ENHANCING BOAR REPRODUCTIVE PERFORMANCE FOR PURPOSES OF ARTIFICIAL INSEMINATION

by

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ABSTRACT

The objectives were to: 1) determine if im treatments of Lutalyse expedited the training of sexually inexperienced boars for semen collection and increased spermatozoal output, and 2) determine the effects of dietary L-carnitine supplementation on boar libido, semen quality, sperm production, and maintenance of sperm motility during liquid storage. Experiment 1 utilized lean-type, terminal-line boars (National Pig Development, Roanoke Rapids, NC) (n = 40; 177.4 ± 2.4 d of age and 112.8 ± 2.0 kg body weight) that had not previously experienced natural mating. Boars were individually moved twice weekly for 6 weeks (total of 12 training sessions) to a semen collection room equipped with an artificial sow. Upon entering the semen collection room, boars received im treatments of either deionized water (4 mL, n = 10) or Lutalyse at doses of 5 mg (n = 10), 10 mg (n = 10), or 20 mg (n = 10), and subsequently received a libido score of 1 to 5 (1 = no interest in the artificial sow; 5 = mounting the artificial sow and allowing semen collection). The percentages of boars successfully trained for semen collection during the experimental period were similar ($P > 0.05$) for controls (20%) and boars receiving 5 mg (30%), 10 mg (20%), or 20 mg (10%) of Lutalyse. Average libido score for boars receiving 10 mg Lutalyse (2.35 ± 0.08) was greater ($P < 0.05$) than for controls (2.14 ± 0.06). Libido score for the 20 mg treatment group were (1.78 ± 0.06) lower ($P < 0.05$) compared to the other treatment groups. Characteristics of ejaculates (volume, gel weight, sperm concentration, total spermatozoa) from control boars and boars treated

with Lutalyse at doses of 5, 10, or 20 mg were similar ($P > 0.05$). For Exp. 2, the same group of boars was utilized in two similar trials (Trial 1, 1a, 1b: n = 9 for control and L-carnitine-treated boars; Trial 2, 2a, 2b: n = 10 for control and L-carnitine-treated boars). Boars were fed a fortified, corn and soybean meal-based diet at a rate of 2 kg/d. Boars that were randomly selected for L-carnitine treatment received the same diet mixed with L-carnitine to achieve supplementation of 500 mg/d. For 16 wk, semen was collected weekly via the gloved hand method and was analyzed for gel-free volume, gel weight, sperm concentration, sperm per ejaculate, and characteristics of sperm motility. Time to ejaculation (reaction time), duration of ejaculation, and number of false mounts were also recorded for each collection. Trials 1a and 2a were conducted during weeks 16 and 17 for each respective trial. Boars were collected once on 4 consecutive days, allowed 4 d of rest, and then collected again, to estimate daily spermatozoal production. At the end of 16 wk, a semen sample was also processed and extended in Beltsville Thawing Solution (BTS) to achieve a dilution of 3×10^9 spermatozoa/100 mL-dose for Trials 1b and 2b. The extended semen was stored in plastic bottles at 18°C and motility was evaluated daily for 7 d post collection. L-carnitine supplementation for 16 wk had no effects on semen volume, gel weight, total number of sperm cells per ejaculate, reaction time, or sperm motility ($P > 0.1$). Boars receiving the L-carnitine-supplemented diet displayed an increase in the number of false mounts before ejaculating and an increase in sperm concentration ($P < 0.05$) in Trial 2. A treatment by week interaction was detected for sperm concentration in Trial 2 ($P < 0.005$). Increased sperm concentrations in L-carnitine-treated boars were demonstrated after only one week of feeding the respective diets. Given that the production of a mature sperm cell requires 7 to 8 wk in boars, it is

therefore difficult to conclude that differences in sperm concentration were due solely to treatment. Daily spermatozoal production was similar between control boars and boars supplemented with L-carnitine ($P > 0.1$) for both Trials 1a and 2a. L-carnitine supplementation did not affect percent motility in Trials 1b and 2b or sperm progressive motility in Trial 2b during 7 d storage ($P > 0.1$). A treatment by day interaction was determined for sperm velocity ($P < 0.05$) in Trial 2b. L-carnitine supplementation decreased mean sperm velocity significantly after 2 d of storage. Overall, L-carnitine had no beneficial effects on boar libido, semen quality, sperm production, or maintenance of sperm motility during liquid storage. However, Lutalyse increased libido scores, but did not affect the number of boars trained for semen collection or number of spermatozoa ejaculated.

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INTRODUCTION

Over the past decade, the U. S. swine industry has embraced artificial insemination (AI) as a tool for improving reproductive efficiency. During this time, the benefits of AI have been elucidated and the utilization of AI is increasing among swine producers. This is creating a higher demand for quality boars that produce quality semen and allow semen collection by a technician. The availability of overnight shipping and the production of quality boar semen extenders allows producers to order semen and have it available for breeding whenever necessary. This, in some cases, has eliminated or decreased the number of on-farm boars housed, but has also caused the development of additional and larger boar stud facilities supplying fresh semen. These boar stud facilities are seeking innovative technologies to optimize the reproductive capacity of boars, which could be achieved through decreasing the amount of time and labor required to train a boar for semen collection and by increasing the number of quality spermatozoa ejaculated.

Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) and $PGF_{2\alpha}$ analogs have been demonstrated to increase the libido of boars in some scenarios. By increasing the libido of a boar, it may potentially expedite the training process and allow the boar to become a part of the breeding herd more quickly. Furthermore, $PGF_{2\alpha}$ has been observed to increase the output of spermatozoa in several species including the boar. This effect could potentially increase the number of AI doses produced by a boar in a single ejaculate. The only commercially available $PGF_{2\alpha}$ analog approved for use in swine in the U.S. is Lutalyse.

Another possible way of increasing the spermatozoal output of boars is through diet supplementation. Being able to influence semen characteristics through manipulation of the diet offers producers an uncomplicated means of improving the reproductive efficiency of boars. L-carnitine is a vitamin-like compound synthesized by the liver, kidney and brain with high concentrations present within the epididymis and spermatozoa. Supplementing L-carnitine has increased spermatozoal output in roosters, men, and limited data reveals similar effects in boars.

Therefore, the intent of this thesis project was to enhance boar reproductive efficiency for purposes of AI, with specific objectives to determine as follows:

1. The effect of Lutalyse on the training of sexually inexperienced boars for semen collection.
2. The effect of Lutalyse on spermatozoal output of boars trained for semen collection.
3. The effect of L-carnitine supplementation on boar libido, semen quality, sperm production, and maintenance of sperm motility during liquid storage.

CHAPTER I: REVIEW OF LITERATURE

Swine Breeding

In modern, confinement swine operations, the majority of sows and gilts are bred via hand-mating, artificial insemination (AI), or a combination of both techniques. Both breeding methods first require detection of estrus, which is facilitated by use of mature, "heat-check" boars. When a female exhibits lordosis in the presence of a boar, she is mated. With hand-mating, a herdsman supervises a boar naturally servicing a female in estrus. Once copulation is completed, the two hogs are removed to their respective pens. With AI, a sow or gilt in estrus is inseminated with an aliquot of collected and processed boar semen, using a catheter that "locks" into the cervix of the female. The semen is gradually deposited into the uterus to minimize any back-flow. After semen deposition, the catheter is removed and the artificial mating is complete. The simplicity of swine AI, along with other advantages, has led to the recent increase in its usage throughout the U. S.

Swine AI in the U. S.

There was relatively little use of AI throughout the swine industry in the U. S. until this past decade. Before 1990, less than 5% of females were bred using AI (1). In 1991, AI accounted for approximately 8% of all females bred (2). During the mid-1990s, AI became a more popular tool for swine producers and by 1997, approximately 47% of all pigs born in the U.S. resulted from AI (3, 4). A recent survey revealed that nearly 70% of the litters farrowed in 2000 were sired through AI (4). At this rate, by the year

2005, nearly 85% of all U.S. litters produced will be a result of AI. This usage would be consistent with some larger pork-producing European countries (2).

Advantages of AI in Swine

There are significant benefits to be gained by swine producers who implement AI into their breeding programs. Applying AI to a breeding scheme allows a producer to increase boar power and the use of genetically superior sires, reduce sexually transmitted diseases, and mate animals of different sizes, while decreasing time and labor requirements.

Artificial insemination decreases the number of boars required to breed a large number of females, which results in an increase in boar power (5). Boar power is defined as a ratio, i.e., the number of breeding females to boars on a specific farm. Hand-mating schemes require that for every 4 breeding females, at least 1 to 3 sexually active boars be housed depending on the age of the boars, the number of services a female receives during estrus, the number of females to farrow, and the expected farrowing rate (6). It is recommended that a young boar (8 to 12 months of age) incorporated into a hand-mating system be used no more than once daily and 5 times weekly (7). A hand-mating scheme requires at least one younger boar for every 2 females to be bred per week when females are serviced twice per estrus (6). A mature boar (> 12 months of age) should be limited to 2 daily and 7 weekly matings in a hand-mating scheme (7). Therefore, a mature boar would be limited to 6 weekly matings if he was incorporated into a hand-mating scheme in which females are serviced twice per estrus (6). In natural service breeding systems, limiting the number of matings per boar may favorably influence farrowing rates and

litter sizes. High conception rates and litter sizes are obtained by accurate timing of insemination with an AI program, not by limiting the number of females per boar (8). Current AI techniques (e.g., once daily detection of estrus, sows bred 0, 24, and possibly 36 h post heat detection and gilts bred 0, 12, and possibly 24 h post heat detection) require a total of 2 to 3 x 10^9 sperm/dose (9, 10). Therefore, a successful breeding of a female via AI utilizes only 4 to 6 x 10^9 spermatozoa. This represents only a small fraction of a typical ejaculate, thus allowing a single boar ejaculate to be diluted and used to inseminate a high number of females, greatly increasing boar power. A typical AI program would require that for every 20 to 250 breeding females at least 1 sexually active boar be housed depending upon the number of doses inseminated, total spermatozoa per dose, turnover rate of boars, number of females to farrow, and expected farrowing rate (10). The increase in boar power with AI reduces the number of on-farm boars required by a producer who houses boars, thus decreasing overall cost of production. Producers then may be able to acquire boars that are genetically superior and utilize higher-quality genetics throughout their herds.

Artificial insemination also provides producers the option of synchronizing and inseminating a greater number of females in a short span of time. This is achieved most easily by group weaning piglets from sows, which will typically result in sows exhibiting estrus within 3 to 7 d post-weaning. A large number of sows, on any day estrus is exhibited post-weaning, may be bred with available fresh semen. A natural mating scheme would not provide this option for a producer unless they housed a high number of boars to potentially breed every sow individually. Furthermore, natural matings with older and larger boars may be impossible and hazardous to achieve with young gilts or

small sows; however, AI allows producers to utilize the superior genetics of larger boars since the sire and dam never actually come into contact and decreases the risk of injury both to sows and to humans.

Artificial insemination also reduces the risk of some sexually transmitted diseases between animals. The development of AI in most species was primarily for the prevention and elimination of venereal diseases (11). A plethora of pathogens exist in boar semen and are capable of being transmitted during mating. The testes, epididymis, vas deferens, vesicular, bulbourethral, and prostate glands, urinary bladder, ureters, kidneys, urethra, penis, preputial membranes, preputial diverticulum, and fecal material of the boar are all potential sources of contamination (12). Pathogens found in boar semen may be in the form of bacterias, mycoplasmas, yeasts, and viruses (12).

Minimizing the chance that new diseases will be introduced into a herd is a compelling reason for implementing AI (5). Incorporating AI into breeding schemes can be an integral part of a disease control program. It is true that some important diseases such as Brucellosis, Porcine Reproductive and Respiratory Syndrome, and Parvovirus can be transmitted through semen. But other diseases such as Pseudorabies, Foot and Mouth Disease, Swine Vesicular Disease, African Swine Fever, Leptospirosis, Chlamydia, and Yeasts can only be transmitted from boar to sow through contact during natural mating (7, 11, 12). Boars utilized in AI schemes normally exhibit an active immune system and are a part of stringent vaccination programs. These high health standards help reduce the transmission of some harmful microorganisms. Also, collecting semen using the gloved-hand technique from boars mounting an artificial sow, and the use of clean equipment, greatly decreases the risk of infection or transmittance of some pathogens as compared to

natural mating (12). These practices represent standard procedures of boar stud facilities supplying fresh semen to producers and clearly offer advantages to utilizing AI. While the health advantages (e.g., transmission of diseases from boar to sow) of AI are greatest when semen is obtained off-site from a boar stud, these aforementioned procedures also provide guidelines for producers utilizing semen from on-farm boars.

Implementing AI saves time and money. Flowers and Alhusen (13) conducted a study comparing the labor requirements between swine hand-mating and AI breeding systems. They concluded that if 4 or more females were to be bred on a single day, then AI required less time per animal than natural service. The labor involved in this AI system included detection of estrus, collection of semen, processing it into appropriate aliquots, breeding the sows or gilts, and cleaning all AI equipment. This was in contrast to the labor required to achieve a hand-mating that consisted of detection of estrus and supervision of animals while breeding.

Characteristics of Boar Semen

Boar semen is composed of seminal plasma and spermatozoa. Seminal plasma consists of various ionic constituents and organic compounds (Table 1). Semen characteristics are highly variable among boars and are influenced by breed, age, season, and frequency of collection (14). Total volume of an ejaculate ranges from 75 to 400 mL containing 20 to 100 x 10^9 spermatozoa (15). Seventy to 90% of the total spermatozoa exhibit normal morphometry and progressive motility (16). A typical AI dose contains 2 to 3 x 10^9 spermatozoa (17); therefore, a single boar ejaculate could yield approximately

6 to 50 AI doses. The reproductive efficiency of boars used for AI would be enhanced even further if the total number of sperm cells per ejaculate was increased.

Table 1. Concentration of substances in boar seminal plasma[a]

Constituent	Concentration[b]
Sodium	125-252
Potassium	17-46
Calcium	1.5- 4.6
Magnesium	2.5-24
Chloride	85-105
Phosphate	0.4
Fructose	0.5
Glucose	0.06-0.3
Sorbitol	0.4
Inositol	28
Lactic acid	2.2
Citric acid	2.6-10.4
Glutamic acid	2
Glycerophosphocholine	4
Phosphocholine	0
Glycerophosphoinositol	0.26
Arginine	0.01
Creatine	0.03
Ergothionine	0.7
Protein (mg/mL)	30
L-carnitine[c]	318
Acyl carnitine[c]	46

[a] adapted from (18).
[b] Concentration is measured in mM unless stated otherwise.
[c] Average of 7 split ejaculate fractions, adapted from (19).

Factors Limiting Reproductive Efficiency in AI Programs

One problem encountered by producers utilizing AI is training boars to mount an artificial sow for semen collection, which can sometimes be a labor-intensive process. Ejaculation of potentially fertile spermatozoa in boars occurs between 24 to 28 wk of age (20); therefore, training should also commence at this time. Boars commonly display

interest in mounting stationary objects, such as an artificial sow, which simulate a female exhibiting lordosis (21). The artificial sow should be located in a room separate from where boars are normally housed and free of any distractions, thus allowing the boar to fully concentrate on the artificial sow. When the boar is first exposed to the artificial sow, adequate time to become acquainted to the new environment may be necessary. Usually several exposures to the artificial sow are necessary before any mounting behavior is observed. Rubbing the preputial sheath of the boar while in the collection room can stimulate thrusting and the displaying of an erection (21). Seasoning the artificial sow with the saliva and semen of other boars, and/or the urine of a female in estrus can provide further stimulus. If the boar fails to display any mounting behavior after numerous exposures, a female in estrus that is within sight and smell of the collection room may arouse the boar and induce him to mount the artificial sow. Allowing the boar to mount a female in estrus within the collection room, begin ejaculation, and then be positioned onto the artificial sow as the female is removed from the collection room may be attempted to aid in training. Repeating this several times may be required before the boar customarily mounts the artificial sow without the presence of a female in estrus. These procedures may be difficult to accomplish.

