

EFFECTS OF DIETARY OCTACOSANOL ON RAM SEMEN QUALITY
PARAMETERS

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ABSTRACT

The fate of sheep production operations is predicated by ewe conception rates. Dietary octacosanol has demonstrated several effects on different metabolic and reproductive functions across various species. Twenty-four spring-born ram lambs were placed in this study investigating the chemical compound's effects on testosterone secretion and semen quality, and observing any differences in effects between rams of different breeds; Rambouillet and Suffolk. Lambs were divided randomly between control and treatment pens at the Management, Instruction and Research Center, where control pens were fed an ad libitum base ration, Ram20, and treatment pens were fed the same ad libitum base ration, Ram20 plus octacosanol at an inclusion rate of 0.25% of the total ration. Semen samples were collected utilizing the QwikCheck Gold automated sperm quality analyzer. Preliminary results suggest octacosanol may serve as an effective means to improve the quality of semen, however results may vary depending on the breed being treated.

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INTRODUCTION

The fate of a sheep production operation is predicated, at the most basic level on ewe conception rates. Modern animal science has devoted ample scrutiny to the female's contribution to reproductive efficiency and has developed a large body of work dedicated to understanding and improving this contribution. Though many new methods, artificial and natural, have been developed to maintain and even enhance the ovine female's ability to reproduce, it seems that less attention has been afforded to the male of the species. Undoubtedly, the means for properly managing rams and maintaining their reproductive effectiveness have been well established. Tests such as breeding soundness exams and microscopic evaluation of sperm cells offer insight into the existing quality of semen in rams. Still, there are a few options available for the enhancement of the ram's influence on conception rates. In an endeavor to consider ways to facilitate such an improvement, one might begin with the hormone that regulates the reproductive function of the male, testosterone. One might also consider the vehicle for the ram's contribution to conception, semen. If there was a way to increase testosterone secretion, and improve semen quality, this method could possibly serve as an enhancement to the ram's reproductive efficiency. The present study proposes to investigate the chemical compound octacosanol, which has been demonstrated to have the potential to influence testosterone secretion and semen quality in some species of animals. Octacosanol presents itself as a long chain alcohol which is found in the natural wax product stemmed from wheat germ oil (WGO), various fruits and leaves of plants, and rice bran oil (Long et al. 2015). In recent years, this compound has been the focus

of several studies examining the substance's effect on metabolic and reproductive functions of several vertebrate species as well as some insects. There also exists a large body of work conducted prior to the isolation and identification of octacosanol. In these studies, WGO was the experimental treatment under examination. Many of the experiments yielded data showing a metabolic or reproductive response to WGO, but researchers of that period were not capable of identifying what component or mechanism of WGO was responsible. More recent studies suggest that the unidentified component was octacosanol based on the similarity in results between the studies examining WGO, and those investigating isolated octacosanol. The present study relies on later works utilizing isolated octacosanol as well as those earlier studies, which employed WGO.

LITERATURE REVIEW

Little is known about the mechanisms through which octacosanol exerts its various effects. However, there is some understanding of how the compound is metabolized, distributed, and stored particularly when administered orally. After several consecutive administrations to rats, the greatest accumulation of octacosanol is found in the liver, followed closely by adipose tissue (Kabir et al. 1989). When administration is ceased, octacosanol content in the liver decreases but remains at high level in adipose tissue and tends to accumulate there suggesting that it is redistributed over time (Kabir et al. 1989). Octacosanol was found in rats up to 9 days after the last administration, which explains why it continues to improve the endurance of exercising rats days after treatments end (Kabir et al. 1989). Because the compound is known to increase the stamina of exercising rats, it is speculated that octacosanol could be converted into fatty acids to be used for energy (Kabir et al. 1989). Large amounts of octacosanol are also distributed to skeletal muscle in rats (Kabir and Kimura, 1994). It seems that the compound can be stored there as in adipose tissue and then later mobilized when demands for energy increase (Kabir and Kimura, 1994). It is also possible that octacosanol can stimulate the utilization of free fatty acids in the tissues of muscle and fat to increase energy production in the body during physical stress or exercise (Kabir and Kiumura 1994).

There have been numerous reports on a variety of effects of octacosanol exerted on several different aspects of metabolism and growth. There is evidence of octacosanol decreasing the weight of perirenal adipose tissue of rats fed a high-fat diet (Long et al. 2016).

