed promising results but did not truly compare a control set of sows against CCK immunized sows. Furthermore, it did not addressed potential implications on post weaning reproductive performance.

Other aspects of the problem that need to be addressed are the possibility that vaccinated animals could compensate the sequestering of free-CCK by CCK-Ab by increasing production or decreasing clearance. As a result, the free-CCK concentration on a given time could remain the same between immunized and non immunized animals, and the anorexic effect would remain unchanged. This hypothesis could explain why in some studies immunization against CCK failed to achieve greater feed intake than control animals (Trout et al., 1989; Spencer, 1992). Effects of passive immunity against CCK on nursery pigs by transfer of immunity from sows to piglets through colostrum is another aspect of the problem in need of study. Weaning piglets at less than three weeks of age (Early Weaning) has become a standard practice in swine production because of health considerations and improved growth performance. Early-weaning main advantage is to stop bacterial and viral spread from the sow to the piglets. Piglets are passively immunized against disease. Sows immunized against CCK transfer

their immunity to CCK to their piglets as well (Nelson, 1996), and thus, could produce an improvement in piglets feed intake by stopping CCK action on satiety.

Model

The model proposed in this study for the mechanism of action of CCK is shown in Figure 1. The model shows CCK action in the periphery. This study will focus in the peripheral action of CCK. The stop symbol "⃠" shows the sections of the model which could be affected by CCK immunization.

When food enters the small intestine, it triggers a response on CCK-producing cells, which increase production and release it into circulation. Systemic CCK reaches receptors that trigger a signal, which in turn, is relayed to the brain trough the peripheral nervous system. The signal reaches the area of the brain in charge of control of feed intake and the animal stops eating.

Immunization against CCK could produce antibodies against the hormone. The antibodies could sequester free peripheral CCK and stop it from binding to the CCK receptors. As a consequence, the signal triggered by free-CCK would be less strong in immunized animals and they would continue eating.

Hypothesis

The hypothesis proposed for each experiment are as follows.

Experiment 1. Sows actively immunized against CCK will increase feed intake during lactation when compared to control sows vaccinated with a placebo.

The immunized sows produce anti-CCK antibodies which sequester the hormone and prevent it from binding to the receptor and exert its action on feed intake.

Experiment 2. Sows actively immunized against CCK have the same eating pattern (number of meals, duration of meals, and amount of feed eaten) than sows vaccinated with a placebo.

Free CCK concentration remains the same for sows immunized against CCK or vaccinated with a placebo. The CCK-immunized sows compensate sequestering of free-CCK by anti-CCK antibodies by increasing production.

Experiment 3. Passively immunized nursery pigs increase feed intake and growth performance when compared to pigs from non-immunized sows.

Antibodies against CCK present in immunized piglets sequester free-CCK in circulation and stop it from exerting its effect on feed intake.

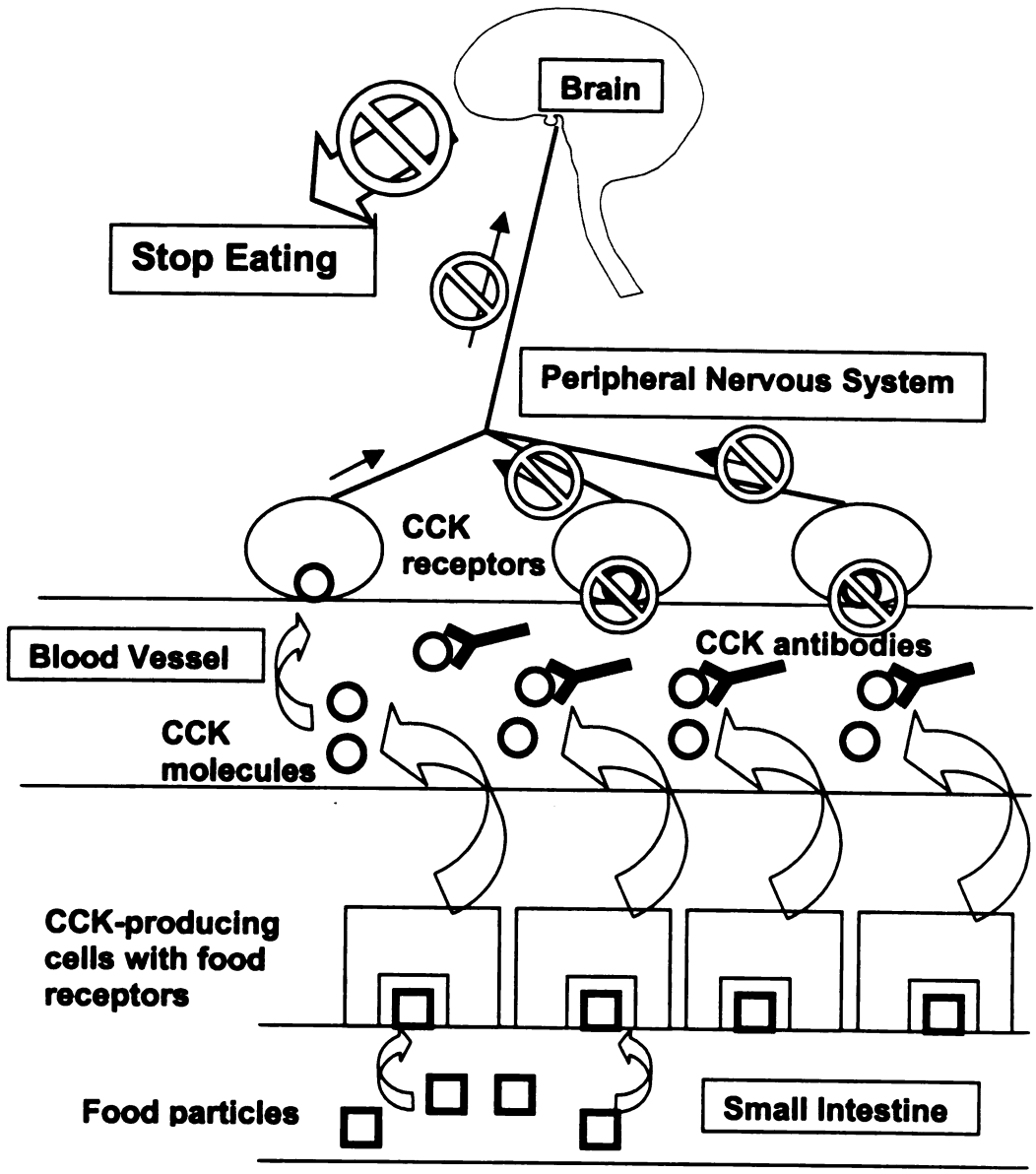

Figure 1. Suggested mechanism of immunization against CCK and its effect on feed intake.

Chapter 2

ACTIVE IMMUNIZATION OF GILTS AGAINST CHOLECYSTOKININ (CCK) AND ITS EFFECT ON PARITY-ONE LACTATION PERFORMANCE, POSTWEANING RETURN-TO-ESTRUS AND PLASMA CCK CONCENTRATION

Abstract

Two experiments were designed to test the effect of active immunization against Cholecystokinin (CCK) on parity-one sow productivity (Exp 1) and plasma CCK concentrations during lactation (Exp 2). In Exp 1, seventy-two gestating, Yorkshire x Landrace, gilts were vaccinated against CCK or with a placebo (Control). Sow performance was monitored the parity-one lactation (26 ± 1 d). A similar protocol was employed in Exp 2 with fourteen gilts, however, in addition to performance measurements, feed intake pattern was studied and females were catheterized to analyze for free-CCK and total-CCK concentration in plasma. Anti-CCK antibody titer, free and total CCK concentration in plasma were analyzed by RIA.

In both experiments, RIA analysis showed a great range of anti-CCK titer for sows in the CCK group (log serum titer values ranged between 0 and 4.15). There were no differences (P>.05) between CCK and Control sows, respectively, in ADFI (kg) in week 1 (4.26 ± .149 vs 4.25 ± .169) week 2 (5.00 ± .150 vs 4.93 ± .170), week 3 (5.70 ± .179 vs 5.46 ± .204), and for the total duration of the experiment (4.99 ± .134 vs 4.88 ± .152). Likewise, there were no differences

(P>.05) in piglet ADG (CCK vs. Control, respectively) in week 1 (.17 ± .009 vs .17 ± .008), week 2 (.23 ± .006 vs .24 ± .007), week 3 (.22 ± .007 vs .22 ± .008) and for the duration of the experiment (.21 ± .005 vs .21 ± .006). Weaning-to-estrus interval (days) was similar for sows in CCK and Control groups (4.84 ± .21 vs 5.20 ± .25, respectively). In Experiment 2, RIA analysis of plasma free-CCK showed sows immunized against CCK had similar (P>.05) peak values as control sows (12 ± 3 pmol/L CCK vs 19 pM/L ± 5 Control). Feed intake pattern was not altered by CCK vaccination. Active immunization against CCK did not improve the sow's lactation and reproductive performance in parity one. Sows may compensate for the sequestering of endogenous CCK by increasing synthesis of free-CCK.

Key words: CCK, Active Immunization, Sows, Lactation.

Introduction

Cholecystokinin (CCK) is a hormone involved in regulation of appetite and feed intake. In a series of studies with growing pigs (Pekas and Trout, 1990; Pekas, 1993; Pekas, 1996), active immunization against CCK stimulated feed intake and growth in proportion to the anti-CCK antibody (CCK-Ab) titer elicited by the animal. Because of the sows general inability to consume enough feed in lactation to maintain body condition, Nelson (1996) actively immunized parity one sows against CCK to try to improve sow feed intake. He observed a correlation between feed intake and CCK-Ab titer.

While the report on active immunization against CCK in parity one lactating sows (Nelson, 1996) showed promising results, the study utilized a regression model approach and a relatively small number of animals. A control set of sows were not included and immunization resulted in widely-variable titer responses. Lastly, the study did not address potential implications on post-weaning reproductive performance.

Because immunization against CCK has produced inconsistent titers in sows and has failed to elicit greater feed intake in sheep (Trout et al., 1989; Spencer, 1992), effects of this technique need further study. In particular overproduction of CCK as a feed back mechanism could explain results of studies in which immunization against CCK failed to achieve greater feed intake.

The objectives of this study were, in Exp 1, to determine the effect of active immunization against endogenous CCK on the voluntary feed intake, lactation productivity, and weaning-to-estrus interval of primiparous sows; in Exp 2, to evaluate systemic concentrations of free-CCK to test for possible overproduction of free-CCK by sows immunized against CCK.

Materials and Methods

All procedures for this study were approved by the Michigan State University Committee for Animal Use and Care.