Thus, training boars can be a timely, labor-intensive process often requiring multiple personnel. Boars that initially display low libido lead to further inefficiencies. The utilization of females in estrus to assist in training would be impossible for most boar stud facilities that lack access to gilts or sows. Intact boars may also be aggressive and dangerous, which increases the difficulty of handling them. The reproductive efficiency

of boars would be enhanced if sexual behavior could be induced for purposes of expediting the training process.

Another problem associated with some AI programs is the production by boars of low quality semen (e.g., low sperm concentration, sperm viability, sperm motility, etc.), which decreases the number of potential insemination doses per ejaculate. Increasing the number of potential females to be bred from a single ejaculate would improve the reproductive efficiency of boars. In AI programs, this can theoretically be accomplished in two ways: 1) decreasing the number of sperm necessary in each insemination dose to achieve acceptable fertilization rates, or 2) increasing the number of viable sperm cells in the ejaculate. Currently AI doses contain 2 to 3 x 10^9 spermatozoa in 80 to 100 mL of diluted seminal plasma (9, 17). Semen is deposited within the anterior portion of the cervix and the uterine body of the female. Depositing semen at the tip of the uterine horn could potentially decrease the number of spermatozoa and volume of extender required to inseminate a female. Surgical deep-intrauterine-insemination techniques have revealed that as few as 10 million sperm in 0.5 mL of diluted seminal plasma are sufficient to breed swine without compromising litter size, or pregnancy or farrowing rates (9, 22). Non-surgical techniques have also been developed to utilize a fiberoptic endoscope for deep intra-uterine insemination of 5 to 10 mL of semen containing 50 x 10^6 spermatozoa, without affecting any fertility characteristics (23, 24, 25). However, these techniques are still being developed and are not currently very practical for commercial swine producers.

Increasing the total number of viable spermatozoa in an ejaculate would increase the potential number of AI doses that could be prepared. This would allow for AI of an increased number of females, which would augment boar power.

Prostaglandins

Prostaglandins are lipids consisting of 20-carbon unsaturated hydroxy fatty acids (26). Synthesis of prostaglandins from arachidonic acid, which is incorporated within cellular phospholipid membranes, can take place in all tissues (27, 28). Prostaglandins exist ubiquitously in the body with a significant role in many physiological processes. Numerous biologically active prostaglandins exert effects on circulation via blood pressure, vasoconstriction, and vasodilatation (28). Prostaglandins also contribute to platelet aggregation, respiration, reproduction, inflammatory responses, intestinal motility, inhibition of gastric secretion, a cytoprotective function on the gastro-intestinal tract, and neurotropic effects that either inhibit or increase adrenergic neurotransmission (28). Various prostaglandins regulate the amount of cyclic-adenosine monophosphate (cAMP), a messenger released by neurons that signals a tissue to perform a specific function (27). Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) specifically contributes to increasing blood pressure, vasoconstriction, bronchoconstrictor effects, regressing corpora lutea (CL), stimulating uterine contractions, and increasing adrenergic neurotransmission (26, 27, 28).

Prostaglandins are very potent molecules with as little as one billionth of a gram producing a measurable physiological response (28). The effects of prostaglandins are usually localized to areas where they are produced, with many of the different

prostaglandins having antagonistic effects on each other. Once prostaglandins enter the systemic circulation, they are rapidly metabolized by the action of dehydrogenase enzymes predominantly in the lungs, and to a lesser extent in the liver and kidneys (27, 28). Prostaglandin synthesis can be suppressed by aspirin and many other non-steroidal inflammatory agents, which inhibit their endogenous formation, innately controlling inflammation (27).

Role of Prostaglandins in Female Swine Reproduction

Prostaglandin-$F_{2\alpha}$ has luteolytic effects (29), and plays a major role in controlling the life of swine CL; therefore, prostaglandins contribute to controlling the length of estrous cycles and gestation. Between days 12 to 15 of the estrous cycle, the non-gravid swine uterus produces $PGF_{2\alpha}$ that is carried to the CL via the utero-ovarian vein, causing regression of the CL (30). Parturition is in part initiated by uterine release of $PGF_{2\alpha}$ induced by increasing concentrations of fetal cortisol (31). This has lead to the practical utilization of $PGF_{2\alpha}$ for inducing abortions or inducing parturition (31, 32, 33, 34, 35). The only commercially available $PGF_{2\alpha}$ in the U.S. approved for use in inducing parturition in swine is Lutalyse® (Pharmacia & Upjohn Company, Kalamazoo, MI).

Effects of Prostaglandin-$F_{2\alpha}$ on Boar Sexual Behavior

The efficiency of swine AI programs could be increased if techniques were developed to expedite the training of boars to mount an artificial sow and allow semen collection. Treatment with $PGF_{2\alpha}$ has been demonstrated to increase the libido of boars in some scenarios.

Szurop et al. (36) demonstrated the effects of a $PGF_{2\alpha}$ analog (Enzaprost; Chinoin, Budapest, Hungary) on the training of young boars for semen collection and on libido in mature boars. Boars were of Dutch x Belgium Landrace or Large White x Duroc breeding. Pubertal boars (n = 156), 210 to 225 d of age and weighing approximately 110 kg, were administered 25 mg of Enzaprost 30 min prior to a training session. A training session was considered successful when a boar displayed an erection and allowed the start of semen collection in less than 5 to 7 min after entering the collection pen. Enzaprost resulted in greater than 90% and 95% success rates during the first and second training sessions, respectively. Previous observations in the same boar stud had revealed only a 70% success rate after 4 or 5 sessions with reaction times (defined as the time taken by an experienced boar from entry of the collection pen to mounting an artificial sow and achieving an erection) varying from 20 to 30 min. Mature boars (n = 120), approximately 3 yr of age and weighing approximately 200 kg, that were exhibiting a loss of libido or a prolonged reaction time were also treated with 25 mg of Enzaprost 30 min prior to an attempted collection. A restoration in libido and reduced reaction times were observed in 95% of the treated boars.

Wettemann et al. (37) attempted to stimulate sexual behavior with Lutalyse in boars that were pre-determined to possess low libido. At 180 d of age, Hampshire boars were exposed to a gilt in estrus and were evaluated weekly for 4 wk to identify boars that would not mount. Ten boars were identified as lacking libido (characterized by failing to mount an estrous gilt) and placed into 1 of 3 treatment groups. Boars were administered either saline (n = 4) im 1 min before being introduced to a gilt in estrus, 10 mg Lutalyse (n = 4) im 1 min before being introduced to a gilt in estrus, or 25 mg Lutalyse (n = 2) im

30 min prior to exposure to an estrous gilt. Exposure to estrous gilts lasted 15 min and the number of ano-genital sniffs, nose-to-nose contacts, nosing the flanks, proper mounts, and matings were recorded. Treatment with Lutalyse did not influence any of the observed activities between boars and gilts. Wetteman et al. (37) concluded that the treatment of low-libido, Hampshire boars with Lutalyse did not enhance mating behavior.

Levis et al. (38) tested the effect of a pharmacological dose of Lutalyse (5 mg $PGF_{2\alpha}$/mL), 1.4 to 2.0 mL/boar, on boars with no previous sexual experience. Large White x Landrace boars (n = 18), approximately 297 d of age, and 137 kg body weight were utilized in two experiments. The sexual behavior traits assessed were elapsed time to first mount, time spent sniffing the ano-genital region, time mounted with penis not exposed, time nosing the side of the gilts, elapsed time until the penis was first exposed out of the sheath, time mounted with penis exposed, elapsed time to copulation, duration of copulation, total number of mounts, and time spent scratching. For Exp. 1, boars received im injection of either 1.0 mL saline or 1.4 to 2.0 mL Lutalyse. Seven days following the first treatment, boars received the reciprocal treatment. Boars received the treatments as they entered the test pen that contained a gilt and sexual behavior was recorded for 20 min. For Exp. 2, the same treatment regiment was used but boars did not enter the test pen until 30 min post-treatment. For Exp. 1, the only behavior traits found to be different were time spent nosing the side of the gilts (Lutalyse treated boars, 52% less time), time mounted without penis exposed (Lutalyse treated boars, 50% less time), and number of mounts (Lutalyse treated boars, 59% less mounts). The proportion of boars that mated was 66.7% for both treatment groups. For Exp. 2, Lutalyse affected none of the sexual behavior traits assessed, including the proportion of gilts mated. Levis

et al. (38) concluded that a pharmacological dose of Lutalyse does not enhance sexual behavior traits in boars.

Estienne and Harper (39) reported positive effects of Lutalyse in training for semen collection boars that had not experienced natural mating. Six litter-mate, Landrace x Yorkshire boars, 289 d of age and approximately 192 kg body weight, were allowed 15 min access to an artificial sow twice weekly for 5.5 wk, for a total of 11 training sessions. Sessions 1 through 9 (Period A) was considered the control period and boars received no treatment. The tenth training session (Period B) commenced with all boars receiving 10 mg Lutalyse im upon entering the collection pen. For the final, eleventh training session (Period C), boars received no treatment. During each session, the libido of each boar was assessed and was assigned a score of 1 to 5 (1 = no interest in the artificial sow, 2 = slight interest in the artificial sow, but no attempts to mount, 3 = mounting the artificial sow, but not displaying an erection, 4 = mounting the artificial sow and displaying an erection, but not allowing semen collection, and 5 = mounting the artificial sow and allowing semen collection). Average libido score was significantly affected and for Period A was 1.7 ± 0.8, compared to Periods B and C which were both 5.0 ± 0.8. Prior to Lutalyse treatment (Period A) boars displayed only a little more than no interest in the artificial sow. The treatment of 10 mg Lutalyse at the beginning of the Period B enticed all boars to mount the artificial sow and allow semen collection. All boars also allowed semen collection during the next training session, Period C.

Estienne and Harper (40) also reported the effects of Lutalyse on the training of sexually active boars that had experienced natural mating but had never been exposed to an artificial sow. Four Hampshire, 4 Landrace, and 6 Yorkshire boars ranging in age

from 1 to 4 yr, were exposed twice weekly for 4 wk to an artificial sow for a maximum of 15 min. Boars were divided into two treatment groups, balanced for both breed and age, with 7 boars receiving 10 mg Lutalyse im and 7 boars receiving 2 mL of deionized water im immediately as they entered the collection pen. Reaction time (defined as the elapsed time from entering the collection pen until the start of ejaculation), duration of ejaculation, and the number of false mounts were recorded for each training session. During the first exposure to the artificial sow, 86% (6/7) of the Lutalyse-treated boars mounted the artificial sow and allowed semen collection, compared to only 29% (2/7) of the control boars. After 4 training sessions, 100% of the $PGF_{2\alpha}$-treated boars were considered trained while only 57% (4/7) of the control boars became trained after 8 sessions. At the conclusion of the study, the 3 remaining untrained, control boars were allowed access to the artificial sow and were administered 10 mg of Lutalyse upon entry to the collection pen. Two of these boars mounted and allowed semen collection within 15 min. Furthermore, Lutalyse decreased the number of false mounts per training session compared to boars receiving deionized water (by 84%). The reaction time of Lutalyse-treated boars was also decreased compared to boars receiving deionized water (by 57%). The duration of ejaculation was similar between treatment groups. Estienne and Harper (40) concluded that Lutalyse has potential for accelerating the training of sexually active boars to mount an artificial sow for semen collection.

Effects of Prostaglandin-$F_{2\alpha}$ on Ejaculate Characteristics

For purposes of AI, reproductive efficiency of males would be significantly increased if the total number of ejaculated spermatozoa were increased. Increasing the

total number of spermatozoa per ejaculate would allow for a greater number of AI doses per collection. Equivocal effects of $PGF_{2\alpha}$ on increasing output of spermatozoa have been observed in several species, including the boar.

Hafs et al. (41) increased sperm numbers within the deferent duct of rabbits through the administration of $PGF_{2\alpha}$ Tham salt (Upjohn Company, Kalamazoo, Michigan). The first preliminary experiment involved the removal of a testis, epididymis, and deferent duct from rabbits (n = 9). The excised organs served as the controls for the experiment. Five mg of $PGF_{2\alpha}$ Tham salt mixed with 0.2 mL saline was administered within the tunica vaginalis around the remaining testis and epididymis. At 10, 30, or 60 min post-$PGF_{2\alpha}$ Tham salt treatment, the remaining testis, epididymis, and deferent duct were extracted (n = 3 for each time). Before each organ was extracted, the deferent duct was ligated at the urethra and at the epididymis to prevent movement of sperm out of that organ. Sperm numbers in the deferent duct, cauda epididymis, and caput-corpus epididymis were determined. Sperm content before and after treatment (10, 30 or 60 min) were similar, but following injections of $PGF_{2\alpha}$ Tham salt, 8 of the 9 rabbits had more sperm in the deferent duct (71×10^6 sperm) than the excised, control organs (33×10^6). However, sperm content of the caput-corpus and cauda of the epididymis were not affected by treatment.

In the second experiment (41), a testis, epididymis, and deferent duct were removed from rabbits (n = 16). The excised organs served as the controls for the experiment. The rabbits then received either 5 mg $PGF_{2\alpha}$ Tham salt mixed with 1.0 mL saline (n = 8) sc or 1.0 mL saline (n = 8) sc. At 10 or 30 min post-$PGF_{2\alpha}$ Tham salt or saline treatment (n = 4 for each time), the treated deferent duct was ligated at the

epididymis and the rest of the reproductive tract was excised. Sperm numbers in the deferent duct, cauda epididymis, and caput-corpus epididymis were determined. Total spermatozoa within the head and tail of the epididymis were unchanged by treatment. At 10 min post-treatment, sperm numbers within the deferent duct were similar for both treatment groups, but at 30 min post treatment with $PGF_{2\alpha}$ Tham salt, the deferent duct sperm content had increased 2.5-fold to 89 x 10^6 spermatozoa.

In Exp. 3 (41), unanesthetized rabbits received either 0.7 mL saline (n = 4) sc or 10 mg $PGF_{2\alpha}$ Tham salt in 0.7 mL saline (n = 4) sc 3 times at 20-min intervals. All rabbits were killed 20 min post-final treatment and their reproductive tracts were removed. Data for the right and left sides of the reproductive tract were combined for data analysis. Sperm content of the deferent ducts and the caput-corpus and cauda regions of the epididymis were analyzed. Total spermatozoa within the caput-corpus and cauda of the epididymis were unaltered by treatment with $PGF_{2\alpha}$ Tham salt; however, the number of sperm in the deferent duct of treated rabbits was nearly threefold greater than that in the controls.

In Exp. 4 (41), anesthetized rabbits (n = 20) received either 0.2 mL saline (n = 10) sc or 1 mg $PGF_{2\alpha}$ Tham salt in 0.2 mL saline (n = 10) sc 5 times at 12-min intervals. All rabbits were killed 12 min post-final treatment with reproductive tracts being analyzed as in Exp. 3. $PGF_{2\alpha}$ Tham salt had no effect on sperm numbers within the caput-corpus or cauda of the epididymis, but increased deferent duct spermatozoal content by approximately threefold. Hafs et al. (41) concluded from these trials that treatment of rabbits with a $PGF_{2\alpha}$ Tham salt results in a 2 to 3-fold increase of spermatozoa density within the deferent ducts.

Hafs et al. (42) conducted further experiments testing the effects of $PGF_{2\alpha}$ tromethamine salt on the sperm output of both rabbits and bulls. Rabbits (n = 7) were ejaculated 4 times at 20 min intervals, once weekly. For the first week, rabbits received either 2.5 mg of $PGF_{2\alpha}$ tromethamine salt or saline sc at 4 and 2 hr prior to the first ejaculation, with treatments being reversed during the second week. For the third week, rabbits were administered either 2.5 mg of $PGF_{2\alpha}$ tromethamine salt sc or saline sc at 8 and 4 hr prior to the first ejaculation, with treatments being reversed during the following week. They concluded that the first ejaculate contained 150% more sperm cells than controls with no influence of $PGF_{2\alpha}$ tromethamine salt on the subsequent ejaculations.