Octacosanol has also been shown to decrease low-density lipoproteins, triacylglycerol and cholesterol levels in mice and humans (Long et al. 2016). Reports of antioxidant qualities in octacosanol have also been reported (Long et al. 2016). A great deal of research has indicated that octacosanol also displays some anti-fatigue properties in exercising rats and humans as well as an ability to effect growth performance in rats and some species of livestock (Long et al. 2015). In regard to growth, octacosanol has been shown to increase thyroid hormone secretion in pigs which can stimulate cell differentiation, organ growth, and suppress some negative effects of stress (Long et al. 2015). Octacosanol also causes an increase in growth hormone, GH secretion in pigs, which is particularly significant given the hormone's role in regulating metabolism, promoting protein synthesis, and accelerating lipolysis during times of stress (Long et al. 2015). This suggests that octacosanol can promote growth, especially during times of stress by increasing protein deposition. Increasing the rate of lipolysis would also contribute to protein deposition, as energy requirements would be met by lipid metabolism rather than protein metabolism when an energy deficit occurs. Moreover, octacosanol has been shown to enhance glycogen storage in the muscle (Long et al. 2016). There is some evidence that dietary octacosanol can also stimulate mRNA expression of glycogen synthase in muscle which provides a possible explanation for how the compound can regulate glycogen metabolism in muscle tissue (Long et al. 2016). Research has shown that octacosanol further effects lipid metabolism by reducing the use of muscle and blood glucose in exercising rats by modifying the animal's metabolism to rely more on the oxidation of fat in muscle for energy (Long et al. 2015). This is likely achieved by altering

the levels of glucagon and adrenaline secreted in the test subjects as some studies has shown that dietary octacosanol caused an increase in the secretion of both of these hormones in pigs and rats (Long et al. 2015). Octacosanol has also been shown to up-regulate the expression of the GLUT-4 gene in the liver and muscle of pigs (Long et al. 2015). This effect was cited as one possible mechanism through which octacosanol facilitates an improvement in growth performance of weaning pigs because an increased expression of GLUT-4 would presumably increase glucose uptake, gluconeogenesis, and glycolysis (Long et al. 2015). This would improve the animal's efficiency in maintaining their energy balance thus allowing for enhanced growth performance. Similarly, octacosanol also appears to up-regulate the gene expression of activated protein kinase, AMPK in the liver and muscle of pigs which also promotes glucose uptake further improving the animal's ability to maintain its energy balance (Long et al. 2015). The modifications of metabolism essentially result in an increase in ATP availability because the described gene regulations allow for a greater access to glucose and less ATP is used for cholesterol and fatty acid synthesis.

There is a significant amount of research suggesting that octacosanol does have some effect on the female reproductive system. This has been demonstrated in several species. Dukelow (1966b) fed octacosanol extracted from WGO to ewes and cows 3 weeks prior to the beginning of breeding season and continued the treatment until the animals experienced two oestrous cycles. The authors found that ewes treated with octacosanol gave birth to a higher number of lambs than the control group of an equal number of ewes. The same study discovered that cows treated with octacosanol in the same method required fewer services

before achieving conception when compared to control cows. There were 6 cows in the treatment group that had required 3 to 7 services in previous pregnancies. After receiving the octacosanol drench, 5 of them were impregnated on the first service and the sixth cow was successfully impregnated on the second service.

Watson (1936) recorded similar results in women who previously experienced habitual abortions before receiving WGO. In this study, 28 women who had experienced 2 or more abortions began ingesting WGO daily after the onset of pregnancy continuing through birth or termination of the pregnancy. Of these 28 women, only 3 had successfully given birth in a previous pregnancy. After receiving the WGO treatment, 21 of the 28 subjects maintained their pregnancies and gave birth to healthy offspring. The same study also used WGO as a treatment for 15 women who experienced a threatened abortion according to the known symptoms of abortion at that time. Eleven of these pregnancies resulted in a healthy birth after WGO treatment. These studies indicate that octacosanol could have some antisterility effect in females, but the mechanism by which this occurs has not been effected. However, feeding octacosanol to rats has shown an increase in oxygen uptake in body cells (Kabir et al. 1989). An increase in oxygen available to cells in the uterus could produce a healthier uterine environment that is more advantageous to a growing embryo (Dukelow 1966b). If this ability to increase cellular oxygen uptake does exist in octacosanol, and it could exert this effect on the cells in the testicle, it could prove influential to the male's reproductive capacity.