General Procedures for Pigs

Experiment 1. Seventy-two gilts, divided in nine different groups, were used from March 1996 to June 1998. Gilts were observed for signs of estrus twice daily and artificially inseminated 12 hrs after being observed in standing heat, and at 12 hours interval thereafter for a maximum of three inseminations. Gilts were housed in individual gestation crates over partially slotted floors throughout gestation, and were offered 2.5 kg per head daily of a standard corn-soybean meal diet (table 1). Room temperature ranged between 20°C and 25°C, heat was provided by gas heaters and ventilation by manually-controlled wall fans. Artificial light was provided between 8 am and 7 pm each day. On about d 64 of gestation (as an average of breeding dates for the entire group) gilts were randomly divided into two treatments: a) control or vaccinated with placebo and b) CCK or vaccinated with cholecystokinin, and administered the primary vaccination (see vaccine preparation below). The primary dose contained KLH-KLH conjugate for control sows and CCK-8-KLH conjugate for CCK sows. Both conjugates were emulsified in a solution of 50% Freund's complete adjuvant and 50% phosphate buffer (Pekas and Trout, 1990). Each 1 mL dose contained 1mg of antigen. The dose was administered subcutaneous on one side of the neck. On d 78, 92 and 106 of gestation gilts were administered booster vaccines (Figure 1) containing the same amount of either conjugate as the primary vaccine, except administered in Freund's incomplete adjuvant. Booster doses were alternatively administered on the opposite side of the neck. Gilts were also vaccinated prebreeding for parvovirus, leptospirosis and erysipelas; prefarrowing

for bordetella, *E. coli*, pasteurella, Transmisible Gastroenteritis, erysipelas and clostridium. Prior to each CCK vaccination, and also on d 7, 14, and 21 of lactation, blood samples were taken from sows via external jugular vein puncture. Serum was harvested and frozen for future anti-CCK titer analysis. One week before scheduled farrowing time, gilts were moved to partially slotted farrowing crates. Room temperature ranged between 20°C and 25°C, heat was provided by gas heaters and ventilation by manually-controlled wall fans. Artificial light was provided between 8 am and 7 pm each day.

During lactation (26 ± 1 d), sows were provided ad libitum access to a standard lactation corn-soybean meal diet (Table 1) and water. Feed disappearance during lactation was recorded daily. Sows were weighed at about d 110 ± 2, 12 h post-farrow and on d 7, 14 and 21 of lactation. The return-to-estrus interval was recorded following weaning. Sows were observed for estrus twice daily (morning and evening).

Because suckling stimulus is known to drive feed intake and may stimulate CCK release (Linden et al., 1990), litter size was standardized at 11 pigs per litter by d 2. Non-experimental sows which farrowed at the same time as the test gilts provided additional piglets as needed. Creep feed was not offered to piglets during lactation.

Stillborn and live piglets were individually weighed within 12 h of birth and the number of mummified fetuses was recorded. Piglets were weighed on d 7, 14 and 21 of lactation. Litter gains were used to assess sow milking ability.

Experiment 2. Fourteen gilts (n=7 for each treatment), were used. In addition to the procedures mentioned above, blood samples were taken on d 18 of lactation for total (bound to CCK-Ab + free) and free-CCK determination. On the evening of d 17 of lactation, gilts were catheterized through the ear vein using a 0.1 mm diameter catheter (Tygon® ID 0.1mm Fisher Cat # 14170-15D). Gilts were fasted until the next morning. On d 18 of lactation (sampling day), a pre-prandial blood sample was taken, and immediately afterwards, gilts were fed 6.8 kg of their lactation diet. Gilts were monitored for number of meals taken, duration of each meal, amount of feed eaten in first meal, and total amount of feed eaten during a 6 h period. In addition, blood samples were obtained at 15 min intervals during the 6h period. Blood samples were collected (5 mL) into a ice-chilled syringe and transferred into a polypropylene tube containing EDTA (1mg/mL of blood) at 0°C. Blood was centrifuged at 1600xg for 15 min at 4°C and plasma was collected and stored at –70°C until laboratory analysis.

Vaccine Preparation and Laboratory Analysis

The CCK immunogen was purchased from Cambridge Research Biochemicals (Cambridge, U.K.). It was produced by conjugating the desulfated C-terminal octapeptide of cholecystokinin (CCK-8) to keyhole limpet hemocyanin (KLH) via glutaraldehyde condensation. Although the sulfated form of CCK-8 is needed for biological activity, antibodies have been raised to the unsulfated form (Pekas and Trout, 1990) at much lower expense. For this reason the desulfated form was used. The placebo immunogen consisted of only KLH-KLH conjugate.

On the same day of vaccination, the antigen was dissolved in phosphate buffer and emulsified with Freund's adjuvant using a tissue homogenizer (Ultra Turrax T25, Janke and Kunkel®, Frankfurt, Germany).

Determination of serum, anti-CCK titers was performed using the radioimmunoassay method described by Pekas and Trout (1990) as revised by Pekas (1996). Bolton-Hunter ^{125}I-labeled CCK8S (sulfated; 2200 Ci/mmol; Dupont, Boston, MA) was the radiolabeled antigen. Each assay tube contained 4,885 dpm or 1 fmol of Bolton-Hunter ^{125}I-labeled CCK8S. Antiserum titers were computed from the specific binding of Bolton-Hunter ^{125}I-labeled CCK8S at four dilutions (1:50, 1:325, 1:2113, and 1:13731) in phosphate buffered saline (.01 M, pH 7.5) containing .05% gelatin (Sigma cat # G-2500). The antiserum titer is defined as that serum dilution that gives 50% specific binding of Bolton-Hunter ^{125}I-labeled CCK8S.

Determination of free-CCK in Experiment 2 was performed by filtering 1.5 mL of plasma (Centriplus 100 filter, cat # 4414 Amicon Inc., MA) using a centrifuge with a 34-degree fixed-angle rotor at a speed of 3000 xg for 2 h at 4°C. Alliquots of 0.7 mL of filtered plasma were extracted with 1.4 mL of 100% ethanol. Samples were stored at room temperature for 30 min after vigorous vortexing for 10 s. The mixture was centrifuged for 30 min (1500 x g) at 4°C. The supernatant was decanted into clean 12 x 75 mm polypropilene tubes and then evaporated in a Speed Vac Concentrator (Heto VR-1, ATR, Laurel, MA) at room temperature. The dried extracts were reconstituted to original volume with assay

buffer and analyzed for CCK (RIK-7181, Peninsula Laboratories, Belmont, CA) following manufacturer's instructions.

Determination of total CCK was performed by extracting 0.4 mL of plasma with 0.8 mL of 100 % ethanol and following the same procedure as described for free-CCK determination with the exception of the filtering step.

Statistical Analysis

Experiment 1. Data was analyzed as a randomized block design using the GLM procedures of SAS (1996). Sows were blocked by time (group). Analysis of Variance was used to test immunization treatment (CCK and Control) on the independent performance variables (sow ADFI and piglet ADG during wk 1,2,3, and for the total 3 wk experimental period, weaning-to-estrus interval, number of piglets born alive, litter weight at birth, litter size at d 21 of lactation, and litter weight at d 21 of lactation). The mean differences between treatments were detected by comparison of least square means. Percent of sows expressing estrus by d 7 postweaning was also analyzed using Chi-square. Regression analysis was used to correlate ADFI and log mean dilution titer during wk 1, 2, 3 and for the total 3 wk experimental period. Difference between means was considered significant at $P<.05$, and $P<.1$ was considered a trend.

Experiment 2. Statistical analysis was performed using the GLM procedure of SAS (1996). Analysis of Variance was used to test immunization treatment (CCK and Control) effect on peak free-CCK concentration, number of meals, average duration of meal, duration of first meal, amount of feed eaten in first meal, and

amount of feed eaten for the duration of the experimental period (6h). The mean differences between immunization treatment (CCK and Control) were detected by comparison of least squares means. Difference between means was considered significant at $P<.05$, and $P<.1$ was considered a trend.

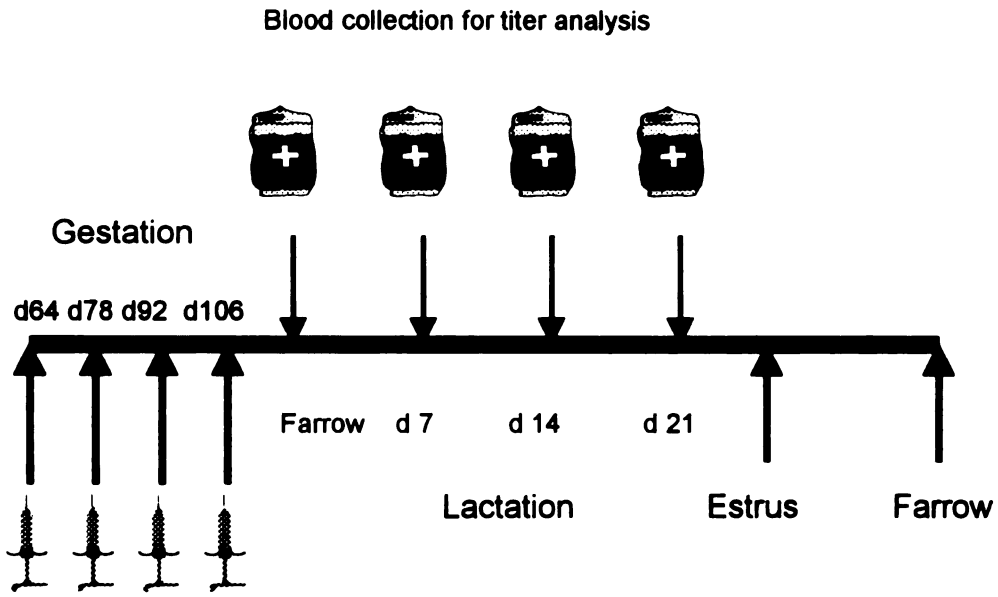

Figure 2. Exp 1 time sequence of vaccination and sample collection.

Table 1. Diet Composition (as fed)

Diets	Gestation	Lactation
Ingredients	% in diet	
Corn dent yellow	68.32	63.92
Soybean meal 44%	14.59	28.97
Wheat Bran	10.00	—
Choice White Grease	2.00	2.00
Ca Phos Monocal 21%	1.88	2.05
Vitamin Premix[a]	0.60	0.60
Salt	0.50	0.50
Limestone	1.30	1.15
Trace Min premix[b]	0.50	0.50
Sow Pack[c]	0.30	0.30
Calculated Analysis		
Lysine %	0.65	1.00
Ca %	0.90	0.90
Total P %	0.80	0.80

[a] Supplied per kilogram of premix: vitamin A, 918583 IU; vitamin D_3, 91858 IU; vitamin E, 11023 IU; vitamin K (as menadione sodium bisulfite complex) 735 mg; riboflavin, 735 mg; pantothenic acid 2939 mg; niacin, 4409 mg; vitamin B_{12}, 5512 mcg; thiamin, 184 mg; pyridoxine, 165 mg; Ethoxyquin, 0 .15%; Mineral Oil, 2 %.

[b] Supplied per kilogram of premix: Zn, 2000 mg; Cu, 2000 mg; Fe, 2000 mg; Mn, 2000 mg; I, 30 mg; Se, 60 mg, mineral oil 2%.