In a separate trial (42), mature dairy bulls (n = 7) were ejaculated once on each Tuesday and Friday for 7 wk. When collected during the first and last weeks, bulls were ejaculated after sexual preparation implemented to maximize sperm output. The sexual preparation consisted of allowing the bull to make 1 to 3 false mounts over a planned duration of 5 to 10 min. For the intervening 5 wk, bulls were ejaculated a total of 10 times (2/wk) without any sexual preparation 30 min after receiving 0, 7, 20, 40 or 80 mg of a $PGF_{2\alpha}$ tromethamine salt im so that each bull received one dose/wk and a different dose for every week. The 40 and 80 mg doses of $PGF_{2\alpha}$ tromethamine salt resulted in the highest spermatozoal output without sexual preparation (33% more than 0, 7, or 20 mg $PGF_{2\alpha}$ tromethamine salt). Values were still 30% lower than the spermatozoal output achieved after sexual preparation.

Berndtson et al. (43) tested the influence of $PGF_{2\alpha}$ Tham salt on semen characteristics in yearling Angus and Hereford bulls. Bulls (n = 14) were collected via electroejaculation twice on each Tuesday and Saturday for 8 wk. The first ejaculate was

collected 1 hr after receiving either 30 mg of $PGF_{2\alpha}$ Tham salt im (n = 7) or no treatment (n = 7) and the second ejaculate was collected 10 min later. Ejaculates were evaluated for volume of pre-sperm and sperm-rich fractions, spermatozoal concentrations, percentage of progressively motile sperm, and total number of sperm cells per ejaculate. They concluded that there was no effect of $PGF_{2\alpha}$ Tham salt on any of the semen characteristics analyzed but noted that the use of electroejaculation may have contributed to the lack of an effect.

Cornwell et al. (44) demonstrated the effects of $PGF_{2\alpha}$ on seminal characteristics of mature stallions (n = 6). Ejaculates were collected once every 3 d from the stallions 1 and 4 h following an im treatment of 10 mg $PGF_{2\alpha}$ (n = 3) or sterile saline (n = 3). Treatments were reversed after the stallions had been collected on 4 separate days. Ejaculates were evaluated for gel-free volume, gel volume, progressive motility, sperm concentration, total sperm cells per ejaculate, and pH. Treatment with $PGF_{2\alpha}$ did not affect gel-free semen volume, gel volume, progressive motility, or pH in either the first or second ejaculates collected. However, treatment with $PGF_{2\alpha}$ resulted in total sperm cells per first ejaculate being increased over 206% from 4.6 x 10^9 in controls to 9.5 x 10^9 for $PGF_{2\alpha}$-treated stallions and sperm concentration being increased 125% from 213.8 x 10^6/mL in controls to 268.3 x 10^6/mL for $PGF_{2\alpha}$-treated stallions. Treatment with $PGF_{2\alpha}$ did not increase total sperm numbers or sperm concentration in the second ejaculate collected 4 h post-administration.

In two experiments, Hemsworth and colleagues (45) studied the effects of $PGF_{2\alpha}$ (Upjohn Ltd., England) on sperm output in boars. In both experiments, 3 Large White and 3 Large White x Berkshire boars were utilized. For both experiments, semen was

collected via the gloved-hand method, but only for the second experiment was the sperm-rich fraction separated from the whole ejaculate at the time of collection. In Exp. 1, boars were collected weekly for 3 weeks to stabilize sperm numbers before being collected four times over a period of 8 d throughout the following weeks. On days 1, 4, 6, or 8 following the 3 wk stabilization period, 30 min prior to collection all boars received either no treatment, 20 mL of saline im, 10 mg of $PGF_{2\alpha}$ in 20 mL of saline im, or 20 mg of $PGF_{2\alpha}$ in 20 mL of saline im. Each boar received a different treatment each day so that every boar received each treatment over the 8 d period. This protocol resulted in 149% greater sperm concentration in ejaculates following treatment with 20 mg of $PGF_{2\alpha}$ compared to saline. For Exp. 2, boars were collected weekly for 6 wk. During week 5 boars received either 20 mL of saline im or 20 mg of $PGF_{2\alpha}$ in 20 mL of saline im 30 min prior to collection, with treatments being reversed for the sixth week. The sperm-rich fraction of ejaculates collected after administration of 20 mg $PGF_{2\alpha}$ im had 49% more total sperm and 43% more volume as compared to saline; however, total numbers of spermatozoa in the whole ejaculate were not affected by $PGF_{2\alpha}$ treatment.

Estienne and Harper (40) demonstrated that treatment of boars with Lutalyse increased the concentration of spermatozoa, leading to a trend for greater numbers of total sperm cells in ejaculates compared to boars receiving no treatment. Four Hampshire, 4 Landrace, and 6 Yorkshire boars ranging in age from 1 to 4 yr, received either 10 mg Lutalyse im (for a total of 9 ejaculates) or 2 mL deionized water im (for a total of 4 ejaculates) immediately prior to semen collection. Lutalyse-treated boars had 28% higher sperm concentration and 26% more total sperm per ejaculate.

Hashizume and Niwa (46) evaluated the effect of $PGF_{2\alpha}$ (Fuji Chemical Industries Ltd., Japan) on the properties of boar semen. Semen was collected from 1 Landrace x Large White and 2 Landrace boars using an artificial vagina once every 3 d for 28 d. Semen collected on days 1, 7, 13, 19, and 25 served as the controls. Semen collected on days 4, 10, 16, 22, and 28 was 1 hr after im administration of 12 mg of $PGF_{2\alpha}$. During all collections, the sperm-rich fraction was separated from the whole ejaculate. Semen volume and sperm count was analyzed in both the sperm-rich fraction and whole ejaculate. The volume and total number of sperm cells in the sperm-rich fraction increased by 28 and 44%, respectively, due to treatment with $PGF_{2\alpha}$. In the whole ejaculates, sperm concentration and total number of spermatozoa increased by 23 and 34%, respectively following $PGF_{2\alpha}$ treatment. Hashizume and Niwa (46) suggested that the main effect of $PGF_{2\alpha}$ was to cause an increase in the volume and total number of spermatozoa in the sperm-rich fraction.

Mechanism of Action of Prostaglandin-$F_{2\alpha}$

Hafs et al. (41) demonstrated the ability of $PGF_{2\alpha}$ Tham salt to increase the number of spermatozoa in the deferent ducts of rabbits. They attributed this response to the known ability of $PGF_{2\alpha}$ to initiate smooth muscle contractions (27) and proposed that the $PGF_{2\alpha}$ Tham salt was possibly increasing the rate of sperm movement out of the epididymis. This could take place in the distal corpus and caudal regions of the epididymis where smooth muscle is more developed. Other researchers, who observed similar effects of $PGF_{2\alpha}$ on spermatozoal output, also attributed this effect to $PGF_{2\alpha}$ increasing the movement of sperm out of the epididymis (42, 45). However, this effect of

$PGF_{2\alpha}$ on epididymal smooth muscle has not been formally tested and warrants further scrutiny.

The mechanisms by which $PGF_{2\alpha}$ affects sexual behavior are not as easily explained. The hormonal profiles of animals exhibiting sexual behavior differ among species. Borg et al. (47) measured cortisol, growth hormone, and testosterone concentrations during mating behavior in both the bull and the boar. The blood of Angus bulls (n = 6), 21 to 22 mo of age was sampled every 15 min for 2 hr prior to a 30 min exposure to a restrained cow in estrus. During the 30 min exposure time, blood samples were taken every 5 min. Blood sampling continued for every 15 min 2 hr post-female exposure. Bulls that mounted the cow more than 8 times experienced increased concentrations of cortisol but concentrations of testosterone did not change throughout the experiment. Bulls that serviced the cow (intromission and ejaculation) had increased concentrations of growth hormone compared to bulls that failed to mate.

In an experiment (47) involving Duroc boars (n = 6), 11 mo of age, the protocol was similar to that of the bull experiment, except exposure time was restricted to 15 min and the sow in estrus was not restrained. Exposure to the sow in estrus increased serum testosterone concentrations prior to either successful or unsuccessful matings. However, boars that successfully mated with the sow had increased serum testosterone concentrations following copulation, compared to boars that did not mate. Also, all boars experienced higher concentrations of cortisol during the exposure period, while growth hormone concentrations remained stable throughout the experiment.

These results (47) reveal that cortisol concentrations increased in both the bull and boar at times of heightened sexual activity. Bishop et al. (48) confirmed this data for the

boar by showing that cortisol concentrations were elevated by exposure to an artificial sow, even when an ejaculation did not take place. Moreover, serum testosterone concentrations increased during copulation in boars. However, when $PGF_{2\alpha}$ was administered to bulls and boars, varying hormonal profiles were displayed compared to those demonstrated by Borg et al. (47).

Haynes et al. (49) and Berndtson et al. (43) tested the effect of $PGF_{2\alpha}$ on the plasma concentration of testosterone in bulls. Haynes et al. (49) administered $PGF_{2\alpha}$ tromethamine salt im at doses of 0, 5, 15, 30, and 60 mg to Friesian bulls (n = 5) that were 5 to 9 yr of age over a 5 d period. Each bull received a different dose each day so that all bulls received each treatment over the 5 d period. They concluded that doses of 30 and 60 mg $PGF_{2\alpha}$ tromethamine salt increased peripheral testosterone concentrations for a longer duration compared to doses of 0 and 5 mg $PGF_{2\alpha}$ tromethamine salt. The 15 mg dose of $PGF_{2\alpha}$ tromethamine salt increased peripheral testosterone concentrations for a longer duration compared to the 0 mg dose. Berndtson et al. (43) found that 30 mg $PGF_{2\alpha}$ tham salt im 1 h prior to collection by electroejaculation increased blood testosterone concentrations two-fold. In these trials (43, 49), $PGF_{2\alpha}$ increased testosterone concentrations in the bull. If $PGF_{2\alpha}$ had a similar affect on the boar, it could possibly enhance boar sexual behavior. Borg et al. (47) demonstrated that testosterone concentrations in the boar increased while being exposed to a sow in estrus.

Fonda et al. (50) analyzed the effect of $PGF_{2\alpha}$ on serum luteinizing hormone, testosterone, prolactin, and cortisol in boars (n = 6) that were 8 to 9 mo old. Blood samples were taken every 0.5 hr for 12 hr beginning at 0630 hr. Immediately following the 0700 hr blood sampling, boars were administered either 20 mg of $PGF_{2\alpha}$ Tham salt

im (n = 3) or 4 mL of saline im, and blood sampling continued every 0.5 hr until 1830 hr. For the duration of the experiment, boars remained within their normal dwelling space and were not exposed to any sexual stimulus such as an artificial sow or estrous female. Serum luteinizing hormone and testosterone concentrations were similar for both treatment groups. Serum prolactin and cortisol concentrations both increased within 30 min of $PGF_{2\alpha}$ administration and then decreased to similar concentrations of the controls within 2 to 2.5 hr. Extension of the penis and ejaculation was observed in all 3 $PGF_{2\alpha}$-treated boars within 10 min of administration, suggesting that $PGF_{2\alpha}$ might possibly affect mechanisms involved in stimulating erection of the penis.

Increasing Boar Spermatozoa per Ejaculate Through Nutrition

Compared to the vast number of studies conducted on female swine, little work has been done on the effects of nutrition on boar reproduction. Low protein concentrations [7% crude protein (CP)] detrimentally affected semen volume and libido; the latter effect was correlated to a reduction in circulating estradiol-17β concentrations (51). However, increasing both protein and energy intakes to 18.1 g/d of lysine and 7.7 Mcal/d of metabolizable energy (ME) increased sperm output by 38% compared to boars receiving 7.7 g/d of lysine and 6.1 Mcal/d of ME (52). Boars receiving 7.7 g/d of lysine and 6.1 Mcal/d of ME also experienced a decrease in libido characterized by a 60% decrease in mounting activity (52). The National Research Council (NRC) (53) recommends that sexually active boars receive 12 g/d of lysine, 3.2 g/d of methionine, 13% CP and 6.53 Mcal/d of ME. Moon and Kim (54) determined that increasing daily lysine intake from NRC recommended levels to 18 and 21 g/d increased both the total

number of sperm cells and ejaculate volume by 27 and 19%, respectively. Moon and Kim (55) also concluded that increasing daily methionine intake from NRC recommended levels (3.2 g/d of methionine) to 10.2 g/d increased both semen volume and total number of spermatozoa in the ejaculate by 24 and 13%, respectively.

Manipulating mineral concentrations has also proved to benefit some traits of sexual development and fertility in boars. Selenium supplementation of 0.5 ppm/d from time of weaning, 28 d, to 18 mo of age increased testicular sperm reserves at 18 mo of age by increasing the number of Sertoli cells, round spermatids, and secondary spermatocytes (56). A fortified selenium diet of 0.5 ppm/day was also shown to increase fertilization rate of oocytes and the number of accessory sperm penetrating the zona pellucida compared to boars receiving 0.0 ppm/d (57). Being able to influence semen characteristics through manipulation of the diet offers producers an uncomplicated means of enhancing the reproductive efficiency of boars.

L-carnitine Biosynthesis and Transportation

L-carnitine, chemically known as β-hydroxy-γ-trimethylaminobutyric acid, is a vitamin-like compound synthesized by the liver, kidney, and brain (58). L-carnitine is the product of two essential amino acids, lysine and methionine, being converted through five reactions as described by Hulse et al. (59), Bremer (60), and Owen et al. (61). The first step in the biosynthesis requires protein-bound lysine to acquire three methyl groups from the methionine intermediate S-adenosylmethionine, creating 6-N-trimethyl-lysine. In the following step, α-ketogluterate, ascorbate, ferrous ions, and oxygen, hydroxylate 6-N-trimethyl-lysine to 3-hydroxy-6-N-trimethyl-lysine with the byproducts succinate

and carbon dioxide being produced. The coenzyme pyridoxal phosphate then cleaves 3-hydroxy-6-N-trimethyl-lysine into γ-Butyrobetaine aldehyde and glycine. The γ-butyrobetaine aldehyde is then oxidized by nicotinamide adenine dinucleotide (NAD^+) to γ-butyrobetaine. The final hydroxylation step again involves α-ketogluterate, ascorbate, ferrous ions, and oxygen to synthesize L-carnitine via the action of butyrobetaine hydroxylase producing succinate and carbon dioxide as by-products. The final enzyme required in the synthesis of L-carnitine, butyrobetaine hydroxylase, is found in the liver of all species and to varying degrees in the kidneys and brains in a selection of other species (60).

Endogenously synthesized L-carnitine is transported throughout the circulatory system and enters cells by either active or passive transport through the cellular membrane (62). The ease by which L-carnitine diffuses through tissues may be attributed to the fact that it is a small quaternary amine with a molecular weight of 162 Da that is classified as a zwitterrion (62). L-carnitine is also highly polar and water soluble (62).

Function of L-carnitine

L-carnitine plays a vital role in energy metabolism. It functions as a transporter of short-, medium-, and long-chain fatty acids towards or across the inner mitochondrial membrane hence facilitating β-oxidation (63, 64). β-oxidation is the process by which fatty acids are subsequently oxidized and cleaved at the resulting β-carbon, producing a two-carbon structure, acetyl-CoA, which is capable of then entering the tricarboxylic acid cycle and being further broken down into two carbon dioxide molecules and yielding adenosine triphosphate (ATP) as energy (65). L-carnitine allows fatty acids to be

shuttled across the inner mitochondrial membrane after converting them to acyl carnitine derivatives (61, 62). The acyl carnitine derivatives are permeable to the inner mitochondrial membrane through the action of a specific carnitine-acylcarnitine translocase (CAT) protein located on the inner mitochondrial membrane and also within the endoplasmic reticulum and peroxisomes of the cell (61, 62). Once inside the mitochondrial matrix, the acyl carnitine derivatives are then converted back to carnitine and a fatty acid CoA derivative ready to undergo β-oxidation (61, 62). The translocation enzyme exists in two forms, carnitine palmitoyl-transferase I (CPT-I) which forms the acyl carnitines on the cytoplasmic side of the inner mitochondrial membrane, and carnitine palmitoyl-transferase II (CPT-II) which converts the acyl carnitine back to a fatty acid CoA derivative and carnitine once inside the mitochondrial matrix (61). The resulting carnitine molecule within the matrix is then exchanged for an acyl carnitine derivative across the inner mitochondrial membrane and transported back out into the intermembrane compartment to repeat its role as a transporter of fatty acids.