Another possibility is that octacosanol possesses some hormone-like quality or an ability to enhance the function of some hormones related to reproduction (Watson, 1936). The fact that octacosanol can be fed prior to, and during the earliest stages of pregnancy and still exert its effects suggests that the mechanism is active during implantation (Dukelow 1966b). This would support the theory that an improved uterine environment due to increased oxygen uptake is responsible for the improved fertility. However, it is also possible that octacosanol could improve implantation success by acting in a way that mimics or enhances the effects of progesterone. However, a study conducted by Dukelow (1966b) revealed that feeding WGO to female minks caused the subjects to achieve mating faster than control animals. One could speculate that an earlier readiness for mating is an estrogenic effect (Dukelow, 1966b). This provides further support for a relationship between octacosanol and sex hormones. However, this example indicates a potential interaction with gonadotropic hormones rather than a luteal hormone which does not directly support the theory of octacosanol exerting a humoral effect on implantation and pregnancy maintenance.

There is further evidence of octacosanol having an estrogenic effect. One study found that female rats injected with WGO reached sexual maturity earlier than control females (Levin et al. 1951). A separate experiment within the study found that rats injected daily with WGO beginning at 21 days of age had significantly heavier uterine and ovarian weights (Levin et al. 1951). Furthermore, estrus was induced in ovariectomized rats by treating them with WGO through feeding the substance orally and through injection. The uteri of hypophysectomized rats treated with WGO displayed increased hyperplasia in the

myometrium, improved vascularity of the stroma and a greater incidence of progestational proliferation when compared to control females that were also hypophysectomized (Levin et al. 1951). Wheat germ oil also stimulated the formation of corpora hemorrhagica on the ovaries of hypophysectomized rabbits which also implies a gonadotropic effect in that species as well (Levin et al. 1951). The fact that all of these developments occurred even in the absence of the pituitary gland indicates that octacosanol had a gonadotropic effect, apparently acting in same way the hormones that would have been secreted by the pituitary in a normal female would have.

Just as octacosanol has been shown to affect female fertility, there are also examples of octacosanol affecting male fertility. One such study focused on a particular strain of rat in which the majority of males achieved fertility at a normal age but then became sterile at the age of 5 months (Evans, 1925). This sterility was marked by a gradual degradation of the seminiferous epithelium and regression of 8fect cells. Males in the treatment groups received either one drop or three drops daily of the ether extract of WGO which is known to contain octacosanol. The control group received only the base ration and all subjects became sterile by the end of the fifth month. Those subjects treated with one drop of the extract retained their fertility 5 months longer than the control group. At 13 months these animals were found to have normal testes with no apparent degradation of the seminiferous epithelium while the seminiferous epithelium in the control group was found to be absent. The subjects receiving 6 drops of the extract on a daily basis maintained fertility until they were sacrificed at 13 months of age. Their testes were also normal with no deterioration of

the seminiferous epithelium. Furthermore, some males who experienced the onset of sterility were treated with the WGO extract and their fertility was restored. At the onset of sterility, these animals' seminiferous epithelium had been severely deteriorated. However, it appears that the treatment allowed for the retention of some of the tissue allowing for regeneration of the epithelium and the eventual restoration of fertility. The mechanism by which the extract maintained the integrity of the seminiferous epithelium in the treatment groups is not known, but it appears that the extract corrected some deficiency present in the diet used in the study. This could suggest a possible ability of octacosanol to stimulate cellular proliferation in the testes. This is decidedly different than the potential mechanisms discussed in regard to octacosanol's effect on female fertility in that it appears to be more closely associated with nutrition rather than hormone regulation or oxygen uptake. However, there is some evidence of interaction between octacosanol and the endocrine system in males of various species.

Levin et al. (1951) evaluated the androgenic effect of WGO by comparing the differences in weight of the seminal vesicles between orchietomized rats injected with WGO, testosterone propionate, or sesame oil which served as a control. The study found rats injected with WGO had significantly heavier seminal vesicles than those receiving testosterone propionate or the control group. Because development of the seminal vesicles is largely dictated by testosterone secretion, it appears that WGO had a similar androgenic effect as testosterone and, in some subjects, exceeded the effect of testosterone. When the pituitaries of these animals were examined, those taken from the WGO possessed a much lower number of "castration cells" in the anterior lobe when compared to the control group.