[c] Supplied per kilogram of premix: Vit A, 918583 IU; Biotin, 73487 mcg; Choline, 128602 mg; Folic Acid, 551 mg; Ethoxyquin, 0.15%; Mineral Oil, 2%.

Results

Experiment 1. Three sows did not finish the experiment because of health considerations (two of them, one per treatment, did not farrow, and the other, Control, failed to milk). Thus, data was obtained from sixty-nine sows.

Serum titer analysis by RIA yielded a great range of titer on farrow (d0), d7, d14, and d21 of lactation. Log dilution values ranged between 0 and 4.15. Variation was similar on any sampling day. Coefficient of Variation for each sampling day were .247, .250, .244, .258 for samples at farrow, d7, d14, and d21 of lactation, respectively. Samples from eleven CCK sows did not reach 50% of specific binding activity at the lower dilution (1:50, definition of titer).

Analysis of lactation and reproduction data showed no differences between treatments in sow ADFI in wk 1, 2, 3 of lactation, and for the total duration of the experiment (Table 2). There were no differences between treatments on ADG of piglets for wk 1, 2, 3 and for the total duration of the experiment (Table 3). Number of piglets born alive, litter birth weight, litter size at d 21 of lactation and litter weight at d 21 of lactation were similar for both treatments (Table 3). Differences among sow groups (replicates) were identified for ADFI in wk 1, 2, 3 and for the total duration of the experiment (Table 7, Appendix D). There were also differences in ADG of piglets among replicates in wk 2, 3, and for the total duration of the experiment (Table 7, Appendix D). Analysis of weaning-to-estrus interval data showed no differences between treatments (Figure 3), but some differences ($P<.05$) among replicates (Table 8,

Appendix D). Percentage of sows returning-to-estrus by d 7 postweaning were similar between treatments (Figure 3).

Data was also analyzed without including values belonging to sows that did not develop a titer against CCK. This was done to rule out the potential confounding effect of animals that, belonging initially to the immunized group (CCK treatment), did not raise antibodies against CCK. Again, there were no differences either between treatments in any of the variables analyzed.

A regression analysis was also performed, correlating ADFI for each sow, during individual wk 1, 2, 3, and the total 3 wk lactation period, with log titer values of sows for those weeks. There were no correlations ($P>.05$) during wk 1, 3 and total 3 wk lactation. There was a significant ($P<.05$) r^2 for ADFI in wk 2 and average log titer for that period, with r^2 explaining only 15% of variation.

Experiment 2. There were no differences between treatments for free-CCK values (12 ± 3 pmol/L CCK vs 19 pM/L ± 5 Control). Immunized animals had greater total CCK (CCK-Ab bound and free) than free CCK (70 ± 9 vs. 12 ± 3 pmol/L, respectively). Animals on the control group had the same values for free and total CCK (Figure 4). There were no differences between treatments in number of meals, total eating time, average meal time, duration of first meal, feed intake of first meal (Table 4). Sows immunized against CCK tended to eat more ($P<.1$) than controls during the 6 h observation period (3.97 ± .41 CCK vs 2.89 ± .45 Control).

Table 2. Effect of immunization against CCK on sow's lactation performance[a].

Item	Treatment		Level of
	Control	CCK	Significance
n	34	35	NS[b]
Sow Pre-Farrow Weight (kg)	180.34 ± 5.04	180.34 ± 4.07	NS
Sow Weight Change from Farrow to d 21 (kg)	11.52 ± 1.83	11.05 ± 1.48	NS
Sow ADFI (kg)			
Wk 1	4.25 ± .169	4.26 ± .149	NS
Wk 2	4.93 ± .170	5.00 ± .150	NS
Wk 3	5.46 ± .204	5.70 ± .179	NS
Total 3 wk	4.88 ± .152	4.99 ± .134	NS

[a] Least Square Means ± SE, n=69
[b] NS = Non-Significant

Table 3. Effect of immunization against CCK on sow's reproductive performance[a]

Item	Treatment		Level of Significance
	Control	CCK	
n	34	35	NS[b]
Piglets Born Alive	8.06 ± .421	7.72 ± .385	NS
Litter Birth Weight (kg) Before Cross-foster	12.44 ± .63	11.57 ± .58	NS
Piglet ADG (kg)			
Wk 1	.176 ± .009	.176 ± .008	NS
Wk 2	.236 ± .007	.233 ± .006	NS
Wk 3	.217 ± .008	.219 ± .007	NS
Total 3 wk	.210 ± .006	.209 ± .005	NS
Litter Size at d 21 (number of pigs)	10.58 ± .18	10.62 ± .16	NS
Litter Weight at d 21 (kg)	62.70 ± 1.67	62.00 ± 1.53	NS

[a] Least Square Means ± SE, n=69
[b] NS = Non-Significant

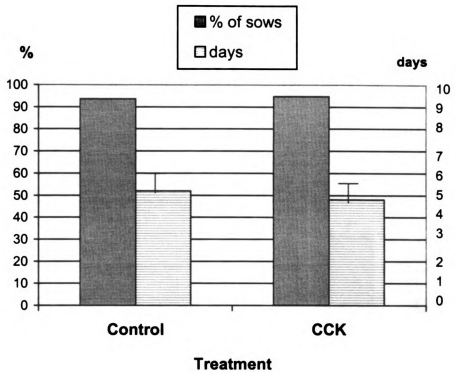

Figure 3. Effect of immunization against CCK on weaning-to-estrus interval.

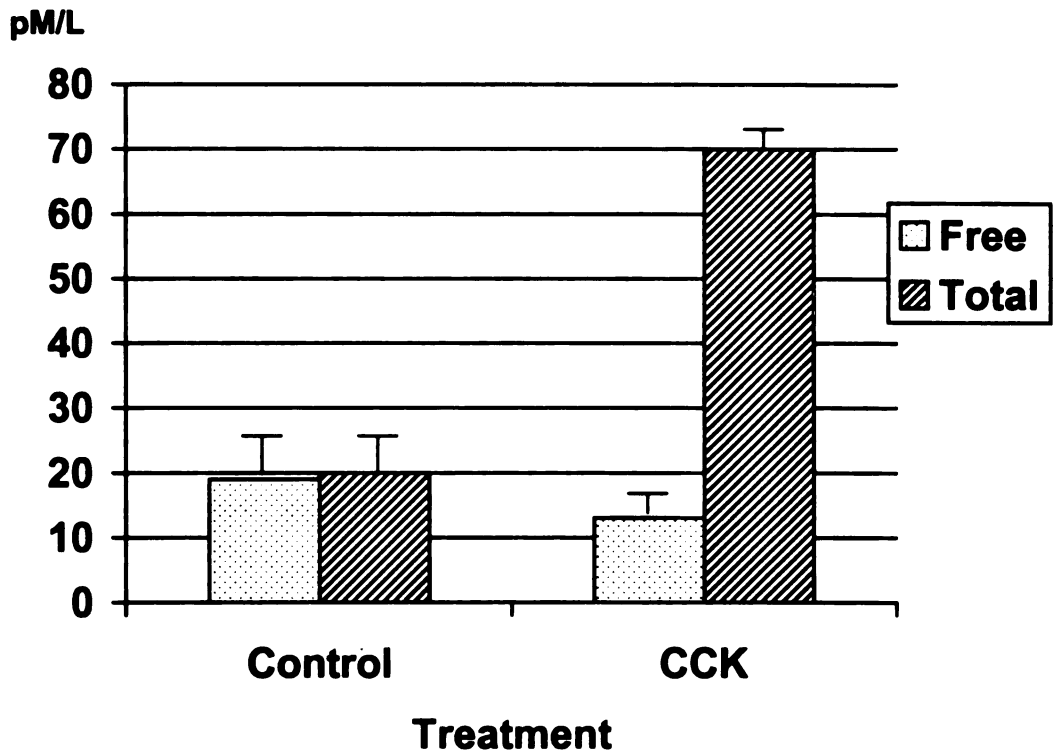

Figure 4. Effect of immunization against CCK on free and total CCK concentration in plasma.

Table 4. Effect of immunization against CCK on eating behavior[a].

Item	Treatment		Level of Significance
	Control	CCK	
n	7	7	NS[b]
Number of meals	4.14 ± .44	4.48 ± .40	NS
Total time eating (minutes)	54.48 ± 10.81	61.41 ± 9.81	NS
Average time per meal	1.82 ± .41	1.87 ± .37	NS
Duration of 1st meal (minutes)	30.49 ± 5.87	27.72 ± 5.32	NS
6 h feed intake (kg)	2.89 ± .45	3.97 ± .41	†
Feed intake 1st meal (kg)	1.82 ± .41	1.88 ± .37	NS

[a] Least Square Means ± SE.
[b] NS=Non-Significant.
† Indicates P<.1 (trend).

Discussion

The great variation in titer values was expected based on results obtained by Nelson (1996). Results from Exp 1, with no differences across treatments for any of the lactation performance variables, indicate that there's no advantage of vaccinating sows against CCK. Our results are in agreement with those in sheep (Trout et al., 1989; Spencer, 1992) in which there were no feed intake differences between vaccinated and control animals. The possibility of vaccinated animals not achieving greater performance simply because they did not develop a titer, is ruled out by the analysis of data only from animals with a titer against controls. Again in this case, there were no differences in performance between animals with a titer and controls.

Regression analysis results demonstrated also that there's no advantage in vaccinating against CCK. Our regression analysis included ADFI during wk 1, 2, 3 and the total 3 wk lactation period, with log titer value for the same period, respectively. Nelson (1996) obtained significant r^2 values (r^2=.7) when correlating ADFI during week 3 with a combination of log titer values including gestation, lactation and colostrum. However, the biological reason for establishing a relationship between ADFI with titer values from periods of sow production as far as 64 days before is weak.

Results of Exp 2 help to explain those of Exp 1. Sows immunized against CCK appear to overproduce CCK in response to the sequestering of free-CCK by anti-CCK antibodies. This is shown by the fact that total CCK values were larger than free-CCK values in CCK-immunized animals (Figure 4). Total and

free-CCK values for control animals did not differ (Figure 4). A reasonable explanation would be that, in animals vaccinated against CCK, CCK-Ab sequestered almost 2.5 times the amount of free-CCK hormone. The animal then, sensing a decrease of free-CCK, responded with a feed-back mechanism by producing more free-CCK in order to achieve a normal (before vaccination) free-CCK concentration in blood. This was suggested by others (Spencer, 1992).

Our values for free-CCK are in agreement with other reports on free-CCK values in pigs (Clutter et al., 1998). Free-CCK values are no different for both treatments. This would explain the lack of response in performance across treatments to CCK immunization in both Exp 1 and Exp 2.