Effect of L-carnitine on Swine Performance

L-carnitine is found in low concentrations within feed ingredients of plant origin and high concentrations in feed ingredients of animal origin (61). L-carnitine is not considered an essential nutrient since it is endogenously synthesized from two essential amino acids, lysine and methionine (61). However, supplementing the diets of pigs with L-carnitine has proved beneficial. The only U.S. commercial source of L-carnitine for supplementation of swine diets is provided by Lonza Inc. (Fair Lawn, NJ) as Carniking®. L-carnitine appears to be crucial to proper metabolic development and the functional

development of the liver, skeletal muscle, and cardiac muscle in neonatal pigs (66, 67). L-carnitine supplemented to diets at rates of 500 and 1000 mg/kg of feed of early-weaned pigs (19 ± 2 d of age) for 14 d increased gain to feed ratios and carcass lipid accretion rates by 12 and 15%, respectively (68). Feeding L-carnitine during the growing-finishing phase of pigs at a rate of 56 to 59 mg/kg of feed decreases tenth-rib backfat by 16% (69), and feeding L-carnitine to growing pigs at a rate of 500 mg/kg of feed increased average daily gain and crude protein accretion rate by 7.3 and 9.0%, respectively (70). Growing-finishing pigs also responded to 50 mg L-carnitine per kg of feed supplementation with an increase in longissimus muscle area (71, 72). Furthermore, L-carnitine added at a rate of 10 mg/kg of feed decreased the time to market weight by two days (73).

Carnitine also positively affects reproduction in female swine. Carnitine supplemented to multiparous sows during gestation and lactation resulted in enhancing sow and litter performance. Musser et al. (74) demonstrated that sows fed 100 mg of L-carnitine/d during gestation displayed increased body weight gain and last rib backfat depth by 16 and 38%, respectively. This rate of supplementation during gestation also increased total litter weight (by 5.8%), individual pig birth weights (by 2.6%), and litter weaning weight (by 8.2%). Feeding L-carnitine throughout gestation (100 mg/d), lactation (50 mg/kg of feed), or both, increased the number of pigs born alive in the following parity by 9.5, 9.1, and 15.6%, respectively.

Effects of L-carnitine on Ejaculate Characteristics

Carnitine has a crucial role in sperm energy metabolism by shuttling fatty acids across the inner mitochondrial membrane. Spermatozoa first come into contact with L-

carnitine within the lumen of the epididymis as the cells mature into potentially fertile gametes. Epididymal plasma L-carnitine content increases from 20 nmol/mg protein in the distal caput to 700 nmol/mg protein in the distal cauda of the boar (75). The spermatozoa first begin to accumulate L-carnitine while in the proximal cauda where the epididymal plasma concentration of carnitine ranges between 200 and 300 nmol/mg protein (75). L-carnitine within the epididymal plasma enters the spermatozoon through its membrane by passive diffusion (75). Once inside the spermatozoon, L-carnitine facilitates the transfer of fatty acids into the mitochondrial matrix to undergo β-oxidation for energy metabolism via acyl carnitine derivatives. Carnitine has also been implicated in buffering the cell against high concentrations of mitochondrial acetyl-CoA by converting it into acyl carnitine (62). Excess acetyl-CoA inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial energy metabolism (62). This action of carnitine may improve the survival of the spermatozoa.

Spermatozoa entering the proximal caput epididymis are immotile but acquire motility during their passage through the epididymis. Rising carnitine concentrations within the epididymis and spermatozoa coincide with acquisition of progressive motility for boar spermatozoa (75, 76). But, boar spermatozoa extracted from the distal caput epididymis, where carnitine concentrations are minimal, do exhibit motility (75). This suggests that carnitine does not initiate motility but may provide necessary buffering and transporting activities crucial to the maintenance of motility.

Boar reproductive efficiency could be increased if the number and quality of spermatozoa per ejaculate were increased. Supplementing the diets of several species, including the boar, with carnitine has resulted in positive effects on semen characteristics.

Palmero et al. (77) demonstrated the effect of L-acylcarnitine (LAC) on oligoasthenospermic-induced, Wistar rats that weighed approximately 500 g when the study commenced. Oligoasthenospermia is characterized by a low sperm count and decreased sperm motility. This condition was induced by dibromochloropropane (DBCP) dissolved in dimethyl-sulphoxide and administered sc (1 mg/0.2mL/100 g body weight). The action of DBCP is directed at the seminiferous tubules causing tubule degeneration and a halt of gamete development at the primary spermatocyte stage. One group of rats receiving one administration of DBCP was labeled DBCP1. A second group of rats received two administrations of DBCP, a week apart, and were labeled DBCP2. Two weeks after the final administration of DBCP, half of the rats in each treatment group then received LAC dissolved in their drinking water (15 mg/kg body weight/d) for 10 wk. This created two separate groups labeled DBCP1-LAC and DBCP2-LAC. Two other separate groups of rats received either L-acylcarnitine dissolved in their drinking water (15 mg/kg body weight/d) or no treatment for 10 wk and were labeled LAC and controls, respectively. During the 10 wk treatment period, epididymal micropunctures were obtained from the rats (n = 6 from each group) and analyzed for sperm concentration and sperm motility at wk 2, 4, 6, 10, and 12 post-DBCP treatment. The DBCP1 and DBCP2 rats exhibited a substantial drop in sperm count compared with the controls during the 10 wk period. Treatment with L-acylcarnitine, exhibited by the DBCP1-LAC rats, increased sperm concentration by 33 and 39% after 6 and 12 wk, respectively, compared to DBCP1 rats receiving no treatment. The DBCP1-LAC rats also experienced increased sperm motility by 42% compared to DBCP1 rats, by 12 wk. The DBCP2-LAC rats were unable to recover from oligoasthenospermia with L-

acylcarnitine treatment. The LAC rats did not experience any change in total sperm count but were 23% higher than any other group. The sperm motility of LAC and control rats was similar. These results lead Palmero et al. (77) to suggest that LAC may act on the spermatogenic process within the seminiferous tubules leading to an increase in sperm production.

In two experiments, Neuman et al. (78) studied the effects of L-carnitine (500 mg/kg of diet) on semen traits of White Leghorn roosters. Experiment 1 utilized older birds (58 to 62 wk of age; n = 24). Experiment 2 employed younger birds (32 to 37 wk of age; n = 84). In each experiment, roosters received ad libitum access to the L-carnitine or control rations for a 5 wk period. Feeding L-carnitine increased sperm concentration and decreased lipid peroxidation in the older birds during wk 3 and 4 of the 5 wk supplementation period. Younger birds supplemented with L-carnitine displayed an increase in sperm concentration and decreased lipid peroxidation during wk 4 and 5 of the 5 wk supplementation period. The younger L-carnitine-supplemented birds also experienced a decline in the percentage of dead sperm during wk 4. Neuman et al. (78) suggested that dietary carnitine has antioxidant properties that may be involved in preserving the integrity of the sperm membranes, extending the life span of the sperm cells.

Costa et al. (79) examined the effects of L-carnitine supplementation on men experiencing unexplained asthenozoospermia (low sperm motility). The subjects (n = 100) were 20 to 40 years of age and had endured infertility for 2 yr that was defined by: (1) concentration of $\geq 10^7$ spermatozoa/mL; (2) motility of 20 to 40% at 2 h post collection; (3) rapid linear progression of sperm < 20%; (4) abnormal morphology of

sperm < 50%; (5) white blood cell (WBC) count in seminal plasma of < 10^6/mL; (6) mean sperm velocity ranging between 20 and 40 μm/s; and (7) sperm linearity index ranging between 2 and 4. Subjects received 3 g/d of L-carnitine for 4 mo orally in 3, 1 g doses following meals. Semen characteristics were analyzed 2 mo prior to supplementation, at the start of treatment, at 2 and 4 mo during treatment, and at 2 mo post treatment (6 mo). L-carnitine supplementation increased concentration of spermatozoa (by 4%), the total number of ejaculated spermatozoa (by 21%), percent motile spermatozoa (by 10%), percent spermatozoa with rapid linear progression (by 7%), concentration of spermatozoa with rapid linear progression (by 4%), and total number of spermatozoa with rapid linear progression (by 14%). The authors concluded that L-carnitine supplementation improved sperm quality in these idiopathic asthenozoospermia patients, however the experiment was confounded by time and there were no untreated control patients.

Vitali et al. (80) conducted a similar study on young human males (n = 47) with idiopathic asthenozoospermia for at least two years and seminal characteristics as follows: sperm concentration > 10 x 10^6/mL; motility < 40% at 2 h post collection; rapid linear progression of sperm < 30%; abnormal morphology of sperm < 50%; and WBC count in seminal plasma < 1 x 10^6/mL. L-carnitine was administered via 3 oral solutions, each containing 1 g of L-carnitine, after meals so that patients received 3 g/d for 3 mo. L-carnitine supplementation resulted in 80% of the subjects experiencing an increase in motile cells (by 99%), sperm concentration (by 80%), and rapid linear motility of sperm cells (by 55%). The authors concluded that L-carnitine might be a potential treatment of

uncomplicated asthenozoospermia, but the findings are tempered by the lack of untreated control patients.

Limited, but promising results have been presented on the effects of L-carnitine on boar semen characteristics. Baumgartner (81) reported the results of a European study that was conducted at the Suisag boar unit in Knutwil in Switzerland. Breeding boars at this unit were divided into a control group and a group that was supplemented with 500 mg/d of L-carnitine (Carniking). Three months prior to the study both groups were producing similar numbers of AI doses. After supplementation of L-carnitine began, the L-carnitine-supplemented group produced ejaculates with a greater number of spermatozoa that increased the number of AI doses per ejaculate by 1 dose. No other semen characteristics or libido characteristics were reported in this study. Increasing the number of AI doses produced by a boar, even by 1 dose, would enhance reproductive efficiency by increasing boar power and positively affect commercial boar semen producers economically.

The Akey Swine Newsletter (82) reported the results of an Akey field trial. This experiment utilized boars (n = 180) of a modern, high lean growth genotype housed in a commercial boar stud. L-carnitine was top-dressed at “low” and “high” levels to a control diet that was formulated as a complete feed containing Akey Boar Plus (Akey, Lewisburg, Ohio). Boars were fed 2.26 to 3.17 kg/d of the diet supplemented with L-carnitine for a 7 wk period prior to test collections to allow for L-carnitine to adequately affect spermatogenesis. Following the 7 wk adjustment period, boars continued supplementation for a 9 wk period during which semen was collected for analysis of sperm motility, percent normal sperm, ejaculate volume/wk, billions of viable sperm/wk,

and number of AI doses/wk. Weekly data within individual boars were averaged so that data could be compared among treatments on a per week basis. L-carnitine supplementation had no effect on percent motile or normal sperm cells. However, a "low" rate of L-carnitine supplementation resulted in a 6.6% increase in ejaculate volume/wk. Supplementing L-carnitine at a "high" rate increased billions of viable sperm/wk by 7.6% and increased the number of AI doses/boar/wk by 2 when compared to control boars. No other semen characteristics or libido characteristics were analyzed. Conclusions from this trial stated that based on an AI dose price of $4.00/AI dose, the benefit to cost ratio of adding L-carnitine to the boar diet is 20:1, representing a highly profitable gain to commercial boar semen producers. This field trial demonstrated the ability of L-carnitine supplementation (at a "high" level) to increase the number of viable sperm cells produced and number of doses per ejaculate, which would enhance both boar power and reproductive efficiency.

In summary, swine AI programs could be enhanced if techniques were developed to expedite the training of boars to mount an artificial sow and allow semen collection. In order to train boars $PGF_{2\alpha}$ and $PGF_{2\alpha}$ analogs have been utilized and have been demonstrated to increase the libido of boars in some scenarios. By increasing the libido of a boar, it may potentially accelerate the training process and allow the boar to become a part of the breeding herd more quickly. Furthermore, $PGF_{2\alpha}$ has been observed to increase the output of spermatozoa in several species including the boar. This effect could potentially increase the number of AI doses produced by a boar in a single ejaculate. Another possible way of influencing the number of spermatozoa in the ejaculate of the boar is through diet manipulation. L-carnitine is a vitamin-like

compound synthesized endogenously with high concentrations present within the epididymis and spermatozoa. Supplementing L-carnitine has increased spermatozoal output in roosters, men, and limited data reveals similar effects in boars.

CHAPTER II: THE EFFECT OF LUTALYSE ON THE TRAINING OF SEXUALLY INEXPERIEINCED BOARS FOR SEMEN COLLECTION[1]

Abstract

The objective was to determine if im treatments of Lutalyse expedited the training of sexually inexperienced boars for semen collection. Lean-type, terminal-line boars (n = 40; 177.4 ± 2.4 d of age and 112.8 ± 2.0 kg body weight) that had not previously experienced natural mating were utilized. Boars were moved individually twice weekly for 6 weeks (total of 12 training sessions) to a semen collection room equipped with an artificial sow. Upon entering the semen collection room, boars received im treatments of either deionized water (4 mL, n = 10) or Lutalyse at doses of 5 mg (n = 10), 10 mg (n = 10), or 20 mg (n = 10), and subsequently received a libido score of 1 to 5 (1 = no interest in the artificial sow; 5 = mounting the artificial sow and allowing semen collection). The percentages of boars successfully trained for semen collection during the experimental period were similar ($P > 0.05$) for controls (20%) and boars receiving 5 mg (30%), 10 mg (20%), or 20 mg (10%) of Lutalyse. Average libido score for boars receiving 10 mg Lutalyse (2.35 ± 0.08) was greater ($P < 0.05$) than for controls (2.14 ± 0.06). In summary, Lutalyse increased libido scores, but did not affect the number of boars trained for semen collection.

Introduction

The use of artificial insemination (AI) by U.S. swine producers has greatly increased during the past decade. In 1989, approximately 190,000 doses of boar semen

[1] This manuscript was submitted to Theriogenology and was published in September, 2002 (Kozink et al., Theriogenology 2002; 58:1039-1045).

were used in the U.S., representing only 8% of all females bred, and only 4.3% of litters registered by purebred organizations were a result of AI (5). In 1997, approximately 47% of all pigs born in the U.S. resulted from AI (3). This continuing trend of increased utilization of AI increases the demand for boars trained to mount artificial sows and allow semen collection. Management protocols that accelerate the training of boars would greatly improve the efficiency and productivity of semen suppliers.

Previous work from our laboratory demonstrated that im treatment with 10 mg Lutalyse decreased the number of sessions required to train sexually active boars (i.e., boars experienced with natural mating) to mount an artificial sow and allow semen collection (40). In that study, the number of false mounts (mounting artificial sow but not allowing semen collection) and reaction time (elapsed time between entering collection pen and start of ejaculation) were also decreased by treatment with Lutalyse (40).

The objective of this study was to extend our previous findings by determining the effects of Lutalyse on the process of training sexually inexperienced (i.e., boars that had not previously experienced natural mating) for semen collection.

Materials and Methods

General. The experiment was conducted at the Virginia Tech-Tidewater Agricultural Research and Extension Center (Suffolk, VA) during the months of April and May and lean-type, terminal-line boars (n = 40) from a commercial source were used. At the beginning of the experiment boars were 177.4 ± 2.4 d of age, weighed 112.8 ± 2.0

kg, and displayed 9.7 ± 0.2 mm back fat over the tenth rib as determined ultrasonically (Lean-meter, Renco Corporation, Minneapolis, MN).