The lack of testosterone in the body should have caused an increase in the number of castration cells. This minimization of castration cell proliferation in the brain indicates a possible androgenic quality of WGO. The control group had more castration cells, presumably due to lower testosterone production as a result of the orchidectomy. It appears that the WGO provided a substitute for the testosterone production lost because of the orchidectomy which explains a castration cell population similar to those found in the rats treated with testosterone propionate.

Levin et. Al. (1951) also examined the effect of WGO on chick comb growth. Like seminal vesicle weight, chick comb growth is thought to be directly related to testosterone secretion. The results revealed chicks treated with WGO experienced similar comb growth when compared to chicks treated with testosterone propionate, both of which surpassed the comb growth of the control group. A later study revisited the chick comb observation but used synthetic octacosanol and octacosanol extracted from WGO rather than WGO in its entirety (Levin, 1963). This data showed that the comb growth in response to 0.00036 μg octacosanol was similar to 11,000 times that amount of testosterone propionate indicating a strong androgenic effect (Levin, 1963). The mechanism of octacosanol in this response is not known but the response was less pronounced when observed in a hybrid breed of chicken suggesting a possible genetic influence on the action of octacosanol.

In a preliminary study conducted by Harris et al. (2018), the effects of dietary octacosanol were observed in young ram lambs during the summer period, a time when rams are typically less fertile due to the stress of the heat. The ram lambs were fed octacosanol ad

libitum as a percentage of their base ration. The objectives of this study were, first, to determine the effects of dietary octacosanol on semen quality parameters in ram lambs, and to determine if there was any interaction between treatment and breed of sheep. Semen samples were collected from subjects and were measured and compared using multiple semen quality parameters. The observations were conducted on Rambouillet and Suffolk ram lambs and compared to allow for identification of effects caused by treatment and interaction between breed and treatment if any exists.

Hypothesis: Dietary octacosanol will increase testosterone production and improve semen quality in ram lambs. Octacosanol has been shown to be readily metabolized, distributed, and stored by a number of mammalian species while also having a wide range of effects on various physiological functions. Of particular interest is the fact that there is evidence of the compound exerting estrogenic and androgenic effects. This could conceivably result in an increase in testosterone secretion. Additionally, octacosanol has been shown to have some influence on cellular respiration and proliferation in the male and female reproductive tracts in some species. It could be possible that dietary octacosanol might interact with cells related to semen production in the testes in way that either enhances their performance or increases their population. One or both of these actions might improve semen quality.

The objectives of this study are, first, to determine the effects of dietary octacosanol on semen quality parameters in ram lambs, and to determine if there is any interaction between treatment and breed of sheep. This will be achieved by collecting semen samples

from subjects and measuring and comparing multiple semen quality parameters. These observations will be conducted on Rambouillet and Suffolk ram lambs and compared to allow for identification of effects caused by treatment and interaction between breed and treatment if any exists.

MATERIALS AND METHODS

There was a total of 39 spring-born ram lambs in this study. Twenty-four lambs were of the Rambouillet breed, and fifteen were of the Suffolk breed. Results of this study included preliminary research data pooled from Harris et al. (2018). This was a completely randomized block design where the subjects were stratified by breed. This allowed for the comparison of the Rambouillet's response to that of the Suffolk subjects. Animals within each breed were randomly assigned to a pen of 3 lambs. There were 5 pens containing 3 Suffolk lambs and 8 pens containing 3 Rambouillet lambs. These pens were randomly assigned to either the control group or treatment group. Within the Suffolk breed there was 2 control pens and 3 treatment pens. Within the Rambouillet breed there were 4 control pens and 4 treatment pens. All pens were housed in the same barn. Each pen contained one gravity flow feeder and one automatic water trough. Bunk space and water troughs were equivalent for all pens. The control pens received a base ration. The base ration, RAM20 was developed at Angelo State University (Table 1). The treatment pens received a diet containing the base ration and octacosanol included at a rate of 0.25% of the total ration. This is the inclusion rate recommended by the manufacturer (Purina Animal Nutrition, Gray Summit, MO).

Table 1. Formulated ration for RAM20 developed at Angelo State University.