In Exp 2, immunized sows tended to eat more during the 6 h period (3.97 ± .41 CCK vs 2.89 ± .45 Control). This could be explained by the free-CCK values for each treatment. Immunized animals had a lower free-CCK concentration (numerically) than Controls (12 ± 3 pmol/L vs 19 pM/L ± 5, respectively). While these values were not significantly different, the fact that that sows on CCK group had lower free-CCK could result in less anti-eating action of CCK, thus showing a greater feed intake.

Implications

Vaccinating parity-one sows against CCK did not improve ,lactation performance, nor it affected return-to-estrus interval. Vaccinated animals may compensate for the sequestering of endogenous CCK by overproducing higher

amounts of free-CCK, as suggested by Spencer (1992). This suggests that the development of different vaccines which produce similar amounts but more consistent titer responses from sows, would be of little benefit. Further studies testing the overproduction hypothesis could be developed by measuring mRNA, an indicator of peptide production, in CCK-producing cells, such mucosal-I cells of the small intestine.

Chapter 3

PASSIVE IMMUNIZATION AGAINST CHOLECYSTOKININ (CCK) AND ITS EFFECT ON GROWTH PERFORMANCE OF NURSERY PIGS

ABSTRACT

Forty-eight, early-weaned crossbred pigs (10 to 12 d of age) from sows vaccinated against CCK or from control sows, which had been vaccinated with a placebo, were used to test the potential benefits of passive immunization on performance of nursery pigs. Animals were housed in pens containing four animals of the same treatment. Individual pig weights were recorded on d 0, 7, 14, 21, 28, and 35. Feed disappearance was monitored daily for each pen. Serum samples were collected via vena cava puncture from each pig on days of age 14, and 21 and anti-CCK titer on was determined by RIA.

There was a difference in ADG (P<.01) between immunized (CCK) and non-immunized (Control) animals during wk 1 (.23 kg vs .20 kg, SE .01). Values for ADG for individual wk 2, 3, 4 and 5 were not different between treatments. There was a difference in ADFI (P<.05) between treatments during wk 2 (.42 kg CCK; vs .35 kg Control; SE .02). Values for ADFI for individual wk 1,3,4 and 5 were not different between treatments. Over the 5 wk experimental period, ADFI (.44 vs. .41, SE .01) was greater (P<.05) and ADG (.67 vs .63, SE .01) tended to be greater (P<.1) for the CCK group. Feed/Gain ratio (F/G) did not differ for any individual week or the 5 wk experimental period. Passive immunization against CCK improved growth performance of early-weaned nursery pigs.

Key words: Pigs, CCK, Passive Immunization, Nursery, Early-Weaning.

Introduction

Cholecystokinin (CCK) is a hormone involved in regulation of appetite and feed intake. In a series of studies (Pekas and Trout, 1990; Pekas, 1993; Pekas, 1996) with growing pigs, active immunization against CCK stimulated feed intake and growth in proportion to the anti-CCK antibody (CCK-Ab) titers elicited in the animal. Nelson (1996) demonstrated that piglets from sows vaccinated with CCK were passively immunized, having a titer at d 7 of age equivalent to that of the lactating sow.

Weaning piglets at less than three weeks of age (Early Weaning) has become a standard practice in swine production because of health considerations and improved growth performance. Early-weaning main advantage is to stop bacterial and viral spread from the sow to the piglets. Piglets are passively immunized against disease. Sows immunized against CCK transfer their immunity to CCK to their piglets as well, and thus, could produce an improvement in piglets feed intake by stopping CCK action on satiety.

This study was designed to determine the effect of passive immunization against CCK on growth performance of early- weaned nursery pigs.

Materials and Methods

All procedures for this experiment were approved by the Michigan State University Committee for Animal Use and Care.

General Procedures for Pigs. A total of 48 early-weaned crossbred pigs Newsham® X (Yorkshire X Landrace)), averaging 11 d ± 1 of age were used.

Piglets were obtained from sows that had been vaccinated against endogenous CCK on d 64, 78, 92 and 106 of gestation (procedure described by Garcia-Sirera, 1999, previous chapter). Gilts were also vaccinated prebreeding for parvovirus, leptospirosis and erysipelas; prefarrowing for bordetella, *E. coli*, pasteurella, Transmisible Gastroenteritis, erysipelas and clostridium.

Processing pigs on day one after birth included: ear notching, clipping of needle teeth, tail docking, iron shots of 150 mg iron and 0.25 mg ceftiofur hydrochloride. One day prior to weaning, pigs were weighed individually for allotment to treatment. At weaning, 150,000 U benzothine penicillin was given for prevention of *Streptococcus suis* infection. Pigs were not vaccinated at weaning.

At weaning, pigs were allotted to treatments based on litter, sex, and passive immunization treatment (non-immunized vs. immunized). Pigs were housed in the Michigan State University Veterinary Isolation Facility, G-barn. Pigs were housed in three rooms (four pens per room). Pens were 1.22 x 0.9 m, with four pigs per pen. Temperature was controlled at each location so that the ambient room temperature remained between 20° and 28° C. Heat lamps and heat pads (1 in each pen) were used for the first week to provide micro environments.

During the first week all pens were fed 50 g per day per pen on 400 cm^2 plastic trays, and also had access to fenceline feeders. Pigs had access to one nipple waterer per pen. During wk 1 waterers were set to drip continually to help avoid dehydration and possible navel sucking. Feed intake and growth performance of piglets was monitored for 5 wk. Individual weights were recorded

on d 0, 7, 14, 21, 28, and 35. Feed disappearance for each pen was monitored daily. Pigs were fed the same diet regardless of treatment (Table 5). The experimental period was divided into four phases. Phase 1 and Phase 2 diets were fed on wk 1 and wk 2, respectively. Phase 3 diet was fed on wk 3 and 4 and Phase 4 was fed on wk 5. Serum samples were collected via vena cava puncture from each pig on days of age 14, and 21 (equivalent to d 2 and d 9 of the feeding trial) . Anti-CCK titer was determined by RIA (Garcia-Sirera, 1999, previous chapter).

Statistical Analysis. Data was analyzed as randomized block design using the GLM procedure of SAS (1996). Pigs were blocked by initial weight and equalized for ancestry and sex. Pen was the experimental unit. The mean differences between immunization treatment (CCK and Control) were detected by comparison of least square means. Differences were considered significant at the level of $P<.05$, $P<.1$ was considered a trend.

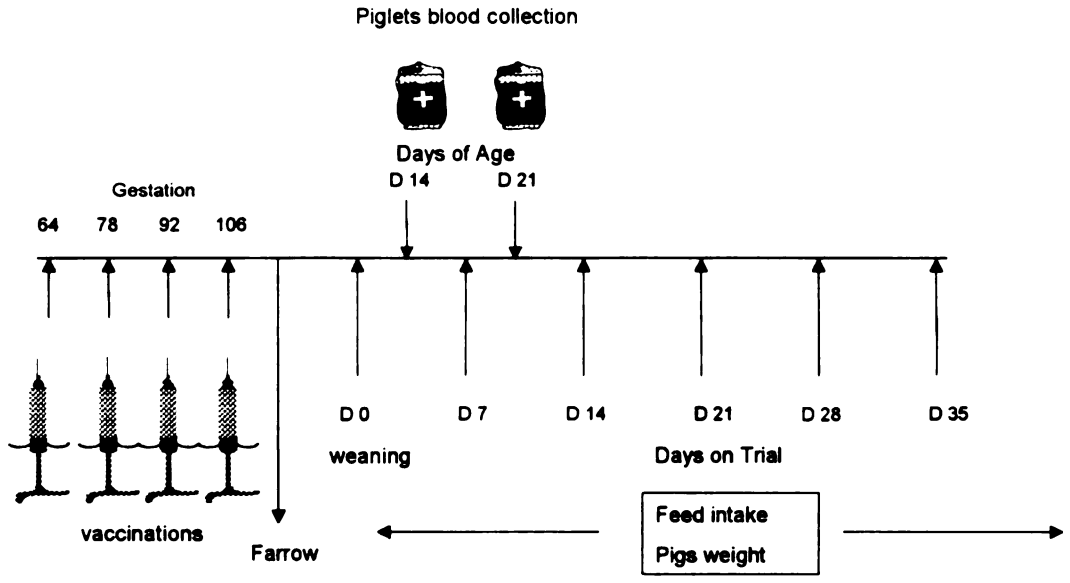

Figure 5. Time sequence of nursery trial showing time of sow vaccinations and sample collection.

Table 5. Composition of diets fed in different phases of nursery (as fed).

Diets	Phase 1	Phase 2	Phase 3	Phase 4
Ingredients %				
Corn dent yellow	29.49	40.53	53.77	60.74
Soybean meal 44%	15.30	17.88	27.63	35.07
Plasma AP920	7.50	2.50	----	----
Edible Grade Lactose	7.50	4.00	----	----
Whey, dried	25.00	20.00	10.00	----
Fish meal	6.62	7.50	5.00	----
Choice White Grease	5.00	4.00	----	----
Mono-Dical Phos	0.62	0.80	1.20	1.45
Vitamin Premix[a]	0.60	0.60	0.60	0.60
Salt	0.50	0.50	0.50	0.50
Zinc Oxide[b]	0.38	0.38	----	-----
Limestone	0.45	0.35	0.45	0.91
Antibiotic[c]	0.25	0.25	0.25	0.25
Trace Min premix[d]	0.50	0.50	0.50	0.50
Copper Sulfate[ef]	0.05	0.05	0.10	0.10
DL-Methionine	0.13	0.06	----	-----
L-Lysine HCl 78.8%	0.10	0.09	----	-----
Calculated Analysis				
ME, kcal / kg	3513.29	3457.36	3396.40	3408.00
CP %	23.70	22.11	19.64	19.19
Lysine	1.70	1.45	1.25	1.15
Met + Cys	0.50	0.45	0.36	0.32
Ca %	0.90	0.88	0.90	0.80
Total P %	0.57	0.53	0.54	0.41

[a] Supplied per kilogram of diet: vitamin A, 5512 IU; vitamin D_3, 551 IU; vitamin E, 66 IU; vitamin K (as menadione sodium bisulfite complex) 4.4 mg; riboflavin, 4.4 mg; pantothenic acid 17.6 mg; niacin, 26.4 mg; vitamin B_{12}, 33 mg; thiamin, 1.10 mg; pyridoxine, 1.0 mg.

[b] Supplied 3000 mg of Zn per kilogram of diet (in addition to that provided by trace mineral premix).

[c] mecadox-10®.

[d] Supplied per kilogram of diet: Zn, 10 mg; Cu, 10 mg; Fe, 100 mg; Mn, 10 mg; I, 0.15 mg; Se, 0.3 mg.

[e] Supplied 125 mg of Cu per kilogram of diet (in addition to that provided by trace mineral premix in Phase 1 and 2).

[f] Supplied 250 mg of Cu per kilogram of diet (in addition to that provided by trace mineral premix in Phase 3 and 4).