Boars were maintained in a passively ventilated curtain-sided building in individual pens that had a combination of concrete and solid steel rod flooring. Animals were fed, at a rate of 2 kg/d, a fortified, corn and soybean meal-based diet that met or exceeded National Research Council (53) recommendations for the various nutrients, and were allowed water on an ad libitum basis via nipple waterers.

Protocol. Boars were moved individually twice weekly for 6 weeks (total of 12 training sessions) to a semen collection room equipped with an artificial sow. Upon entering the semen collection pen, boars received im treatments of either deionized water (4 mL, n = 10) or Lutalyse at doses of 5 mg (n = 10), 10 mg (n = 10), or 20 mg (n = 10). Each individual training session lasted a maximum of 10 min and the order in which animals were brought to the training pen was randomized each day of the experiment. Semen was collected via the gloved-hand technique and was filtered through a double layer of cheesecloth to remove gel. Gel-free semen volume and the weight of the gel were then determined using gravimetric methods. Sperm concentration was determined using a photometer (Spermacue, Minitube of America, Inc., Verona, WI).

The appropriate treatment was administered at the beginning of each training session until the boar mounted the artificial sow and allowed collection of semen. After a successful collection, the boar was again moved to the semen collection pen on the next scheduled day in order to confirm that the boar was actually "trained." Boars received no treatment before the attempt at a second collection. However, if the boar failed to mount the artificial sow and ejaculate within 10 min, the animal received the appropriate

treatment (Lutalyse or deionized water) and an additional 10 min of exposure was allowed. The boar was then moved again to the collection pen on the next scheduled day. This process continued until the boar mounted the artificial sow and allowed semen collection without first receiving a treatment. Boars that had not mounted the artificial sow and allowed semen collection after 12 training sessions were classified as untrained.

During each training session, boars received a libido score of 1 to 5: 1 – boars showed no interest in artificial sow; 2 – slight interest in artificial sow but did not attempt to mount; 3 – mounted the artificial sow but did not display an erection; 4 – mounted artificial sow and displayed an erection, but did not allow semen collection; and 5 – mounted the artificial sow and allowed semen collection. The number of false mounts, defined as mounting the artificial sow with or without an erection but not allowing semen collection, was also recorded.

Data were analyzed using the Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC). The percentages of boars trained for each treatment group were compared using Chi-square procedures. Semen characteristics were compared using one-way ANOVA with dose of Lutalyse as the main effect. Using ANOVA for a repeated measures design, libido score and the number of false mounts were compared for boars receiving various doses of Lutalyse or deionized water. The statistical model included treatment, boar within treatment, training session, and the treatment by training session interaction as possible sources of variation. Individual means were compared using the PDIFF option of the GLM procedure of SAS.

Results

The percentage of boars trained for semen collection during the experimental period was similar ($P > 0.05$) for controls (20%) and boars receiving 5 mg (30%), 10 mg (20%), or 20 mg (10%) of Lutalyse. Semen volume (68.8 ± 12.2 mL), gel weight (19.9 ± 5.0 g), sperm concentration (321.4 ± 43.8 x 10^6 spermatozoa/mL), and total sperm cells (21.0 ± 4.4 x 10^9) were similar ($P > 0.05$) between groups.

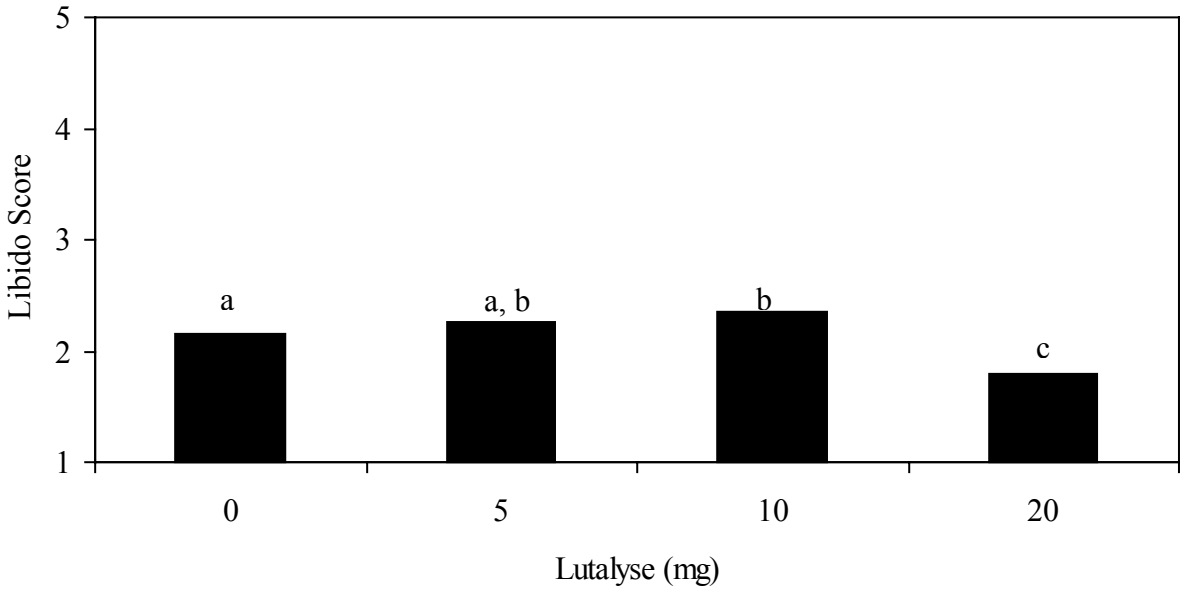

Figure 1. The effect of Lutalyse treatment on mean libido scores (1 = no interest in the artificial sow; 5 = mounting the artificial sow and allowing semen collection). Means without common superscripts (a, b, c) differ ($P < .05$). The standard error was 0.08. The 0 mg dose of Lutalyse was 4 mL of deionized water.

For libido scores, there were effects of treatment ($P < 0.05$) and training session ($P < .01$) but no treatment by training session interaction ($P > 0.05$). Libido scores for controls and boars receiving various doses of Lutalyse are depicted in Figure 1. Across

treatments, libido scores tended to increase as the number of exposures to the artificial sow increased (Figure 2).

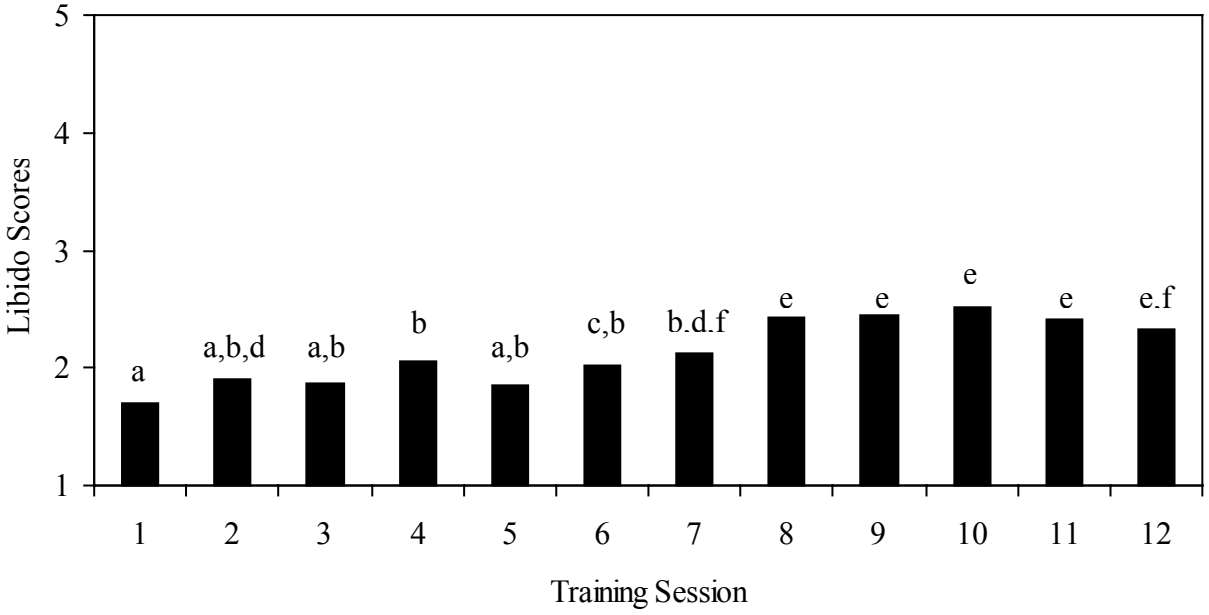

Figure 2. The effect of training session on mean libido scores (1 = no interest in the artificial sow; 5 = mounting the artificial sow and allowing semen collection). The standard error was 0.1. Means without a common superscript (a, b, c, d, e, f) differ ($P < .05$).

There was no effect of treatment or treatment by session on the number of false mounts ($P > 0.05$). The number of false mounts per session for controls was 0.73 ± 0.15 and 0.85 ± 0.15, 0.76 ± 0.20, and 0.13 ± 0.13 for boars receiving 5, 10, or 20 mg Lutalyse, respectively. There was an effect of training session on the number of false mounts ($P < 0.02$). The number of false mounts per session tended to increase with increasing exposures to the artificial sow (Figure 3).

Mild and transient scratching of the face and neck with the hind legs was observed in boars treated with 5 or 10 mg Lutalyse. Boars receiving 20 mg Lutalyse

responded with intense scratching of the face and neck with hind legs followed by a transient state of immobilization while standing. One boar exhibited emesis within 5 min of each administration of 20 mg Lutalyse.

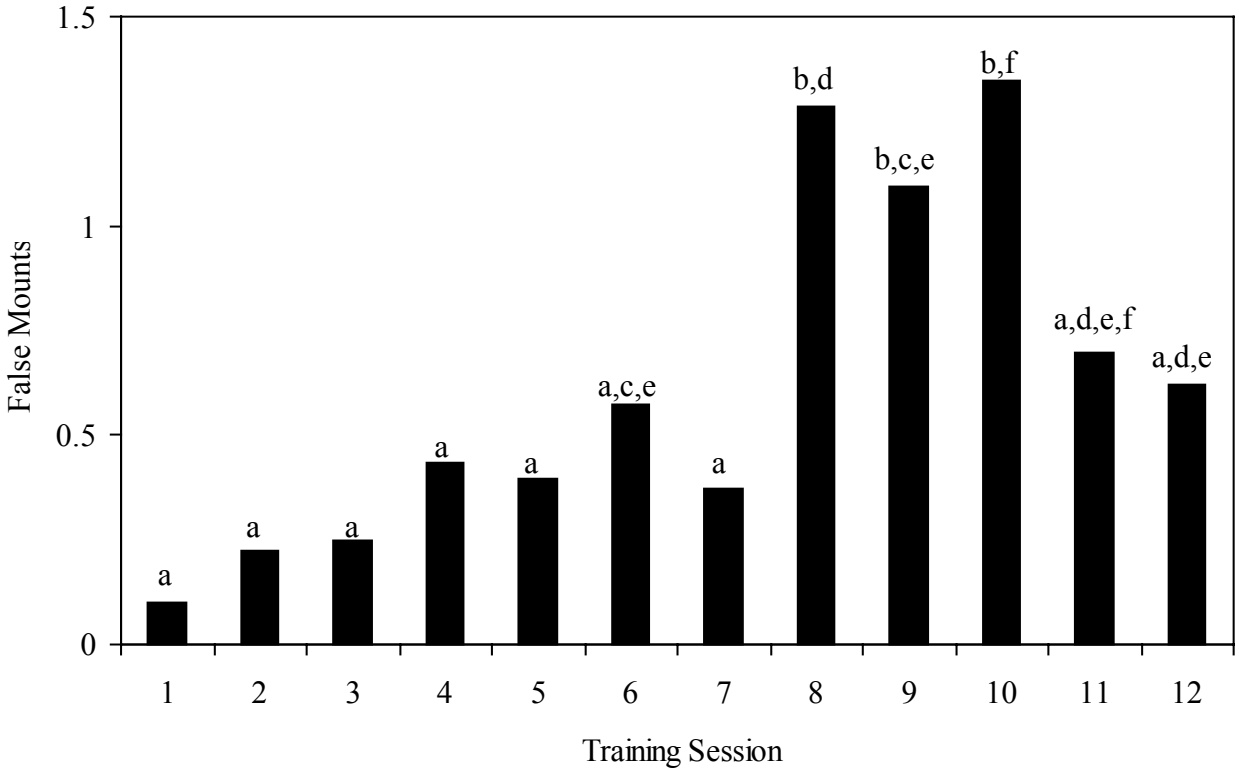

Figure 3. The effect of training session on false mounts. The standard error was 0.26. Means without common superscripts (a, b, c, d, e, f) differ ($P < .05$).

Discussion

Previous results from our laboratory indicated that 10 mg Lutalyse administered im at the beginning of the training session enhanced libido in boars (40). Over 85% of boars mounted and allowed semen collection during the first exposure to the artificial sow, and all boars were trained within a total of 4 training sessions (40). The experimental animals utilized in that study (40) were mature, sexually active boars (range

in age was 1 to 4 yr) that had been employed in a natural mating breeding program, but had no prior experience with an artificial sow and semen collection.

As the use of AI by swine producers expands in the U.S., the demand for boars trained for semen collection will also increase. Young boars without previous sexual experience will need to be trained for semen collection. Boar semen suppliers and producers utilizing on-farm semen collection will require strategies that ensure expeditious training.

The current study examined the effects of Lutalyse in young boars that had not experienced natural mating on the ability to train for semen collection. Lutalyse enhanced libido somewhat, but failed to increase the number of sexually inexperienced boars trained for semen collection during the experimental period. In contrast, Szurop et al. (36), found that treating young, sexually inexperienced boars with a $PGF_{2\alpha}$ analog improved libido and the number of boars mounting an artificial sow during the first or second exposure. The conflicting results could be due to the fact that Lutalyse was used in the current study while Szurop et al. (36) employed Enzaprost, a potent $PGF_{2\alpha}$ analog. However, as noted previously, Lutalyse did expedite the training of sexually mature boars (i.e., boars experienced with natural mating) for semen collection in a previous study (40).

Boars used in the current study and those used in previous research by Szurop et al. (36) differed in genetic composition. For our investigation, lean-type, terminal-line boars from a commercial source were utilized. In contrast, Szurop et al. (36) employed Dutch and Belgium Landrace, Duroc, and large white boars. Moreover, boars used in the current experiment (177 d of age and 113 kg body weight) were younger than boars used

in the study by Szurop et al. (36) (210 to 225 d of age and 110 kg body weight). Perhaps the effectiveness of $PGF_{2\alpha}$ for training for semen collection is influenced by genetics or the age of the treated boar. Age and weight related changes in libido were reported by Esbenshade et al. (83), who reported that boars do not exhibit sexual motivation combined with ejaculation until between the ages of 150 and 270 d of age. Esbenshade et al. (83) also indicated that the majority of the boars in their study did not allow semen collection until they reached a weight of 150 kg, which is approximately 37 kg heavier that the initial weight of boars used in our experiment.

Wetteman et al. (37) reported no effects of $PGF_{2\alpha}$ on mounting behavior in boars. Before beginning the experiment, however, the boars were characterized as lacking libido for failing to mount a sow in estrus. It is doubtful that the lack of an effect of Lutalyse on the number of individuals trained for semen collection was a consequence of a group of inherently low-libido boars used in our experiment. Indeed, after concluding our experiment, semen was eventually collected from 90% of the boars using other husbandry techniques (e.g., allowing boar to mount a female in estrus, beginning semen collection, and then moving boar onto the artificial sow).