Feed Name	Amount %
Corn	27.00
Corn Gluten Feed	14.50
Cottonseed Hulls, Dry	26.00
Alfalfa Pellets	26.00
Molasses	4.00
ASU Ram Premix	2.50

All animals underwent a 1-week acclimation period after being assigned to their respective pens. During this time, all animals were fed the base ration ad libitum and sorghum hay ad libitum. Following the acclimation period, on d 0, treatment groups began receiving the treatment ration ad libitum. Animals remained on this diet for the entirety of the study. Control animals continued to be fed the base ration ad libitum for the entirety of the study. Sorghum hay was provided ad libitum to all groups for the duration of the study.

Beginning on d 7 and continuing on a weekly basis until the study's termination on d 56, BW was measured and recorded for all animals. On d 56, following the final BW measurement, semen was collected from all animals via electro-ejaculation. Semen samples were analyzed for ejaculate volume, concentration, motility, progressive motility, morphology, motile sperm concentration (MSC), progressive motile sperm concentration (PMSC), velocity, total number of sperm, total number of motile sperm, and total number of progressive sperm using QwikCheck Gold automated sperm quality analyzer instrumentation (Medical Electronic Systems).

The experimental unit for this study is pen ($n = 12$). All observations were analyzed using the MIXED model procedures of SAS. The model included fixed effects of treatment, breed, and treatment \times breed interaction. Day 0 weights were used as a covariate. Least squares means were separated using the p diff option with $P \leq 0.05$ considered significant.

RESULTS AND DISCUSSION

VOLUME

Treatment of octacosanol showed various differences and trends across the ram semen parameters. The comparison of volume within breeds and among treatments showed a trend that indicates an increase in volume for the Suffolk breed. While not significant, the table below shows the differences between the treatment and control groups which indicate a trend of increased volume, however the differences were not significant ($P > 0.05$), (Table 2). When observing the differences in volume between the Rambouillet and Suffolk breeds, a significant difference, ($P \leq 0.05$) was recorded, which can be seen in Figure 1. Although the differences between breeds have long been explored, the Suffolk breed showed an increase in volume of semen collected with a mean of 1.1400mL while the Rambouillet breed showed a mean volume collection of 0.337mL. When looking at the differences between the treatment group of Rambouillets and the treatment group of Suffolks, a difference of ($P \leq 0.05$) was observed. It is important to recognize that the collection method used was electroejaculation which could serve as a factor influencing the amount of semen volume collected. Further research can be conducted utilizing additional methods of collection if one was particularly interested in investigating the effects on semen volume.

Table 2. Comparison of semen volume (ml) within breeds among treatments. There was no significant difference. (Rambouillet $P = 0.42$ and Suffolk $P = (0.07)$).

Breed	Treatment	Volume (ml)	SEM
Rambouillet	Control	0.19	0.35
Rambouillet	Treatment	0.48	0.35
Suffolk	Control	0.79	0.38
Suffolk	Treatment	1.48	0.38