Results

Analysis by RIA on d 14 of age (d 2 of feeding trial), showed only 12 pigs with a titer against CCK (mean log titer 2.37 ± .44). All samples from control animals did not show any specific binding as expected. Immunized piglets in the experiment were originally from litters of six sows actively immunized against CCK. Serum from all sibling piglets belonging to one of the six original vaccinated sows, showed very low specific binding (between 15 and 19%) on d 14, and even lower on d 21 (around 10%). At least one piglet in each of the five other litters had serum with specific binding higher than 50% (1:50 dilution, definition of titer) on d 14. With the exception of piglets from the low-titer litter, all other piglets had serum values showing specific binding values beyond 50% or very close to titer definition (higher than 40% but lower than 50%).

Four out of the 12 pigs with a titer on d 14, still had a titer on d 21 (mean log titer 2.54 ± .20). Day 21 of age corresponds to an average of nine days into the feeding experiment. For the remaining 8 pigs with a titer on d 14, the specific binding had dropped beyond titer definition (50%) on d 21. The four pigs with serum samples still showing specific binding higher than 50% on d 21 were from the same original litter. All other samples from immunized animals on d 21 had low specific binding values (around 30%).

Table 6 shows ADFI, ADG and F/G. There was a significant difference between immunized (CCK) and non-immunized (Control) animals for ADFI during wk 2 (.42 vs. .35, respectively; $P < .05$) and the difference tended to be significant for all 5 wk of experiment (.67 vs. .63; $P < .1$). There was also a significant

difference between treatment groups for ADG during wk 1 (.23 CCK vs. .20 Control; P < .01) and for all 5 wk of experiment (.44 CCK vs. .41 Control; P< .05). There were no differences in F/G ratio in any of the 5 weeks individually or when considering the whole experimental period.

Discussion

Overall ADG during the total duration of the experiment was increased in immunized animals vs. controls (.44 vs. .41, P<.05, respectively), and tended to be increased in ADFI (.67 vs. .63, P<.1, respectively). The results on growth performance can be explained with the results of titer analysis.

Analysis of serum titer against CCK showed half the piglets from CCK sows, had a specific binding higher than 50% on d 14 of age, the threshold considered to be called "titer". All the siblings originally from one of the six vaccinated sows that provided piglets for the experiment did not develop any titer. This was, most likely, a result of the sow not developing good immunity and consequently not passing it down to the piglets. On d 21 of age the percentage of piglets with a titer was even lower (only four pigs). This was expected because of clearance of immunoglobulins. This low percentage of animals with a titer may explain why the ADFI and ADG differences between treatments were not even more obvious. Titer in the piglets is determined by the titer in the colostrum of the sow, which can vary greatly (Nelson, 1996) and clearance of immunoglobulins once they are absorbed by the piglet.

It's generally accepted that passive immunity in piglets reaches its peak after absorption of colostrum, at approximately 2 – 3 d of age and decreases rapidly after that. By week 3 of age immunity from colostrum is very low. For this reason passive immunization against CCK was expected to yield any potential benefits during the firsts weeks of the experiment, when the piglets were still very young. This reasoning was reflected, to a point, by the improved ADFI and ADG of immunized vs. control animals during the first two weeks of the experiment. The fact that ADFI was different between treatments during wk 2 and not wk 1 could be explained by the stress from weaning and adaptation to the experiment diets tended to equalize feed intake across treatments during wk 1. During wk 2, with the animals well adapted, the treatment effect of passive immunization was evident. It is important to notice also that, numerically, CCK-immunized animals outperformed Control animals in each individual week of the experiment for ADFI and ADG (Table 6). Good performance in the nursery is very important for future productivity of pigs, as a rule of thumb, one kilogram of improved weight gain during the nursery phase results in three total days less to achieve market weight.

Implications

Passive immunization against CCK improved growth performance of early-weaned nursery pigs. Development of a sow immunization schedule with emphasis on producing peak titer values at farrowing, in order to increase

passive immunity of the piglets, may further improve results seen in this experiment.

Table 6. Effect of passive immunization against CCK of nursery pigs on growth performance[a].

Item	Treatment		Std Err	Level of Significance
	Control[b]	CCK[c]		
n	24	24		
Start Weight (kg)	4.17	4.16	.13	NS
Average Daily Feed Intake (kg)				
Wk 1	.16	.18	.01	NS
Wk 2	.35	.42	.02	*
Wk 3	.60	.68	.04	NS
Wk 4	.91	.94	.02	NS
Wk 5	1.12	1.13	.02	NS
Total 5 wk	.63	.67	.01	†
Average Daily Gain (kg)				
Wk 1	.20	.23	.01	**
Wk 2	.30	.33	.02	NS
Wk 3	.48	.54	.03	NS
Wk 4	.57	.57	.03	NS
Wk 5	.50	.53	.03	NS
Total 5 Wks	.41	.44	.01	*
Feed/Gain				
Wk 1	.80	.78	.06	NS
Wk 2	1.15	1.28	.08	NS
Wk 3	1.26	1.28	.04	NS
Wk 4	1.63	1.67	.10	NS
Wk 5	2.27	2.18	.14	NS
Total 5 Wk	1.53	1.53	.03	NS

[a] Least Square Means. 6 pens per treatment, 4 pigs per pen
[b] Piglets from non-immunized sows.
[c] Piglets from sows immunized against CCK.
† P < .1 (trend)
* P < .05
** P < .01

Chapter 4

CONCLUSIONS

The potential benefits of immunization against CCK were analyzed in this study. First, the effect of active immunization on lactation performance of sows and weaning-to-estrus interval was tested in Experiment 1. Vaccination against CCK did not improve parity-one productivity of sows, in particular ADFI during lactation. Our results are somewhat in contradiction with those of Nelson (1996) which reported a correlation between ADFI of sows vaccinated against CCK with a combination of titer values including samples taken in gestation, lactation, and colostrum. One could question the validity of such a comparison. One could question the biological reason to try to explain feed intake during a given period of sow production, with titer values from samples obtained as far as 64 d before. The most reasonable correlation under study should be between ADFI and titer values during the same period of production. When analysis was performed under this condition, no correlation could be established between titer and ADFI.

Since the immunized sows involved in Experiment 1 developed antibodies against CCK, several explanations could account for not obtaining any differences in production performance between immunized and non-immunized animals. Considering the results of Experiment 1 only, explanations could be:

(1) The antibodies developed with immunization against CCK do not bind free-CCK, and consequently, do not stop CCK from exerting its action on satiety.

(2) The antibodies bind free-CCK but do not stop the hormone from binding to its receptor. So, in practice, the hormone, while bound, still can exert its action.

(3) When free-CCK decreases, as a result of being sequestered by CCK-antibodies, a feed-back mechanism is turned on, increasing production of free-CCK. In this way, concentration of free-CCK in blood does not decrease and its action on satiety doesn't either.

Results from Experiment 2 refute (1) and (2) and appear to corroborate (3). Free-CCK concentration was the same across treatments. Since total-CCK was greater than free-CCK only in immunized animals, this would indicate that immunized animals had overcompensated for the sequestering of free-CCK by the CCK-antibodies.

Experiment 3 was designed to test for potential benefits of passive immunization against CCK. Overcompensation of free-CCK production observed in Experiment 2 was not believed likely because pigs were passively immunized. It is not likely that piglets would react adversely against their initial source of immunity. The hypothesis was that passive immunization against CCK could improve growth performance of nursery piglets.

Improvement of growth performance in the nursery is very important in overall swine production. Generally, piglets that have an advantage in the early stages of production, keep this advantage until the end, resulting in better productivity.

In Experiment 3, the limitation factor was the initial titer. Most piglets did not have good titer at the beginning of the feeding trial, due to the rapid clearance of antibodies from the circulation. It is reasonable to think that better results would have been achieved if the piglet immunity against CCK at the beginning of the nursery phase had been greater.

In summary, active immunization against CCK does not improve lactation performance of sows, while passive immunization of piglets does improve their performance during the nursery phase of production. Further research in the area of passive immunity should be done to improve results in the nursery phase of production, already observed in this study.

Future Research

The hypothesis of overproduction of free-CCK by immunized animals should be further tested. Studies involving measurement of mRNA could determine if the hypothesis is correct. Elevated levels of mRNA is an indication of increased production. Immunized animals should have elevated mRNA, encoding for CCK, in mucosal I-cells of the duodenum and jejunum, where CCK is mainly produced in the periphery, when compared to controls.

The immunization schedule followed in Experiment 3 was the same than in experiments 1 and 2, because of our experience in previous research. This schedule though, was based on studies by Pekas (1996) and Nelson (1996), and it was designed to achieve a peak immunity during wk 2 of lactation. The reason for this was to maximize lactation performance of lactating sows (the objective of

previous research). Further experiments with passive immunity could test different immunization schedules, possibly moving the initial vaccination earlier in gestation, so peak immunity is achieve at farrowing time, when colostrum is produced, and consequently, a higher immunity could be passed down from the sow to the piglets.

APPENDICES

APPENDIX A

DETERMINATION OF ANTISERA TITER AGAINST CHOLECYSTOKININ

Reagents

0.1% Gel Buffer, pH 7.5 (for dissolving tracer)

2.68 Na_2HPO_4 $7H_2O$ (.01 M)(Sigma cat.# S-9390)

0.37 g EDTA Na_2 (.001 M) (Sigma cat.# ED2SS)

1.0 g Na azide (.1% w/v) (Sigma cat.# S-2002)

8.10 g NaCl (.14 M) (Sigma cat.# S-9888)

1.0 g Gelatin (.1% w/v) (Sigma cat.# G-2500)

Place in a 1000 mL volumetric flask and partially fill to volume with deionized distilled water. Stir on low heat on magnetic stirrer in order to dissolve gelatin. After gelatin is dissolved, solution is cooled to room temperature. Solution is then adjusted to pH 7.5 with 1 N HCL. Finish filling the flask to volume with deionized water. Check to be sure pH is still 7.5.

0.05% Gel Buffer, pH 7.5 (assay buffer)

2.68 Na_2HPO_4 $7H_2O$ (.01 M)(Sigma cat.# S-9390)

0.37 g EDTA Na_2 (.001 M) (Sigma cat.# ED2SS)

1.0 g Na azide (.1% w/v) (Sigma cat.# S-2002)

8.10 g NaCl (.14 M) (Sigma cat.# S-9888)

0.5 g Gelatin (.1% w/v) (Sigma cat.# G-2500)

Place in a 1000 mL volumetric flask and partially fill to volume with deionized distilled water. Stir on low heat on magnetic stirrer in order to dissolve gelatin.

After gelatin is dissolved, solution is cooled to room temperature. Solution is then adjusted to pH 7.5 with 1 N HCL. Finish filling the flask to volume with deionized water. Check to be sure pH is still 7.5.

1.25% Dextran-coated Charcoal in 0.05% gel buffer

1.258 g Norit A charcoal (Fisher cat. # C176-500)

0.125 g Dextran, average molecular weight = 79100 (Sigma cat. # D4751)

100 ml of 0.05% Gel Buffer, pH 7.5

Place in 150 mL beaker and stir at room temperature on magnetic stirrer for 30 minutes. Store in refrigerator. Stir continuously over crushed ice while using.