It is also doubtful that a higher dose of Lutalyse would have increased the number of boars allowing semen collection in our study. The 20 mg dose of the drug caused marked non-sexual behavioral changes and actually decreased libido scores below controls. One boar exhibited emesis within 5 min of treatment with the 20 mg dose during each of the 12 training sessions.

Limited information is available concerning the possible mechanisms by which Lutalyse may stimulate libido in boars. One proposal may be that Lutalyse stimulates

testosterone secretion. However, as reported by Fonda et al. (50), im administration of 20 mg Lutalyse to boars caused an elevation in circulating prolactin and cortisol concentrations but failed to alter secretion of luteinizing hormone or testosterone. Thus, further research is needed to fully explain the mechanisms of exogenous $PGF_{2\alpha}$-induced sexual behavior in boars.

Hashizume and Niwa (46) and Estienne and Harper (40) reported that sperm concentration and total number of spermatozoa tended to increase after im treatment of boars with $PGF_{2\alpha}$. In contrast, in the current study, characteristics of ejaculates from controls and boars treated with Lutalyse at doses of 5, 10, or 20 mg were similar. The low number of boars from which semen was collected in the present investigation and previous studies (40, 46), however, precludes definitive conclusions on the effects of Lutalyse on various measures of semen quality.

In summary, Lutalyse increased libido but not the number of sexually inexperienced boars trained for semen collection or the spermatozoa output of boars. Factors that influence the efficacy with which Lutalyse expedites training of young, sexually inexperienced boars for semen collection and the effects of Lutalyse on spermatozoal output warrant further scrutiny.

Acknowledgements

This research was supported by Hatch funds allocated to the Virginia Tech Tidewater Agricultural Experiment Station (Project No. VA-135620). The completion of this study would not have been possible without the technical assistance of Phillip Taylor.

CHAPTER III: THE EFFECT OF DIETARY L-CARNITINE SUPPLEMENTATION ON SEMEN CHARACTERISTICS AND MEASURES OF LIBIDO IN BOARS USED FOR ARTIFICIAL INSEMINATION

Abstract

The objective of the current study was to determine the effects of dietary L-carnitine supplementation on boar libido, semen quality, sperm production, and maintenance of sperm motility during liquid storage. A commercial source of lean-type, terminal-line boars was utilized in two similar trials (Trial 1, 1a, 1b: n = 9 for control and L-carnitine-treated boars; Trial 2, 2a, 2b: n = 10 for control and L-carnitine-treated boars). Boars were fed a fortified, corn and soybean meal-based diet at a rate of 2 kg/d. Boars that were selected randomly for L-carnitine treatment received this diet mixed with L-carnitine to achieve supplementation of 500 mg/d. For 16 wk, semen was collected weekly via the gloved hand method and was analyzed for gel-free volume, gel weight, sperm concentration, total sperm per ejaculate, percent normal sperm morphology (Trial 1 only), and characteristics of sperm motility. Time to ejaculation (reaction time), duration of ejaculation, and number of false mounts were also recorded for each collection. Trials 1a and 2a were conducted during weeks 16 and 17 for each respective trial. Boars were collected once daily for 4 consecutive days, allowed 4 d of rest, and then collected again to estimate daily spermatozoal production. At the end of 16 weeks for each respective trial, a semen sample was also processed and extended in Beltsville Thawing Solution (BTS) to achieve a dilution of 3 x 10^9 spermatozoa/100 mL-dose for Trials 1b and 2b. The extended semen was stored in plastic bottles at 18°C and motility was evaluated daily for 7 d post collection. L-carnitine supplementation for 16 wk had no effects on semen volume, gel weight, total number of sperm cells per ejaculate,

reaction time, percent normal sperm morphology, or sperm motility ($P > 0.1$). Boars receiving the L-carnitine-supplemented diet experienced an increase in the number of false mounts before ejaculating and an increase in sperm concentration ($P < 0.05$) in Trial 2. A treatment by week interaction was determined for sperm concentration in Trial 2 ($P < 0.005$). Increased sperm concentrations in L-carnitine-treated boars were demonstrated after only one week of feeding the respective diets. Given that the production of a mature sperm cell requires 7 to 8 wk in boars, it is therefore difficult to conclude that differences in sperm concentration were due to an effect of treatment on spermatogenesis. The estimate of daily spermatozoal production was similar ($P > 0.1$) between control boars and boars supplemented with L-carnitine for both Trials 1a and 2a. L-carnitine supplementation did not affect percent motility in Trial 1b and 2b or sperm progressive motility in Trial 2b during 7 d storage ($P > 0.1$). A treatment by day interaction was determined for sperm velocity ($P < 0.05$) in Trial 2b. L-carnitine supplementation decreased mean sperm velocity after 2 d of storage. Overall, L-carnitine had no beneficial effects on boar libido, semen quality, sperm production, or maintenance of sperm motility during liquid storage.

Introduction

The use of AI by U. S. swine producers has greatly increased over the past decade. Before 1990, less than 5% of females were bred using AI (1). In 1991, AI accounted for approximately 8% of all females bred (2). A recent survey revealed that nearly 70% of the litters farrowed in 2000 were sired through AI (4). At this rate, by the year 2005, nearly 85% of all U.S. litters produced will be a result of AI. The increase in

usage of AI will fuel the demand for quality semen from boars trained to mount artificial sows for semen collection.

The employment of AI clearly augments boar power, but could be further enhanced by increasing the total number of sperm per ejaculate produced by boars. Currently, AI doses contain 2 to 3 x 10^9 spermatozoa in 80 to 100 mL of diluted seminal plasma (9). Total volume of an ejaculate ranges from 75 to 400 mL containing 20 to 100 x 10^9 spermatozoa (15). Therefore, a single boar ejaculate could yield approximately 6 to 33 AI doses. It would be beneficial to the swine industry to maximize the number of AI doses produced by boars.

L-carnitine is a vitamin-like compound synthesized within the liver, kidney, and brain through the conversion of two essential amino acids, lysine and methionine (58, 59). L-carnitine plays a vital role in cellular energy metabolism. It functions as a transporter of short-, medium-, and long-chain fatty acids towards or across the inner mitochondrial membrane hence facilitating β-oxidation (63, 64). Spermatozoa first come into contact with L-carnitine within the lumen of the epididymis as the cells mature into potentially fertile gametes. Epididymal plasma L-carnitine content increases from 20 nmol/mg protein in the distal caput to 700 nmol/mg protein in the distal cauda of the boar (75). The spermatozoa first begin to accumulate L-carnitine while in the proximal cauda where the epididymal plasma concentration of carnitine ranges between 200 and 300 nmol/mg protein (75). L-carnitine within the epididymal plasma enters the spermatozoon through its membrane by passive diffusion (75). Once inside the spermatozoon, L-carnitine facilitates the transfer of fatty acids into the mitochondria to undergo β-oxidation for energy metabolism via acyl carnitine derivatives.

Feeding L-carnitine (500 mg/kg of diet) increased sperm concentration and decreased lipid peroxidation of sperm in roosters (78). In humans, L-carnitine supplementation (3 g/d) increased sperm concentration and sperm motility of idiopathic asthenozoospermia patients (79, 80). In a European study, breeding boars received a diet that was supplemented with 500 mg/d of L-carnitine which resulted in an increase in the number of AI doses produced per ejaculate by one (81). Furthermore, a 16-week field trial involving 180 boars housed in a commercial stud revealed that a "high" level of L-carnitine increased the number of viable sperm cells and the number of insemination doses produced per week (82). The objectives of the current study were to determine the effects of L-carnitine supplementation on boar libido, semen quality, production, and maintenance of spermatozoal motility during liquid storage.

Materials and Methods

General. Hamline boars, donated by National Pig Development-USA (Roanoke Rapids, NC) were utilized. Boars were housed at the Virginia Swine Evaluation Station Research Boar Stud in Suffolk, VA in individual pens (4.5 m^2) that had a combination of concrete and solid steel rod flooring. Each pen was fitted with a nipple waterer and boars were floor-fed. The building was curtain-sided and temperature was controlled by gas heaters, stirring fans, and a drip cooling system. Boars were fed, at a rate of 2 kg/d, a fortified, corn and soybean meal-based diet that met or exceeded NRC (53) nutrient recommendations for breeder boars. Boars assigned to the active treatment received the same diet supplemented with L-carnitine (Carniking; Lonza, Inc., Fairlawn, NJ) at a level of 250 mg/kg of diet and fed to provide 500 mg supplemental L-carnitine per boar daily.

The control diet and L-carnitine-supplemented diet were analyzed by a radioenzymatic assay for free L-carnitine content for each trial (Metabolic Analysis Labs, Inc., Madison WI). For Trial 1, 1a, and 1b, the free L-carnitine content of the control diet was 3.0 mg/kg feed and the L-carnitine-supplemented diet contained 249.4 mg free L-carnitine/kg of feed. For Trial 2, 2a, and 2b, the free L-carnitine content of the control diet was 3.5 mg/kg feed and the L-carnitine-supplemented diet contained 256.1 mg free L-carnitine/kg of feed.

Trial 1 Protocol: Effects of L-carnitine on semen quality and libido. Trial 1 took place throughout the months of July to December and employed Hamline boars (n = 18), approximately 270 d of age and weighing approximately 157 kg at the beginning of the study. Boars were assigned randomly to receive a control diet or the control diet supplemented with L-carnitine at a rate of 500 mg/d (n = 9 per treatment). Semen was collected, via the gloved-hand technique, once weekly for 17 wk. During collection, semen was filtered through a double layer of cheesecloth to remove the gel fraction. Semen was analyzed for gel-free volume, gel weight, sperm concentration, total sperm, percent normal sperm morphology, and the percentage of spermatozoa exhibiting motility. Sperm concentration was determined using a Spermacue photometer (Minitube of America, Inc., Verona, WI). A top-loading balance (Acculab, Minitube of America, Inc.) was utilized to determine gel weight and semen volume. Percent morphologically normal spermatozoa were determined (1000x) after being eosin stained and dried. The slides prepared for determining motility were viewed (100x) under an Olympus phase contrast trinocular microscope system (Olympus America, Inc., Melville, NY) with a heated stage (37°C), camera, and video screen (Panasonic, Secaucus, NY). Samples

viewed under the microscope were recorded onto a VHS tape for detailed analysis of motility to be performed at a later time (84).

Slides for motility analysis were prepared by diluting semen with a commercially available extender (Beltsville Thawing Solution, Minitube of America, Inc.). Ten or more independent visual fields of each diluted semen sample were recorded for approximately 45 s. Motility analysis consisted of tracking motility of 10 distinct spermatozoa in each of the 10 or more independent views of the recorded sample. This was accomplished by pausing the tape while playing, marking individual spermatozoa with an "X" on the screen, allowing the tape to play for 3 to 5 s, pausing the tape again, and then assessing if the spermatozoa progressed from the previously marked "X". The same evaluator performed all motility analyses throughout the study.

Reaction time, duration of ejaculation, and the number of false mounts for each boar were also recorded weekly. Reaction time was defined as the elapsed time from the boar being allowed access to the artificial sow until the start of ejaculation. False mounts were defined as mounting the artificial sow but not ejaculating.

Trial 1a Protocol: Effects of L-carnitine on daily sperm production. During wk 16 and 17, daily production of spermatozoa was estimated using the procedure described by Flowers (85). Boars were collected for 4 consecutive days, rested for 4 d, and then collected once immediately after the rest period. The total number of spermatozoa in the ejaculate after the rest period, divided by the number of days rested, provides an estimate of daily sperm production (85). Ejaculates were analyzed as described in Trial 1 along with the number of false mounts for each boar being recorded.

Trial 1b Protocol: Effects of L-carnitine on maintenance of sperm motility. During week 16, collected semen was analyzed and then processed and diluted in a commercially available extender (Beltsville Thawing Solution, Minitube of America, Inc.) to a final dilution of 3 x 10^9 sperm cells/100 mL of seminal plasma and extender. The 100-mL AI doses were stored in plastic bottles at 18°C in a semen storage unit (Minitube of America, Inc.) and motility was evaluated, as described in Trial 1, daily for 7 d post collection. Stored semen was warmed to 37°C for 0.5 h before re-evaluation.

Trial 2 Protocol: Effects of L-carnitine on semen quality and libido. Trial 2 took place during the months of March through July and utilized Hamline boars (n = 20), approximately 525 d of age at the beginning of the study. Boars were randomly assigned to receive either a control diet or the control diet supplemented with L-carnitine at a rate of 500 mg/d (n = 10 per treatment). Semen was collected, via the gloved-hand technique, once weekly for 17 wk. During collection, semen was filtered through a double layer of cheesecloth to remove the gel fraction. Semen samples were analyzed for gel-free volume, gel weight, sperm concentration, total sperm cells, percentage of spermatozoa exhibiting motility, percentage of spermatozoa exhibiting progressive motility (minimum path velocity of 45 μm/s and path straightness greater than 45%), and spermatozoal velocity (μm/s) (average velocity measured over the actual point-to-point track followed by the cell). Prior to analysis, collected semen was diluted in a commercially available extender (Beltsville Thawing Solution) to achieve a ratio of semen to extender of 1 to 30. Sperm concentration, total sperm cells, percentage of spermatozoa exhibiting motility, percentage of spermatozoa exhibiting progressive motility, and spermatozoal velocity were analyzed using a computer-assisted sperm analysis system (Hamilton Thorne

Research, Beverly, MA). A top-loading balance (Acculab, Minitube of America, Inc.) was utilized to determine gel weight and semen volume.

Reaction time, duration of ejaculation, and the number of false mounts for each boar were also recorded weekly. Reaction time was defined as the elapsed time from the boar being allowed access to the artificial sow until the start of ejaculation. False mounts were defined as mounting the artificial sow but not ejaculating.

Trial 2a Protocol: Effects of L-carnitine on daily sperm production. During weeks 16 and 17, daily production of spermatozoa was estimated using the procedure described by Flowers (85). Boars were collected for 4 consecutive days, rested for 4 d, and then collected once immediately after the rest period. The total number of spermatozoa in the ejaculate after the rest period, divided by the number of days rested, is an estimate of daily sperm production (85). Ejaculates were analyzed as described in Trial 2, along with reaction time, duration of ejaculation, and the number of false mounts for each boar being recorded.

Trial 2b Protocol: Effects of L-carnitine on maintenance of sperm motility. During week 16, collected semen was analyzed and then processed and diluted in a commercially available extender (Beltsville Thawing Solution, Minitube of America, Inc.) to a final dilution of 3×10^9 sperm cells/100 mL of seminal plasma and extender. The 100-mL AI doses were stored in plastic bottles at 18°C in a semen storage unit (Minitube of America, Inc.) and motility characteristics were evaluated, as described in Trial 2, daily for 7 d post collection. Stored semen was warmed to 37°C for 0.5 h before re-evaluation.

Statistical Analysis. Semen characteristics, reaction time, duration of ejaculation, and number of false mounts was subjected to analysis of variance for a repeated measures design utilizing the GLM procedure of SAS (SAS Institute Inc., Cary, NC). Boar served as the experimental unit. The statistical model included treatment, boar within treatment, time, and treatment by time interaction as possible sources of variation. If a significant treatment by time interaction was detected, then means between treatments and within treatments across time were compared using the PDIFF option of the LSMEANS statement in the GLM procedure.

Results

Various semen characteristics, reaction time, duration of ejaculation, and number of false mounts for Trial 1 are displayed in Table 2. Supplementing the diets of boars to provide 500 mg L-carnitine/d for 16 wk, had no effect on any of the variables analyzed ($P > 0.05$). However, control boars did display a trend of increased sperm concentration ($P = 0.08$) compared to the L-carnitine-treated boars. There was an effect of time (week) on all parameters ($P < 0.03$) except for the weekly number of false mounts ($P > 0.1$). There was no treatment by week interactions determined for any of the variables analyzed in Trial 1 ($P > 0.1$).

Table 2. Trial 1. Reproductive characteristics of boars receiving a control diet or control diet supplemented with L-carnitine to provide 500 mg/d.