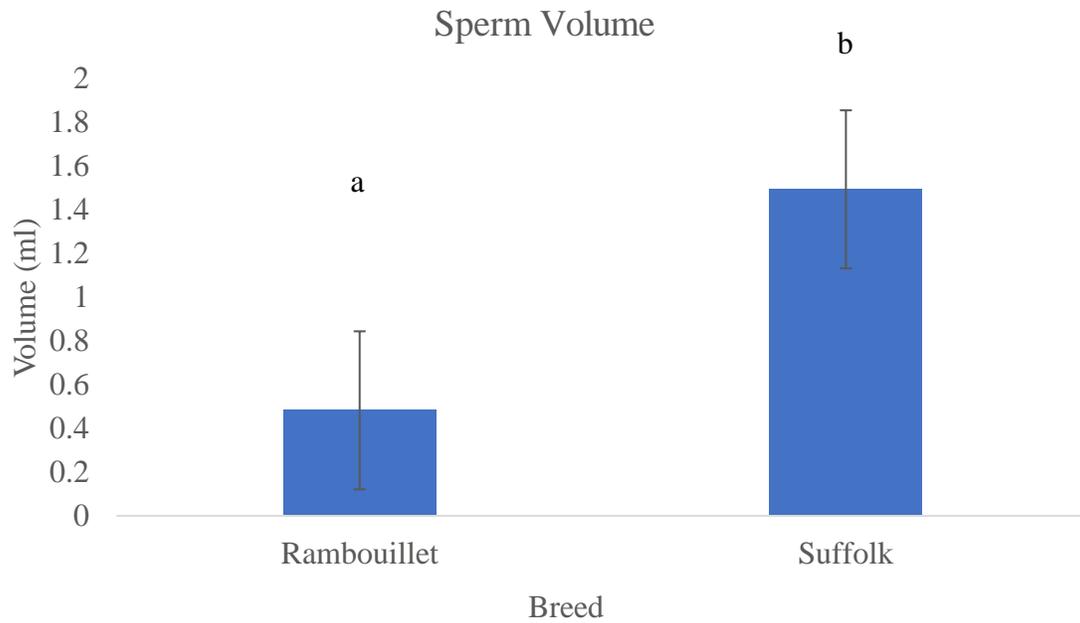


Figure 1. Comparison of volume of sperm (ml) between breeds, Rambouillet and Suffolk. Least squares means with different superscripts differ ($P \leq 0.05$).

CONCENTRATION

Concentration levels between the control and treatment groups were not statistically significant, ($P \geq 0.05$). Though not significant, the treatment groups for both Rambouillets and Suffolks did show an increase in concentration level, demonstrating that octacosanol did have a positive effect on the level of semen concentration. The interaction between the Rambouillet and Suffolk breeds on concentration levels was significantly different ($P \leq 0.05$), with the Rambouillets showing a higher semen concentration level compared to the Suffolks (Figure 2). The comparison and understanding of the Rambouillet breed showing higher semen concentration levels has been long studied and found to be valid in numerous studies. However, the treatment of octacosanol within the Suffolks showed a larger increase in concentration level compared to the treatment Rambouillets. This indicates a possible trend that octacosanol could have which demonstrates a higher potency of octacosanol exerted on the Suffolk breed.

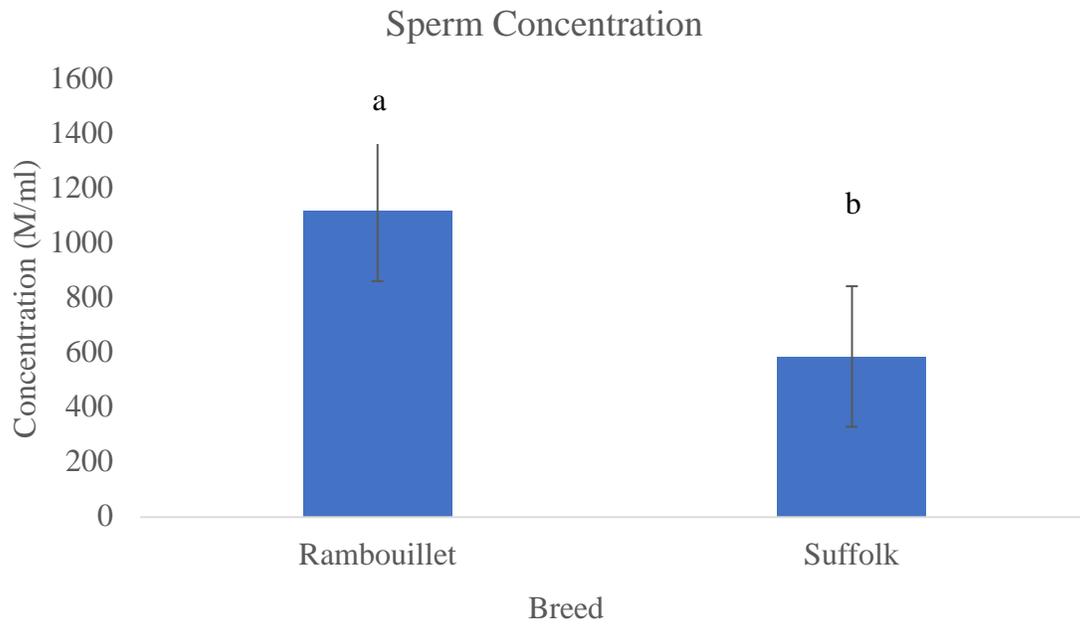


Figure 2. Comparison of sperm concentration between breeds, Rambouillet and Suffolk. Least squares means with different superscripts differ ($P \leq 0.05$).