Tracer

[^{125}I]-CCK-8s-Bolton Hunter labeled (DuPont NEN cat.# NEX-203)

The μL of concentrated tracer used will depend on the size of the assay, but will be diluted with 0.1% Gel Buffer, pH 7.5 to give a final radio concentration of 4884 dpm/100 μL assay (1fmol CCK-8s/100 μL assay). Mix on rocker table for 30 minutes at room temperature. Tracer is then quantitatively transferred to another tube to prevent any bound tracer on walls of tube from leaching into the solution. Fifty μL aliquots are then pippeted into 12x75 mm tubes and counted to determine actual dpm being put into assay tubes.

Reference antisera

For assays in Dr. Pekas lab (USMARC), 25 μL of pig 42307 (Expt. # JP8704, CCK immunized, blood collected on 1/28/88) is diluted in 25 ml of 0.05% gel buffer, pH 7.5 (1:1000 dilution) which gives specific binding of approx. 50%.

Diluted antisera is then aliquoted into 300 μL amounts into 12x75 mm assay tubes and stored at -20°C. These tubes will be used directly in each assay run.

Antisera dilutions

A)	1:50	26 μL of raw antisera + 1274 μL of 0.05% gel buffer
B)	1:325	200 μL of "A" + 1100 μL of 0.05% gel buffer
C)	1:2113	200 μL of "B + 1100 μL of 0.05% gel buffer
D)	1:13731	200 μL of "C" + 1100 μL of 0.05% gel buffer
E)	1:89253	200 μL of "D" + 1100 μL of 0.05% gel buffer
F)	1:580145	200 μL of "E" + 1100 μL of 0.05% gel buffer

Dilutions are made in 2.0 mL screw cap vials and gently vortexed three times between dilutions.

Assay Procedure

Total Counts (TC)	100 μL of tracer
Nonspecific Binding (NSB)	300 μL of 0.05% gel buffer + 100 μL of tracer
Reference Antisera (RA)	300 μL of reference antisera + 100 μL of tracer
Unknowns (antisera dilutions)	300 μL of unknowns + 100μL of tracer

1. Pipette 300 μL of each antisera dilution in triplicate into 12x75 mm glass test tubes. For NSB tubes use 300 μL of 0.05% gel buffer.

2. Add 100 μL of tracer to each tube and gently vortex.

3. Cover racks of assay tubes with aluminum foil and incubate for 48 h at 4^0C.

4. At the end of 48 h, add 250 μL of ice cold dextran-coated charcoal to tubes. Shake rack of tubes vigorously to mix and incubate for 10 min at 4^0C. **Do <u>not</u> add charcoal to TC tubes.**

5. After the 10 min incubation, add 300 μL of ice cold 0.05% gel buffer to tubes and shake vigorously to mix. Incubate an additional 10 min at 4^0C.

6. At the end of the additional 10 min, centrifuge tubes for 5 min in 4^0C centrifuge at 2000 xg.

7. Remove tubes from centrifuge and immediately remove supernatant from charcoal pellet using disposable 9" Pasteur pipettes. Place supernatants in corresponding 12x75 mm glass test tubes. Keep other tubes on ice until ready to be separated.

8. Count both supernatants and charcoals in gamma counter for 2 min.

<u>Calculations</u>

Nonspecific binding = cpm of NSB/cpm of TC

Specific binding = (cpm of unknowns -cpm of NSB) / (cpm of TC - cpm of NSB)

APPENDIX B

EAR VEIN CATHETERIZATION PROCEDURE

The procedure involves three people and the following equipment:

Tape (Elastikon®)

Iodine-scrub (Betadine®) solution

Tourniquet

Wire-guide (TSF-38-145, diameter .038 inch, length 145 cm Cook Veterinary Products, Bloomington, Indiana, Cat # 009845)

16 gauge needle (Becton & Dickinson)

Catheter (Tygon® ID 0.040" Fisher Cat # 14170-15D)

Heparinized saline (20 units/ml)

Syringe (10 ml, luer lock®).

Tubing adapter (18 gauge, Becton & Dickinson).

Hemostats

Scissors

Lighter

Procedure

- One person (A) snares the sow trying to keep it from moving too much.
- Second person (B) shaves the hair on the ear and surrounding area (neck).
- Third person (C) washes the whole ear area with a warm disinfecting iodine-scrub solution.

- (B) uses a tourniquet around the ear to expose the veins.(C) holds the tourniquet in place.
- After selecting the vein, (B) inserts a 16 gauge needle and holds it in place with a piece of tape.
- After making sure the needle is in the vein (there should be a steady drip of blood) (B) inserts the wire-guide.
- (B) makes sure that the wire has gone through the needle, (C) releases the tourniquet. At the same time, (C) is holding the wire-guide, inside its cover to avoid exposure to the air, while (B) is inserting the free end into the vein. After the wire has been inserted 40-50 cm, the needle is removed
- The catheter is inserted at the free end of the wire. A syringe containing heparinized saline is inserted at the free end of the catheter, The syringe is connected to the catheter with the tubing adapter
- The saline is flushed through the catheter inserted on the wire, and run down to the insertion point of the ear. (B) gently starts pushing the catheter into the vein, (C) keeps flushing the catheter using the syringe
- The initial insertion of the catheter through the skin of the ear can become quite difficult. A scalpel can be used to make a small incision to enlarge the insertion point through the skin of the ear.
- When the catheter is inserted so far that the wire-guide is coming out through the free end, the syringe and tubing adapter are removed. At this point (C) holds the wire and pushes the catheter in, trying NOT to pull out the wire. (B) inserts the catheter at the ear vein. The catheter should be inserted at least

40 cms (successful bleedings have been accomplished with only 25 cms inserted).

- Once the catheter is inserted, the wire is removed. The catheter is flushed with heparinized saline, making sure it's operational. The catheter is then held in place with a piece of tape and sealed with a heparinized saline block.
- The sealing is done by holding the tip of the catheter with a hemostat and burning the tip. When the tip burns it has to be pressed between the fingers (wet fingers). To check if there's good seal the tip of the catheter should be burnt all around.

The last step is to wrap the catheter and fixed to the ear with plenty of tape to prevent the sow from breaking it until time of collection of blood samples.

APPENDIX C

FREE AND TOTAL CCK ANALYSIS

Free CCK

Immunized Animals:

1. Filter 1.5 mL of plasma on centriplus 100 (cat # 4414, Amicon Inc.) using a centrifuge with a 34° fixed-angle rotor and a speed of 3000 xg for 2 h at 4°C.
2. Separate the filtered sample in one tube with 0.8 mL (analysis tube) and second tube with the remaining filtered plasma (left -over tube). Use 12 x 75 mm polypropilene tubes.
3. Freeze left-over tube for future analysis.
4. Mix the 0.8 mL filtered plasma with 1.6 mL of 100% ethanol. Vortex for 10 s and store at room temperature for 20 min.
5. Centrifuge the mix for 30 minutes (1500 xg) at 4°C. Decant the supernatant into four (4) 1.5 mL polypropilene tubes and then evaporate in a Speed Vac concentrator at room temperature.
6. Store samples until analysis at –20° C.
7. When ready for analysis, reconstitute all four (4) tubes from the same original sample with 300 μL of assay buffer. Do this by pipetting 300 μL into the first tube, collect the pellet, aspirate the liquid and pipette it into the second tube. Repeat procedure for all four tubes from same original sample. At the end one should have 300 μL of assay buffer containing the pellets from all four tubes from same original sample.

8. Analyze using RIA kit from Peninsula Laboratories using manufacturer's instructions.

Non-immunized animals:

Repeat steps 2 through 8 as described above. Use original plasma (unfiltered).

Total CCK (only for Immunized animals)

1. Mix 0.4 mL of plasma with 0.8 mL of 100% ethanol. Vortex for 10 s and store at room temperature for 20 min.
2. Centrifuge the mix for 30 min (1500 xg) at 4°C. Decant the supernatant into two (2) 1.5 mL polypropilene tubes and then evaporate in a Speed Vac concentrator at room temperature.
3. Store samples until analysis at –20° C.
4. When ready for analysis, reconstitute both (2) tubes from the same original sample with 300 μL of assay buffer. Do this by pipetting 300 μL into the first tube, collect the pellet, aspirate the liquid and pipette it into the second tube. At the end, one should have 300 μL of assay buffer containing the pellets from both tubes from the same original sample.
5. Analyze using RIA kit from Peninsula Laboratories using manufacturer's instructions.

APPENDIX D

EXPERIMENT 1 GROUP DATA

Table 7. Exp 1: Effect of replicate (group) on sow ADFI and piglet ADG[a].

	Sow ADFI			
Group	Week 1	Week 2	Week 3	Total
1	2.013 ± 0.362	3.510 ± 0.365	4.193 ± 0.436	3.238 ± 0.325
2	3.565 ± 0.296	4.623 ± 0.298	4.864 ± 0.356	4.350 ± 0.266
3	4.650 ± 0.362	5.353 ± 0.365	5.668 ± 0.436	5.225 ± 0.325
4	4.747 ± 0.282	5.345 ± 0.284	5.626 ± 0.339	5.240 ± 0.253
5	5.404 ± 0.315	5.278 ± 0.317	5.590 ± 0.379	5.428 ± 0.283
6	4.278 ± 0.362	4.911 ± 0.365	5.396 ± 0.436	4.863 ± 0.325
7	4.141 ± 0.447	5.164 ± 0.451	6.450 ± 0.538	5.250 ± 0.402
8	4.601 ± 0.229	5.023 ± 0.231	5.583 ± 0.276	5.069 ± 0.206
9	4.966 ± 0.401	5.554 ± 0.405	6.868 ± 0.484	5.795 ± 0.361
	Piglet ADG			
1	0.194 ± 0.021	0.230 ± 0.017	0.200 ± 0.017	0.207 ± 0.013
2	0.195 ± 0.016	0.239 ± 0.013	0.194 ± 0.014	0.210 ± 0.011
3	0.171 ± 0.019	0.230 ± 0.016	0.242 ± 0.017	0.213 ± 0.013
4	0.188 ± 0.015	0.254 ± 0.012	0.249 ± 0.013	0.231 ± 0.010
5	0.184 ± 0.017	0.244 ± 0.014	0.233 ± 0.015	0.220 ± 0.011
6	0.190 ± 0.019	0.246 ± 0.016	0.201 ± 0.017	0.213 ± 0.013
7	0.165 ± 0.024	0.253 ± 0.019	0.224 ± 0.021	0.214 ± 0.016
8	0.157 ± 0.012	0.200 ± 0.010	0.187 ± 0.011	0.181 ± 0.008
9	0.140 ± 0.022	0.212 ± 0.017	0.231 ± 0.019	0.195 ± 0.015

[a] Least Square Means ± SE, n=69

Table 8. Exp 1: Effect of replicate (group) on weaning-to-estrus interval.