Variables	Control[a]	L-carnitine[a]	Standard Error	P-value[b]
Boars (n)	9	9		
Semen Volume (mL)	138.0	157.9	2.6	0.32
Gel weight (g)	26.8	33.6	0.6	0.12
Sperm concentration (x 10^6/mL)	422.3	362.8	4.9	0.08
Total sperm cells (x 10^9)	56.1	55.7	0.9	0.95
Motile sperm (%)	84.3	90.1	0.5	0.18
Normal Morphology (%)	82.8	87.8	0.5	0.31
False mounts	2.8	2.5	0.1	0.82
Reaction time[c] (s)	327	280	15	0.63
Duration of ejaculation (s)	252	275	6	0.34

[a] Values are least square means.
[b] A significant effect required $P < 0.05$; $P = 0.1$ to $P = 0.05$ was considered a trend.
[c] Elapsed time after entering collection pen until the start of ejaculation.

Various semen characteristics, reaction time, duration of ejaculation, and number of false mounts for Trial 2 are displayed in Table 3. Supplementing the diets of boars with L-carnitine (500 mg/d) for 16 wk had no effect on semen volume, gel weight, total number of sperm, reaction time, percent of motile sperm, percent of progressively motile sperm, or sperm velocity ($P > 0.1$). Boars receiving the L-carnitine-supplemented diet displayed an increase in the number of false mounts before ejaculating and an increase in sperm concentration ($P < 0.05$). Over the 16 wk treatment period, control boars established a trend to ejaculate for a longer period of time ($P = 0.067$). There was an effect of time (week) on all parameters analyzed ($P < 0.01$) except for semen volume and number of false mounts ($P > 0.1$). The effect of week on the duration of ejaculation was considered a trend ($P = 0.08$). A treatment by week interaction was determined for sperm concentration ($P < 0.005$) (Figure 4). There was a trend for an influence of treatment

over time for duration of ejaculation (P = 0.078) (Figure 5) and sperm velocity (P = 0.079) (Figure 6). No treatment by week interaction was determined for all other variables assessed throughout Trial 2.

Table 3. Trial 2. Reproductive characteristics of boars receiving a control diet or control diet supplemented with L-carnitine to provide 500 mg/d.

Variables	Control[a]	L-carnitine[a]	Standard Error	P-value[b]
Boars (n)	10	10		
Semen Volume (mL)	175.4	154.6	2.0	0.17
Gel weight (g)	29.3	28.4	0.5	0.83
Sperm concentration (x 10^6/mL)	240.6	362.8	5.5	0.02
Total sperm cells (x 10^9)	41.8	53.8	0.8	0.10
Motile sperm (%)	72.8	72.0	0.6	0.87
Progressively motile sperm (%)	41.3	37.9	0.9	0.52
Sperm velocity (μm/s)	135.4	136.0	1.4	0.94
False mounts	0.8	1.7	0.1	0.03
Reaction time[c] (s)	327	395	14	0.53
Duration of ejaculation (s)	288	237	4	0.06

[a] Values are least square means.
[b] A significant effect required P < 0.05; P = 0.1 to P = 0.05 was considered a trend.
[c] Elapsed time after entering collection pen until the start of ejaculation.

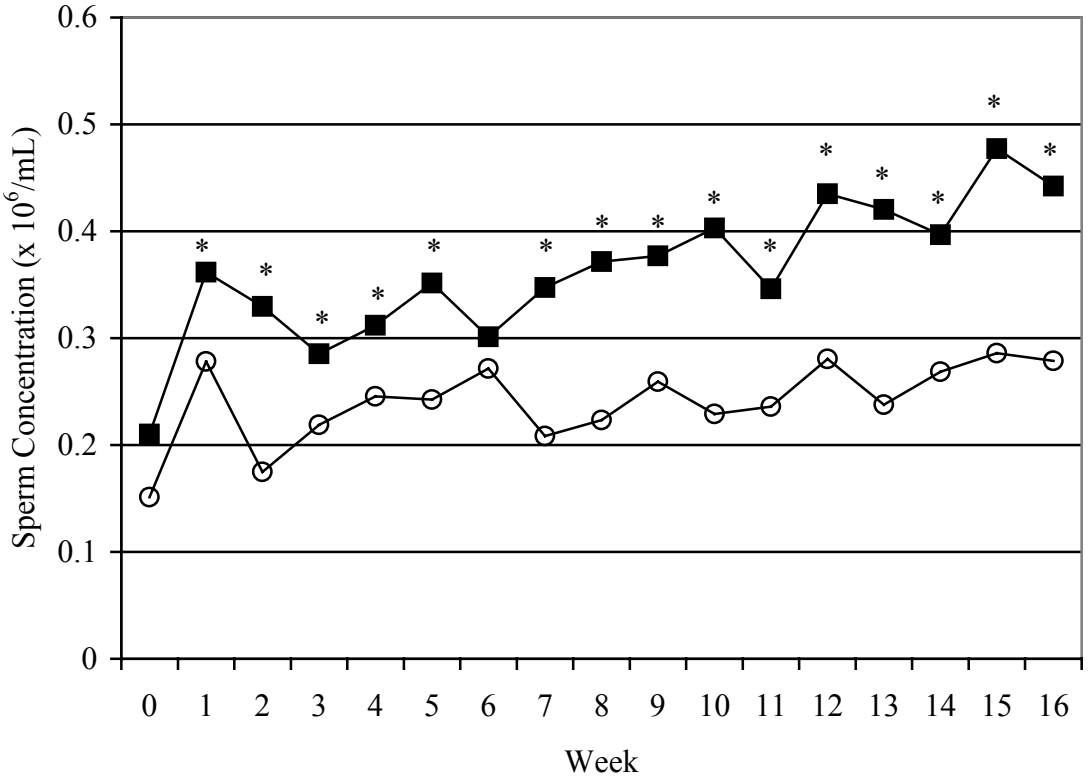

Figure 4. Trial 2. Least square means of sperm concentration (x 10^6/mL) for boars receiving a control diet (○, n = 10) or a control diet supplemented with L-carnitine to provide 500 mg/d (■, n = 10) for 16 weeks. Weekly data series marked with * differ ($P < 0.05$) between treatments. There was a treatment by time interaction ($P = 0.005$). The standard error was 0.023.

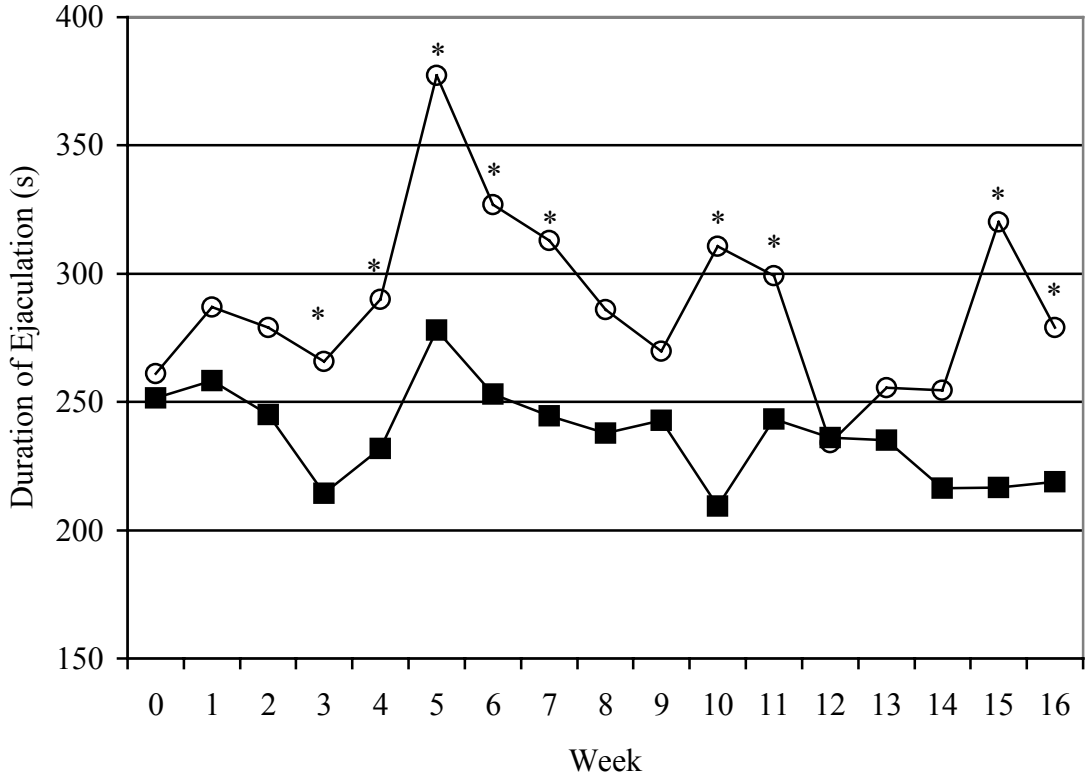

Figure 5. Trial 2. Least square means of the duration of ejaculation (s) for boars receiving a control diet (○, n = 10) or a control diet supplemented with L-carnitine to provide 500 mg/d (■, n = 10) for 16 weeks. Weekly data series marked with * differ ($P < 0.05$) between treatments. There was a trend for a treatment by time interaction ($P = 0.078$). The standard error was 17.67.

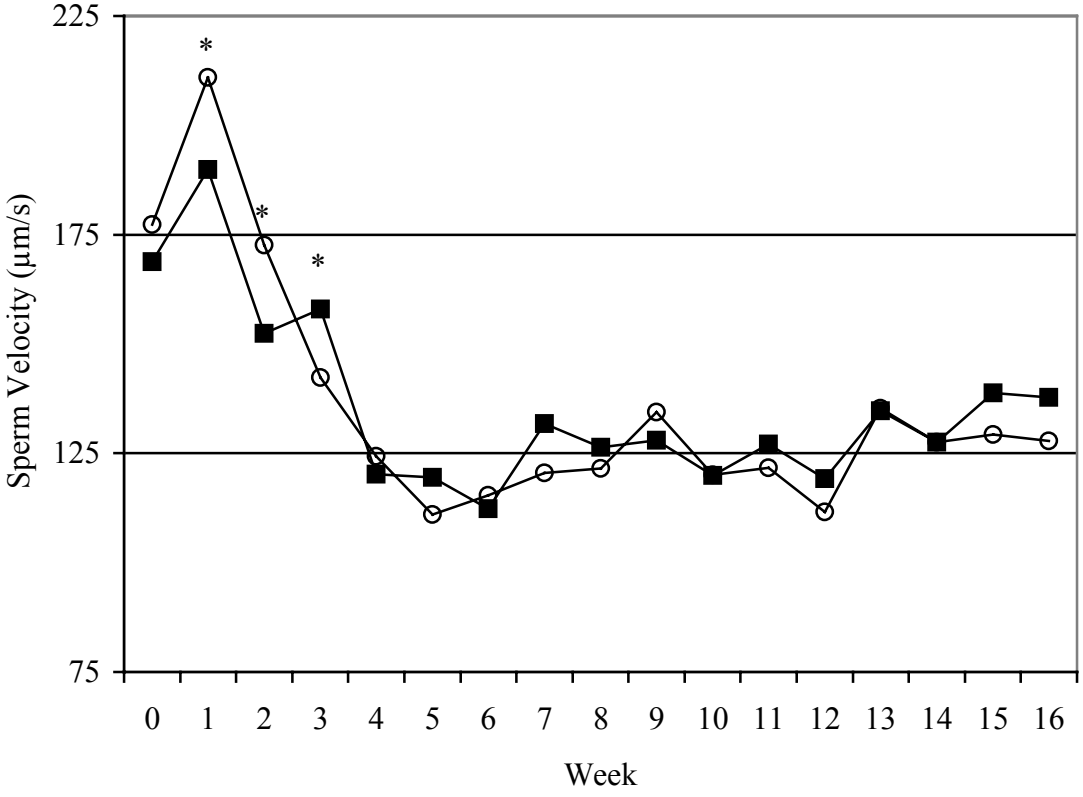

Figure 6. Trial 2. Least square means for sperm velocity (µm/s) of boars receiving a control diet (○, n = 10) or a control diet supplemented with L-carnitine to provide 500 mg/d (■, n = 10) for 16 weeks. Weekly data series marked with * differ ($P < 0.05$) between treatments. There was a trend for a treatment by time interaction ($P = 0.0796$). The standard error was 5.84.

Various semen characteristics over the 4 d depletion period and the estimate of daily sperm production based on the total number of sperm collected after the rest period for Trial 1a are displayed in Table 4. Over the 4 d depletion period, ejaculate volume, total number of spermatozoa, gel weight, and number of false mounts were similar between the control boars and L-carnitine-supplemented boars ($P > 0.1$). Semen collected from control boars had a trend for higher sperm concentration ($P = 0.082$) when collected consecutively for 4 days. There was an effect of time (day) on both sperm

concentration and total numbers of spermatozoa ejaculated ($P < 0.005$), but no effect of day on volume, gel weight, or number of false mounts ($P > 0.1$). No treatment by day interactions were determined for any of the parameters analyzed. The estimate of daily spermatozoa production was similar for L-carnitine-treated boars and control boars ($P > 0.1$).

Table 4. Trial 1a. Reproductive characteristics of boars receiving a control diet or control diet supplemented with L-carnitine to provide 500 mg/d over a 4 day sperm depletion period.

Variables	Control[a]	L-carnitine[a]	Standard Error	P-value[b]
Boars (n)	9	9		
Semen Volume (mL)	162.4	185.3	4.0	0.18
Gel weight (g)	36.3	45.2	1.3	0.15
Sperm concentration (x 10^6/mL)	282.2	228.7	7.3	0.08
Total sperm cells (x 10^9)	45.8	43.0	1.5	0.59
False mounts	2.2	1.7	0.3	0.64
Daily sperm production[c] (x 10^9)	11.3	11.2	0.9	0.94

[a] Values are least square means.
[b] A significant effect required $P < 0.05$; $P = 0.1$ to $P = 0.05$ was considered a trend.
[c] The total number of spermatozoa collected after the rest period and divided by elapsed number of days from the last collection at the end of the depletion period.

Various reproductive characteristics over the 4 d depletion period and the estimate of daily sperm production based on the total number of sperm collected after the rest period for Trial 2a are displayed in Table 5. Supplementing L-carnitine had no effect on gel weight, motile sperm, progressively motile sperm, sperm velocity, or total numbers of spermatozoa ejaculated ($P > 0.1$) compared to control boars when collected consecutively over a 4 d period. Boars supplemented with L-carnitine had a greater number of false

mounts ($P < 0.05$) and a higher sperm concentration throughout the depletion period ($P < 0.01$), and control boars established a trend of ejaculating a greater volume ($P = 0.078$). There was an effect of time (day) on sperm concentration, total sperm numbers, progressively motile sperm, sperm velocity, and duration of ejaculation ($P < 0.05$), but no effect of day on false mounts or gel weight ($P > 0.1$). There was a trend for an effect of day on semen volume ($P = 0.059$) and motile sperm cells ($P = 0.064$). The only variable determined to have a treatment by day interaction was sperm concentration ($P < 0.001$) (Figure 7). The estimate of daily spermatozoa production was similar between control boars and boars supplemented with L-carnitine ($P > 0.1$).

Table 5. Trial 2a. Reproductive characteristics of boars receiving a control diet or control diet supplemented with L-carnitine to provide 500 mg/d over a 4 day sperm depletion period.