MOTILITY AND PROGRESSIVE MOTILITY

Motility and progressive motility levels between the control and treatment groups were deemed to not be statistically different, ($P \geq 0.05$). There was no statistical difference between the Rambouillet breed and Suffolk breed and there was no statistical difference between the treatment group of Rambouillets and the treatment group of Suffolks (Table 3).

Table 3: Comparison of percent motility (%) of Rambouillet and Suffolk rams among treatments. There was no treatment effect among breeds. (Rambouillet $P = 0.40$, Suffolk $P = 0.23$).

Breed	Treatment	Percent Motility (%)	SEM
Rambouillet	Control	13.93	12.51
Rambouillet	Treatment	3.34	12.51
Suffolk	Control	-8.39	13.67
Suffolk	Treatment	8.42	13.67

TOTAL MOTILE SPERM

Though not statistically different, the treatment groups compared to the control groups showed a trend of increased total motile sperm count, ($P \leq 0.1$). There was no statistical difference between breeds or treatment of breeds.

MORPHOLOGY

There was no statistical difference in morphology when comparing the treatment and control groups. Likewise, there was no statistical difference between the Rambouillet and Suffolk breeds on morphology, nor was there a statistical difference between the Rambouillet treatment groups and Suffolk treatment groups.

MSC(motile sperm concentration), PMSC (progressive motile sperm count), VELOCITY

No statistical difference was observed when comparing the control and treatment groups on msc, pmsc, and velocity. Similarly, there was no statistical difference observed between the Rambouillet and Suffolk breeds on msc, pmsc and velocity, nor was there a statistical difference between the Rambouillet treatment groups and Suffolk treatment groups.

TOTAL SPERM

When analyzing the total sperm count, a treatment effect is observed ($P \leq 0.05$) between the treatment groups and the control groups for the Suffolk breed. This significant difference showed octacosanol to have an effect on the total number of sperm present in a

treatment sample for the Suffolk breed (Table 4). It is believed that this increase in total number of sperm is correlated with the increase in volume for the Suffolk breed. While not statistically significant, there is also an increase of total sperm count between the Rambouillet and Suffolk breeds with the Rambouillets showing a mean of 2.0139 and the Suffolks showing a mean of 1.7424. The interaction between treatment Rambouillets and treatment Suffolks were not deemed significantly different, ($P \geq 0.05$).

Table 4. Comparison of total number of sperm (Bil) of Rambouillet and Suffolk rams among treatments. Means with different superscripts differ ($P \leq 0.05$).

Breed	Treatment	Total Sperm (Bil)	SEM
Rambouillet	Control	1.79	0.58
Rambouillet	Treatment	2.23	0.58
Suffolk	Control ^a	0.98 ^a	0.64
Suffolk	Treatment ^b	2.50 ^b	0.64

Least squares means with different superscripts differ ($P \leq 0.05$).

CONCLUSIONS

Although not significant, the increase in total number of sperm and volume for the Suffolk breed provides a glimpse into the enhanced reproductive efficiency potential that octacosanol could deliver to the crossbred commercial industry specifically. A greater number of total sperm per ejaculate directly correlates to an improved semen sample. A larger amount of volume per sample for Suffolks, presumably due to the increased total amount of sperm, translates to better overall semen quality for the Suffolks treated with octacosanol. The added element that octacosanol did not have a significant effect on the morphology, motility, and velocity indicate that there is not an issue with octacosanol negatively harming the shape, movement, or speed of the individual sperm in each sample. It is a good indicator that those with increased sperm count and added volume while simultaneously maintaining adequate morphology, motility, and velocity are more fertile.

For Suffolk or crossbred producers, this could serve as a viable solution for providing fertility improvements to the rams chosen to breed. Because this study was conducted throughout the heat of the Texas summer, a time when rams are known to show lower fertility due to the stress of the heat, it can be said that Suffolk or crossbred producers who have traditionally struggled to maintain fertility in their rams during out-of-season breeding will have a greater chance of success if they add octacosanol to their rams diet during the breeding period.