Group	Days[a]
1[bc]	4.8 ± .55
2[bc]	4.7 ± .41
3[bc]	4.8 ± .50
4[b]	3.9 ± .44
5[b]	4.1 ± .47
6[c]	5.5 ± .50
7[c]	5.8 ± .62
8[c]	5.5 ± .32
9[c]	6.0 ± .56

[a] Least Square Means. n=69
[bc] Rows with different superscripts differ (P<.05).

BIBLIOGRAPHY

Bibliography

Anika SM, Houpt TR, Houpt KA. 1981 Cholecystokinin and satiety in pigs. Am. J. Physiol. 240:R310.

Bado A, Duriex C, Moizo L, Roques BP, Lewin MJM. 1991. Cholecystokinin-A receptor mediation of food intake in cats. Am. J. Physiol. 256:R693-R697.

Buchan A, Polak J, Solcia E, Capella C, Hudson D, Pearse A. 1978. Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. Gut 19:403-407.

Calam J, Ellis A, Dockray CJ. 1982. Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. J. Clin. Invest. 69:218-225.

Chen DY, Deutsch JA, Gonzalez MF, Gu Y. 1993. The induction and suppression of c-fos expression in the rat brain by cholecystokinin and its antagonist L364, 718. Neurosc. Lett. 149:91-94.

Cheng CA, Geogheganm JG, Lawson DC, Berlangieri SU, Akwari O, Pappas TN. 1993. Central and peripheral effects of CCK receptor antagonists on satiety In dogs. Am. J. Physiol. 265:G219-G223.

Clutter AC, Jiang R, McCann JP, Buchanan DS. Plasma Cholecystokinin-8 in pigs with divergent genetic potential for feed intake and growth. Dom. Anim. Endoc. 15:9-21.

Cooper SJ, Dorusih CT. 1990. Multiple cholecystokinin (CCK) receptors and CCK- monoamine interactions are instrumental in the control of feeding. Physiol. Behav.48:849.

Corp ES, McQuade J, Moran TH, Smith GP. 1993. Characterization of type A and Type receptor binding sites in vagus nerve. Brain Res. 623:161.

Covasa M and Forbes JM. 1994. Effects of the CCK receptor antagonist MK-329 on food intake on broiler chickens. Pharmacol. Biochem. Behav. 48:479-486.

Crawley JN. 1985. Comparative distribution of cholecystokinin and other neuropeptides: Why is this peptide different fromn all other peptides?. Ann. NY Acad. Sci. 448:1-8.

Crawley JN, Kiss JZ. 1985. Paraventricular nucleus lesion abolish the inhibition of feeding induced by systemic cholecystokinin. Peptides 6:927.

Crawley JN, Kiss JZ, Mezi E. 1984. Bilateral midbrain transactions block the behavioural effects of cholecystokinin on feeding and exploration in rats. Brain Res. 322:316-321.

Della-Fera MA, Baile CA, Schneider BS, Grinker JA. 1981. Cholecystokinin antibody injected in cerebral ventricles stimulates feeding in sheep. Science 212:687.

Deschenes Rj, Lorens LJ, Haun RS, Roos BA, Collier KJ, Dixon JE. 1984. Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proc. Natl. Acad. Sci. 81:726-730.

Deschodt-Lanckman M. Bui ND, Noyer M, Christopher J. 1981. Degradation of cholecystokinin-like peptides by a crdue rat brain synaptosomal fraction: a study using high pressure liquid chromatography. Reg. Peptides 2:15-30.

Docray GJ. 1976. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 274:711-713.

Docray GJ. 1978. Isolation, structure and biological activity of two cholecystokinin octapeptides form sheep brain. Nature 274:711-713.

Dockray GJ, Gregory RA, Tracy HJ, Zhu WY. 1981. Transport of cholecystokinin-octapeptide-like immunoreactivity toward the gut in afferent vagal fibers in cat and dog. J. Physiol. (Lond.) 314:501-511.

Ebenezer IS and Baldwin BA. 1995. 2-Naphtalenesulphanyl-L-aspartatyl-2-(phenethyl) amide (2-NAP) and food intake in rats: Evidence that endogenous peripheral CCK does not play a major role as a satiety factor. Br. J. Pharmacol. 116:2371-2374.

Ebenezer IS, de la Riva C, Baldwin BA. 1990a. Effects of the CCK receptor antagonist MK 329 on food intake in pigs. Physiol. Behav. 47:145-148.

Ebenezer IS, Thornton SN, Parrot RF. 1996. The effects of the novel CCK_A analogue Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on feeding and cortisol release in pigs. Pharmacol. Biochem. Behav. 54:255-259.

Eberlien GA, Eysselein VE, Goebell G. 1988. Cholecystokinin-58 is the major molecular form in man, dog and cat but not in pig, beef and rat intestine. Peptides 9:993-998.

Eysselein VE, Reeve JR Jr, Shively JE, Miller C, Walsh JH. 1984. Isolation of a large cholecystokinin precursor from canine brain. Proc. Natl. Acad. Sci. USA 81:6565-6568.

Flemming AS. 1976. Control of feed intake in the lactating rat: role of suckling and hormones. Physiol. Behav. 17(5):841.

Gaudreau P, Quirion R, St-Pierre S, Pert C. 1983. Characterization and visualization of cholecystokinin receptors in rat brain using [^{3}H] pentagastrin. Peptides 4:755-762

Gibbs J, Young RC, Smith GS. 1973 Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psych. 84:488.

Gourch A, Orosco M, Pages N, Comoy E, Bohuon C, Rodriguez M, Martinez J, Jacquot C, Cohen Y. 1990. Changes in hypothalamic neuropeptide Y concentrations induced by cholecystokinin analogues. Eur. J. Pharmacol. 187:117-122.

Gubler U, Chua AO, Hoffman BJ, Collier KJ, Eng J. 1984. Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. Proc. Natl. Acad. Sci. 81:4307-4310.

Harper AA, Rapper HS. 1943. Pancreomyzin, a stimulant of secretion of pancreatic enzymes in extracts of the small intestine. J. Physiol. (Lond.) 102:115-125.

Helmerich DL, Thiels E, Sved AF, Verbalis JG, Stricker EM. 1991. Effect of suckling on gastric motility in lactating rats. Am. J. Physiol. 261:R38.

Hewson G, Leighton GE, Hill RG, Hughes J. 1988. The cholecystokinin receptor antagonist L-364,718 increases food intqke in the rat by attenuating the action of endogenous cholecystokinin. Br. J. Pharmacol. 93:79-84.

Hill DR, Campbell NJ, Shaw TM, Woodruff GN. 1987. Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in the rat CNS using highly selective nonpeptide CCK antagonists. J. Neurosci. 7:2967-2976.

Hill DR, Shaw TM, Graham W, Woodruff GN. 1990. Autoradiographical detection of cholecystokinin (CCK-A) receptors in primate brain using [^{125}I]-Bolton Hunter CCK8 and [^{3}H]-MK-329. J. Neurosci. 10:1070.

Hill DR, Singh L, Boden P, Pinnock GN, Hughes J. 1992. Detection of CCK receptor subtypes in mammalian brain using highly selective non-peptide antagonists. In *Multiple cholecystokinin receptors in the CNS*. pp.57-76. Eds. C.T. Dourish, S.J. Cooper, S.D. Iversen and L.L. Iversen. Oxford Univer. Press. Oxford.

Hokfelt T, Herrera-Marshitz M, Seroogy K, Gong J, Staines WA, Holets V, Schalling M, Understed U, Post C, Rehfeld JF, Frey P, Fischer J, Docray G, Hamaoka T, Walsh JH, Godstein M. 1988. Immunohistochemical studies on cholecystokinin (CCK)- immunoreactive neurons in the rat using sequence specific antisera and with special reference to the caudate nucleus and primary sensory neurons. J. Chem. Neuroanat. 1:11-52.

Hokfelt T, Rehfeld JF, Skirboll L, Ivemark B, Goldstein M, Markey K. 1980. Evidence for co-existence of dopamine and CCK in mesolimbic neurons. Nature 285:476-478.

Houpt TR. 1983. the sites of action of cholecystokinin in decreasing meal size in pigs. Physiol. Behav. 31:693.

Innis RB, Snyder SH. 1980. Distinct cholecystokinin receptors in brain and pancreas. Proc. Natl. Acad. Sci. USA 77:6917-6921.

Ivy AC, Oldberg E. 1927. Contraction and evacuation of gall-bladder by a highly purified "secretin" preparation. Proc. Soc. Exp. Biol. Med. 25:113-115.

Ivy AC, Oldberg E. 1928. Contraction and evacuation of gall-bladder by a purified "secretin" preparation. J. Am. Med. Assoc. 90:445-446.

Jorpes JE, Mutt V. 1966. Cholecystokinin and pancreozymin, one single hormone?. Acta Physiol. Scand. 66:196-202.

Kow LM, Pfaff DW. 1986. CCK-8 stimulation of ventromedial hypothalamic neurons in vitro: a feeding-relevant event?. Peptides 7(3):473.

Larsson L-I, Rehfeld JF. 1979. Localization and molecular hteterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res. 165:201-218.

Leibowitz SF. 1986 Brain monoamines and peptides: role in the control of eating behaviour. Federation Proc. 45:1396.

Leibowitz SF, Weiss GF, Yee F, Tretter JB. 1985. Noradrenergic inervation of the paraventricular nucleus; specific role in control of carbohydrate ingestion. Brain Res. Bull. 14:561.

Liddle RA, Goldfine ID, Rosen MS, Taplitz RA, Williams JA. 1985. Cholecystokinin bioactivity in human plasma: molecular forms, responses to feeding, and relationship to gallbladder contraction. J. Clin. Invest. 75:114-1152.

Liddle RA, Goldfine ID, Williams JA. 1984. Bioassay of plasma cholecystokinin in rats: effects of food, trypsin inhibitor, and alcohol. Gastroenterology 87:542-549.

Linden A. 1989. Role of cholecystokinin in feeding and lactation. Acta Physiol. Scan. 137(Suppl 585):1.

Linden A, Eriksson M, Hansien S, Uvnas-Moberg K. 1990. Suckling-induced release of cholecystokinin into plasma in the lactating rat: effects of abdominal vagotomy and lesions of central pathways concerned with milk ejection. J.Endocrin. 127:257.

Matzat PD. 1990. Factors affecting feed intake in lacatting sows and the effect of feed intake in excess of ad libitum on lactation performance in multiparous sows. PhD Dissertation. Michigan State University. E.Lansing.

McLaughlin CL, Baile CA, Buonomo FC. 1985. Effect of CCK antibodies on food intake and weight gain in Zucker rats. Physiol. Behav. 34:277.

McLaughlin CL, Baile CA, Peikin SR. 1983. Hyperphagia during lactation: satiety response to CCK and growth of the pancreas. Am. J. Physiol. 244:E61.