Variables	Control[a]	L-carnitine[a]	Standard Error	P-value[b]
Boars (n)	10	10		
Volume (mL)	176.3	144.5	3.6	0.07
Gel weight (g)	28.5	24.5	0.8	0.35
Sperm concentration (x 10^6/mL)	183.0	268.6	8.0	0.01
Total sperm cells (x 10^9)	33.0	39.0	1.5	0.27
Motile sperm (%)	69.5	68.5	1.3	0.87
Progressively motile sperm (%)	34.1	30.6	1.7	0.52
Sperm velocity (μm/s)	116.4	121.7	2.5	0.46
False mounts	0.3	1.5	0.1	0.03
Reaction time[c] (s)	155	290	31	0.08
Duration of ejaculation (s)	264	214	8	0.13
Daily sperm production[d] (x 10^9)	6.0	6.9	0.8	0.47

[a] Values are least square means.
[b] A significant effect required $P < 0.05$; $P = 0.1$ to $P = 0.05$ was considered a trend.
[c] Elapsed time after entering collection pen until the start of ejaculation.
[d] The total number of spermatozoa collected after the rest period and divided by elapsed number of days from the last collection at the end of the depletion period.

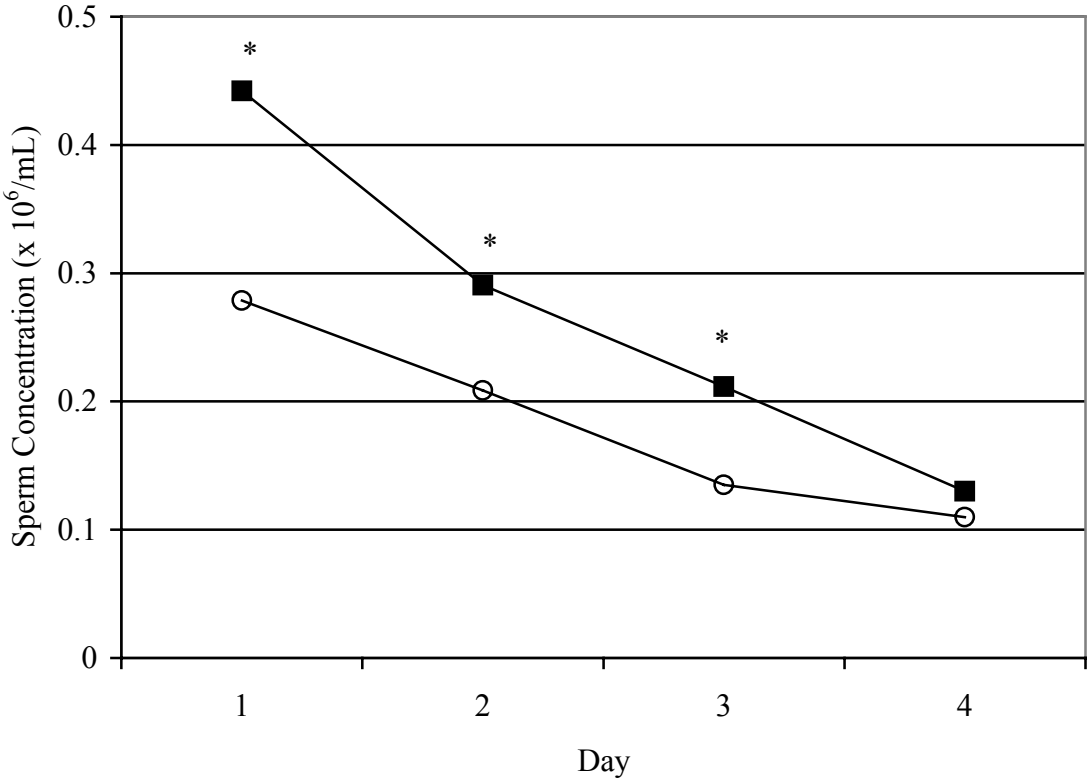

Figure 7. Trial 2a. Least square means for sperm concentration (x 10^6/mL) over a 4 day depletion period of boars receiving a control diet (○, n = 10) or a control diet supplemented with L-carnitine to provide 500 mg/d (■, n = 10) for 16 weeks. Daily data series marked with * differ ($P < 0.05$) between treatments. A treatment by time interaction was determined ($P < 0.001$). The standard error was 0.016.

For Trial 1b and Trial 2b, the sperm motility characteristics over a 7d storage period are shown in Table 6. There was no effect of treatment on sperm motility throughout the storage period of Trial 1b ($P > 0.1$). However, there was an effect of time (day) on sperm motility of boars ($P = 0.0005$), but no treatment by day interaction ($P > 0.1$). Control boars of Trial 2b exhibited a trend for a greater percentage of motile sperm

(P = 0.070) and progressively motile sperm (P = 0.086). Sperm velocity was similar between control boars and L-carnitine-supplemented boars (P > 0.1). There was an effect of day on all motility characteristics observed in Trial 2b (P < 0.005). A treatment by day interaction was determined for sperm velocity (P < 0.05) (Figure 8), but not for sperm motility or sperm progressive motility (P > 0.1).

Table 6. Sperm motility characteristics over a 7 day storage period from boars receiving a control diet or control diet supplemented with L-carnitine to provide 500 mg/d.

	Variables	Control[a]	L-carnitine[a]	Standard Error	P-value[b]
Trial 1b	Boar (n)	9	9		
	Motility (%)	75.2	80.5	1.0	0.42
Trial 2b	Boar (n)	10	10		
	Motility (%)	46.9	31.2	1.6	0.07
	Progressive Motility (%)	21.0	9.8	1.0	0.08
	Velocity (μm/s)	105.8	96.1	1.3	0.29

[a] Values are least square means.
[b] A significant effect required P < 0.05; P = 0.1 to P = 0.05 was considered a trend.

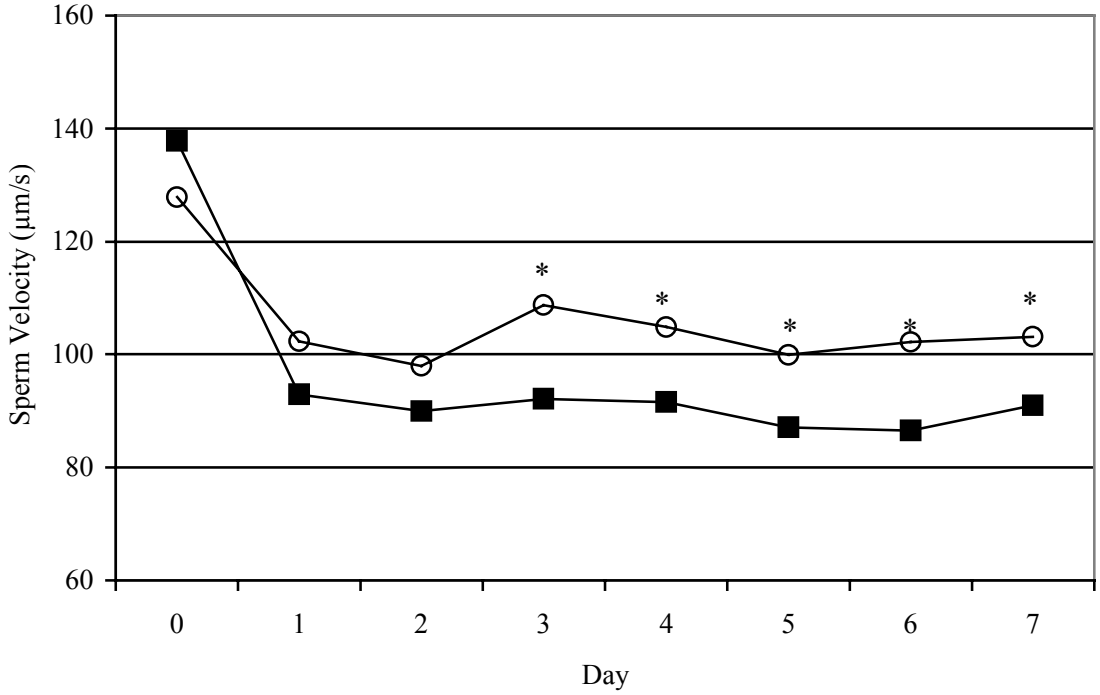

Figure 8. Trial 2b. Least square means of sperm velocity (µm/s) over a 7 day storage period for boars receiving a control diet (○, n = 10) or a control diet supplemented with L-carnitine to provide 500 mg/d (■, n = 10) for 16 weeks. Daily data series marked with * differ (P < 0.05) between treatments. A treatment by time interaction was determined (P = 0.0235). The standard error was 3.85.

Discussion

Previous work has demonstrated beneficial effects of L-carnitine supplementation on semen characteristics. In humans, L-carnitine supplementation (3 g/d) for 3 to 4 mo increased sperm concentration and sperm motility of idiopathic asthenozoospermia patients (79, 80). L-acylcarnitine supplementation (15 mg/kg body weight/d) for 10 wk also increased sperm concentration and percent motile sperm in oligoasthenospermia-induced Wistar rats (77). In these three experiments, all subjects were experiencing below normal sperm concentrations and percent motile spermatozoa before

supplementation commenced. In the current investigation, none of the boars utilized were predetermined to have a low sperm concentration or low percentage of motile spermatozoa. It may be possible that for L-carnitine to positively affect sperm concentration or sperm motility characteristics there must be a pre-existing infertility condition. In Trial 2, supplementing L-carnitine (500 mg/d) in the diet of boars increased the concentration of sperm in the ejaculate. This difference may be partially explained by the ejaculate volume of L-carnitine-treated boars being 11.88% less than the controls throughout the 16 wk experimental period. The possibility of L-carnitine positively affecting spermatogenesis in boars is unlikely. Although data in boars is lacking, concentrations of L-carnitine within the seminiferous tubules of the rat, hamster, and ram have been determined to be low (< 1 mM) (86). Spermatogenesis in boars requires 34 to 39 d and epididymal transport involves another 9 to 12 d (87, 88). Therefore, if a nutritional supplement, L-carnitine, was to have an effect on sperm concentration, an effect would probably not be observed until approximately 7 to 8 wk after supplementation was initiated. In the current investigation, a significant difference was observed within the second week of collections and continued for the majority of the 16 wk study (Figure 4). Furthermore, the estimates of daily sperm production generated in Trial 1a and 2a were similar.

Although it is doubtful that L-carnitine has an effect on sperm concentration through an increase in sperm production, L-carnitine may increase the survivability of spermatozoa within the epididymis. L-carnitine has been implicated as crucial to the survival of sperm cells (62). Increasing the concentration of L-carnitine within the lumen of the epididymis would perhaps increase the amount of L-carnitine within the

spermatozoa. Extracellular and intracellular L-carnitine concentrations of epididymal spermatozoa are in equilibrium even after exogenous supplementation of L-carnitine in vitro (58). If L-carnitine increases the viability of spermatozoa, then possibly fewer dead sperm would be reabsorbed, inherently increasing total spermatozoa output. However, few if any spermatozoa are absorbed from the epididymis or voided in the urine of sexually active boars (89). Supplementing boars with "high" levels of L-carnitine was shown to increase the number of viable sperm cells/ejaculate in an Akey field trial (82). L-carnitine has also been implicated in buffering the cell against high concentrations of mitochondrial acetyl-CoA by converting it into acyl carnitine (62). Excess acetyl-CoA inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial energy metabolism (62). This function of L-carnitine may further improve the survival of spermatozoa and increase the total number of sperm that are ejaculated. However, total spermatozoal output was similar throughout Trial 1 and 2, but the actual numbers of viable sperm cells/ejaculate were not examined.

For swine AI, the frequency of collection is an important factor affecting semen quality (90). During wk 16 in Trials 1a and 2a, boars were collected once daily for 4 d consecutively to deplete epididymal sperm reserves. In both studies, semen characteristics decreased over time. This is similar to other studies with once daily collection frequencies (90, 91). Throughout the 4 d depletion period of Trial 2a, L-carnitine-treated boars exhibited a higher sperm concentration than control boars for the first 3 days of the depletion period. This may be partially explained by the ejaculate volume of the control boars being 18% greater over the 4 d depletion period. As discussed previously, it is doubtful that L-carnitine supplementation has any effect on

boar spermatogenesis or total sperm output, which may have lead to a greater sperm concentration.

The reaction time, defined as the elapsed time from the boar being allowed access to the artificial sow until the start of ejaculation, and number of false mounts, defined as mounting the artificial sow but not ejaculating, were recorded throughout Trials 1, 1a, 2 and 2a as measures of libido. Treatment of boars with L-carnitine had no effect on reaction time for any trials ($P > 0.05$). Boars treated with L-carnitine exhibited a significant increase in the observed number of false mounts before ejaculating throughout Trials 2 and 2a. We have no explanation for this effect of L-carnitine treatment. Perhaps the observed number of false mounts prior to ejaculating is not an appropriate indication of libido in boars already trained for purposes of artificial insemination. The number of false mounts could be influenced by the semen collector and thus would not portray the true libido characteristics of the boar. A more suitable variable to observe would be the response time from entering the collection pen to the first attempt to mount when assessing libido of boars trained for semen collection.

The ability to store boar semen in a liquid state for an extended period of time is essential to the swine AI industry. Overnight delivery systems allow for producers to obtain fresh, quality semen whenever necessary. Numerous commercially available extenders exist for this purpose, but as post-collection time increases, the fertility of liquid stored boar semen decreases (92). However, individual boars exhibit great variation in the ability of their spermatozoa to maintain quality throughout storage (93). One control boar maintained 90% sperm motility throughout the 7d storage period of Trial 2b (data not shown). Motility of ejaculated spermatozoa is an important factor in

determining fertility of semen (92). Boars supplemented with L-carnitine exhibited similar maintenance of motility over the 7 d storage period in both Trials 1b and 2b. The velocity of boar spermatozoa after 2 h of liquid storage has also been implicated as a sufficient measure of fertility (94). In Trial 2b, control boars exhibited a greater velocity throughout the 7 d storage period compared to L-carnitine supplemented boars. It has been postulated that high concentrations of L-carnitine within the lumen of the rat epididymis plays a role in preserving spermatozoa in a quiescent state (95). When 20 Mm of L-carnitine was added to suspensions of ejaculated bovine spermatozoa (96) and rooster spermatozoa (97), L-carnitine was shown to have an inhibitory effect on sperm progressive motility by reducing the oxygen consumption of the spermatozoa. L-carnitine supplementation in the present study, at a rate of 500 mg/boar/d, may have increased the concentration of L-carnitine in the ejaculate of the boar above its normal concentrations of 318 mM (19), thus having a similar affect to what was observed in both the bull (96) and the rooster (97). A reduction in oxygen consumption by the spermatozoa may have contributed to the decrease in velocity of L-carnitine-supplemented boars observed in Trial 2b. If the spermatozoa were experiencing a reduction in oxygen consumption, due to higher concentrations of L-carnitine in the seminal plasma and inherently the extended AI dose, the velocity of the spermatozoa may have been affected.

To the knowledge of the author, this study represents the first scientific report on the effects of L-carnitine supplementation on boar libido, semen characteristics, and maintenance of sperm motility parameters during liquid storage. In summary, the

supplementation of L-carnitine had no significant beneficial effects on boar libido, semen quality, sperm production, or maintenance of sperm motility during liquid storage.

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SUMMARY AND CONCLUSIONS

The objectives of this thesis project were to attempt to enhance the reproductive efficiency of boars. The first study tested the effect of im treatments of Lutalyse on expediting the training of sexually inexperienced boars for semen collection and increasing spermatozoa output. The second study determined the effects of L-carnitine supplementation (500 mg/d) on boar libido, semen quality, sperm production, and maintenance of sperm motility during liquid storage. Lutalyse increased libido but not the number of sexually inexperienced boars trained for semen collection or the spermatozoal output of boars. L-carnitine supplementation increased sperm concentration, but this effect was observed prior to the time in which supplementation would be able to influence concentration through spermatogenesis. Overall L-carnitine had no significant beneficial effects on boar libido, semen quality, sperm production, or maintenance of sperm motility during liquid storage. Factors that influence the efficacy with which Lutalyse expedites training of young, sexually inexperienced boars for semen collection, the affects of Lutalyse on spermatozoa output, and the affects of L-carnitine on boar libido, semen quality, sperm production, and maintenance of sperm motility during liquid storage warrant further scrutiny.

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VITA

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