When trying to understand the significance of the effects on the Suffolks vs. the Rambouillets, further questions need to be explored in distinguishing between the two breeds. Tracing back the roots and origins of the breeds could prove useful in these efforts towards discovering the reasoning behind the contrasting level of effectiveness between the Rambouillets and Suffolks. The Suffolk breed may have more room for fertility improvements compared to the Rambouillets simply due to the Suffolk's origination and adaptation to the objectives given to them. The Rambouillet breed was developed from the Spanish Merino, one of the most prolific breeds, whereas the Suffolk breed was the result of crossing Southdown rams on Northfolk Horned ewes. This may serve as an opportunity for one to retrace how much improvement prospective that the Suffolk breed has the ability to make due to their origination. Studies could be devoted to identifying the difference in heat tolerance for the purpose of determining which breed had a more difficult time maintaining all normal body functions during the summer period. One might collect fecal egg counts to analyze the different parasite loads the two breeds are carrying.

LITERATURE CITED

- Dukelow, W. R. 1966a. Effects of age and strain of female, and of ethylene dichloride extracted wheat germ oil on reproduction in mink. *Reproduction* 11:181–184.
- Dukelow, W. R. 1966b. Effects of extracted wheat germ oil in sheep and cattle. *Reproduction* 11:465–467.
- Evans, H. M. 1925. Invariable Occurrence of Male Sterility with Dietaries Lacking Fat Soluble Vitamine E. *Proceedings of the National Academy of Sciences* 11:373–377.
- Harris, C., Runyan, C., and Dickison, J. 2018. The effects of dietary octacosanol on ram semen quality parameters. *Journal of Animal Science*. 96. 1093:69-70
- Kabir, Y., M. Kawamura, T. Ido, M. Nakata, and S. Kimura. 1989. Biodistribution of ¹⁴C octacosanol in rats after serial doses administration. *Cyclotron and Radioisotope Center Annual Report* 1:140-146.
- Kabir, Y. and S. Kimura. 1994. Distribution of radioactive octacosanol in response to exercise in rates. *Die Nahrung* 38:373-377.
- Levin, E., J. F. Burns, and V. K. Collins. 1951. Estrogenic, androgenic and gonadotrophic activity in wheat germ oil. *Endocrinology* 49:289–301.
- Levin, E. 1963. Effects of Octacosanol on Chick Comb Growth. *Experimental Biology and Medicine* 112:331–334.
- Long, L., W. Shugeng, J. Sun, J. Wang, H. Zhang, and G. Qi. 2015. Effects of octacosanol extracted from rice bran on blood hormone levels and gene expressions of glucose

transporter protein-4 and adenosine monophosphate protein kinase in weaning piglets.
Animal Nutrition 1:293-298.

Long, L., S. G. Wu, F. Yuan, J. Wang, H. J. Zhang, and G. H. Qi. 2016. Effects of Dietary Octacosanol on Growth Performance, Carcass Characteristics and Meat Quality of Broiler Chicks. *Asian-Australasian Journal of Animal Sciences* 29:1470–1476.

Watson, E. M. 1936. Clinical experiences with wheat germ oil (vitamin E). *Canadian Medical Association Journal* 2:134-140.

APPENDIX



ANGELOSTATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

December 19, 2019

Dr. Will Dickison, Associate Professor
Department of Agriculture
Angelo State University
San Angelo, TX 76909

Your proposed project titled, "Effects of Octacosanol on the development of young Rambouillet and Suffolk Ram lambs" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective December 19, 2019 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 2019-109. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

A handwritten signature in black ink that reads "Chase Runyan". The signature is written in a cursive style and is positioned above the typed name and title.

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

VITA

My name is Mikhaila A. Barnett. I graduated high school in Lometa, Texas in 2015, however my family has deep roots imbedded in Mills County where I learned and developed my passion for agriculture. Education, specifically in the field of agriculture, dates back 4 generations in my family. It is safe to say that I was destined to thrive in the educational field not only through continually educating myself, but those who share in my passion as well. Following the completion of my Agricultural Science and Leadership undergraduate degree at Angelo State University in 2018, I accepted a position at Texas A&M AgriLife Extension where I serve as the Youth Sheep and Goat Associate. There I act as a liaison for developing educational programs and workshops geared towards educating our youth about agriculture, specifically the sheep and goat industry. I also serve as an associate for the Texas Sheep and Goat Validation Program, a system dedicated to providing youth opportunities to become engaged and involved in the Texas Youth Livestock Program across the state of Texas. Promoting agriculture and fostering opportunities for others to relish in the joys of our industry is something that I take great pride in.