Mercer JG, Lawrence CB. 1992. Selectivity of cholecystokinin receptor antagonists. MK-329 and L-365, 260, for axonally transported CCK binding sites in the rat vagus nerve. Neurosci. Lett. 177:229.

Moran TH, Ameglio PJ, Peyton HJ, Schwartz GJ, McHugh PR. 1993. Blockade of type A, not type B, CCK receptors postpones satiety in rhesus monkeys. Am. J. Physiol. 265:R620-R624.

Moran TH, Robinson PH, Goldrich MS, McHugh PR. 1986. Two brain cholecystokinin receptors: implications for behavioural actions. Brain Res. 362:175-179.

Morisset J, Levenez F, Corning T, Benrezzak O, Pelletier G, and Calvo E. 1996. Pig pancreas acinar cells posses predominantly the CCK-B receptor subtype. Am. J. Physiol. 271:E397-E402.

Mutt V, Jorpes JE. 1968. Structure of porcine cholecystokinin-pancreozymin. Cleavage with thrombin and with trypsin. Eur. J. Biochem. 6:156-162.

Mutt V, Jorpes JE. 1971. Hormonal polypeptides of the upper intestine. Biochem. J.125:57-58.

Muller JE, Strauss E, Yalow RS. 1977. Cholecystiokinin and its COOH-terminal octapeptide in the pig brain. Proc. Natl. Acad. Sci. USA 74:3035-3037.

Nelson DA. 1996. Dietary Fiber and Active Immunization Against Cholecystokinin (CCK) During Gestation Increases Lactation Feed Intake and Productivity of Sows. PhD dissertation. Michigan State University. E.Lansing.

Ondetti MA, Pluscec J, Sabo EF, Sheehan UT, Williams NJ. 1970. Synthesis of cholecystokinin-pancreomyzin. I. The C-terminal dodecapeptide. J. Am. Chem. Soc. 92:195-199.

Parrot RF. 1994. Central effects of CCK ligands in pigs making operant responses for food. Pharmacol. Biochem. Behav. 49:463-469.

Parrot RF and Baldwin BA. 1981. Operant feeding and drinking in pigs following intracerebroventricular injection of synthetic cholecystokinin octapeptide. Physiol. Behav.26:419-422.

Parrot RF and Baldwin BA. 1982. Centrally administered bombesin produces effects unlike short-term satiety in operant feeding pigs. Physiol. Behav. 28:521-524.

Parrot RF, Ebenezer IS, Baldwin BA, Forsling ML. 1991. Central and peripheral doses of cholecystokinin that inhibit feeding in pigs also stimulate vasopresin and cortisol release. Exp. Physiol. 76:525-531.

Parrot RF and Forsling ML. 1992. CCK-A receptors mediate the effect of cholecystokinin on vasopressin but not on cortisol in pigs. Am. J. Physiol. 262:R1154.

Passaro E, Debas M, Oldendorf W, Yamado T. 1982. Rapid appearance of intraventricularly administered neuropeptides in the peripheral circulation. Brain. Res. 241: 355-340.

Pekas JC. 1985. Animal growth liberation from appetite suppression. Growth. 49:19.

Pekas JC. 1993. Association of cholecystokinin (CCK) antisera with food intake in swine. Fed. Amer.Soc. Exper. Biolog. J. 7(3):A206.

Pekas JC. 1996. Immunogenicity of cholecystokinin octapeptide conjugated antigens in pigs. J. Anim. Sci. 74(8):1953.

Pekas JC, and WE Trout. 1990. Stimulation of food intake and growth of swine by cholecystokinin immunization. Growth. 54:51.

Pelto-Huikko M. Persson H, Schaling M, Hokfelt T. 1989. Immunohistochemical demonstration of cholecystokinin-like immunoreactivity in spermatozoian epididymis. Acta Phisiol. Scand. 137:465-467.

Persson H, Ericson A, Schalling M, Rehfeld JF, Hokfelt T. 1988. Detection of cholecystokinin in spermatogenic cells. Acta Physiol. Scand. 134:565-566.

Pisegna JR, de Weerth A, Huppi K, Wank SA. 1992. Molecular cloning of the human brain and gastric cholecystokinin receptor: structure, functional expression and chromosomal localization. Biochem. Biophys. Res. Commun. 189:296-303.

Polak J, Pearse A, Bloom S, Buchan A, Rayford P, Thompson J. 1975. Identifification of cholecystokinin-secreting cells. Lancet 2:1016-1021.

Rehfeld JF. 1978. Immunochemical sutdies on cholecystokinin II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J.Biol. Chem. 253:4022-4030.

Rehfeld JF. 1986. Characterization of preprocholecystokinin products in the porcine cerebral cortex. Evidence for different processing pathways. J. Biol. Chem. 261:5832-5840.

Rehfeld JF, Holst JJ, Lindkaeer Jensen S. 1982. The molecular nature of vascularity released cholecystokinin from the isolated perfused porcine duodenum. Regul. Pept. 3:15-28.

Riley JE. 1989. Recent trends in pig production: the importance of intake. In:The voluntary food intake of pigs. Eds. JM Forbers, MA Varley and TLJ Lawrence. Pp 1-6. Edimburg.

Robberecht P, Deshodt-Lanckman M, Vanderhaeghen JJ. 1978. Demonstration of biological activity of brain gastrin-like peptideic material in the human: its relationship with the COOH-terminal octapeptide of cholecystokinin. Proc. Natl. Acad. Sci. USA 75:524-528.

SAS. 1996. SAS User's Guide: Statistics (Version 6 Ed.). SAS Ins. Inc., Cary, NC.

Schick RR, Schusdziarra V, Nussbaumer C, Classen M. 1991. Neuropeptide Y and food intake in fasted rats: effect of naloxone and site of action. Brain Res. 552:232-239.

Schick RR, Schusdziarra V, Yaksh TL, Go LW. 1994. Brain regions where cholecystokinin exerts its effect on satiety. NY Acad. Sci. 713:242-254.

Shively J, Reeve JR Jr, Eysselein VE, Ben-Avram C, Vigan SR, Walsh JH. 1987. CCK-5: sequence analysis of a small cholecystokinin from canine brain and intestine. Am. J. Physiol. 252:G272-G275.

Silver AJ, Flood JF, Song AM, Morley JE. 1989. Evidence for a physiological role for CCK in the regulation of food intake in mice. Am. J. Phusiol. 256:R646-R652.

Sjolund K, Sanden G, Hakanson R, Sundler F. 1983. Endocrine cells in human intestine: an immunocytochemical study. Gastroenterol. 85:1120-1130.

Skirboll L, Hokfelt T, Rehfeld J, Cuello AC, Docray G. 1982. Coexistence of substance P and cholecystokinin-like immunoreactivity in neurons of the mescephalic periaqueductal central gray. Neurosci. Lett. 28:35-39.

Smith GP, Gibbs J. 1992. The development and proof of the CCK hypothesis of satiety. In Multiple cholecystokinin receptors in the CNS. Pp. 166-182. Eds. CT Dourish, SJ Cooper, SD Iversen and LL Iversen. Oxford Univ. Press. Oxford.

Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ. 1981. Abdominal vagotomy blocks the satiety effects of cholecystokinin in the rat. Science213:1036-1037.

Smith GP, Jerome C, Norgrenm R. 1985. Afferent axons in abdominal vagus mediated satiety effect of cholecystokinin in rats. Am. J. Physiol. 249:R638-R641.

Spencer GSG. 1992. Immunization against cholecystokinin decreases appetite in lambs. J.Anim. Sci. 70:3820.

Stallone D, Nicolaidis S, Gibbs J. 1989. Cholecystokinin-induced anorexia depends on serotoninergic function. Am. J. Physiol. 256:R1138.

Strube JH, Goriseen J. 1980. Meal patterning in the lactating rat. Physiol. Behav.25(5):775.

Tatemoto K, Carlquist M, Mutt V. 1982 Neuropeptide Y – A novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296:659-660.

Tatemoto K, Jornvall H, Siimesmaa S, Hallden G, Mutt V. 1984. Isolation and characterization of cholecystokinin-58 (CCK-58) form porcine brain. FEBS lett. 174:289-293.

Trout WE, Pekas JC, Schanbacher BD. 1989. Immune, growth and carcass responses on ram lambs to active immunization against desulfated cholecystokinin (CCK-8) J.Anim.Sci. 67:2709.

Tsai S, Passaro Jr HE, Lin MT. 1984. Cholecystokinin acts through catecholaminergic mechanisms in the hypothalamus to influence ingestive behaviour in the rat. Neuropharm. 23(11):1351.

Van der Kooy, D. 1984. Area Postrema: Site where cholecystokinin acts to Decrease food intake. Brain Res. 295:345-357.

Vanderhaeghen JJ, Schiffmann SN. 1992. Distribution of brain neuronal CCK. An *in situ* hybridization study. In *Multiple cholecystokinin receptors in the CNS.* Pp. 38-56. Eds. C. T. Doursih, S. J. Cooper, S. D. Iversen and L. L. Iversen. Oxford Univ. Press. Oxford.

Vanderhaeghen JJ, Signeu JC, Gepts W. 1975. New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 257:604-605.

Wager-Srdar SA, Morley JE, Levine AS. 1986. The effect of cholecystokinin, bombensin and calcitonin on food intake in virgin, lactating and postweaning female rats. Peptides 7(5):729.

Walsh JH, Lamer CB, Valenzuela JE. 1982. Cholecystokinin octapeptide-like immunoreactivity in human plasma. Gastroenterology 82:438-444.

Wank SA, Harkins R, Jensen RT, Shapira H, de Weerth A, Slattery T. 1992a. Purification molecular cloning, and fuctional expression of the cystokinin receptor from rat pancreas. Proc. Natl. Acad. Sci. USA 89:8691-8695.

Wank SA, Pisegna JR, de Weerth A. 1992b. Brain and gastrointestinal cholecystokinin receptor family: Structure and fuctional expression. Proc. Natl. Acad. Sci. USA. 89:8691-8695.

Wank SA, Pisegna JR, de Weerth A. 1994. Cholecystokinin receptor family. molecular cloning, structure and fuctional expression in rat, guinea pig, and human. NY. Acad. Sci. 713:49-66.

Weatherford SC, Chiruzzo FY, Laughton WB. 1993. Satiety induced by endogenous and exogenous CCK is mediated by CCK-A receptors in mice. Am. J. Physiol. 262:R574.

Weller A, Smith GP, Gibbs J. 1990. Endogenous cholecystokinin reduces feeding in young rats. Science 247:1589-1591

Zhou ZZ, Eng J, Pan YCE, Chang M, Hulmes JD, et al. 1985. Unique cholecystokinin peptides isolated from guinea pig intestine. Peptides 6:337-641.

Zuzel KA, Rose C, Schwartz JC. 1985. Assessment of the role of "enkephalinase" in cholecystokinin inactivation. Neurosci. 15:149-158.