Effects of different applications of pyrethrins and cyfluthrin, a synthetic pyrethroid, on bull reproductive parameters[*]

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Abstract

The use of pyrethroid insecticides has been reported to affect semen quality and steroidogenesis in many mammals. Recent experiments with bulls demonstrated no effects on semen quality when the pyrethroid insecticide, cyfluthrin, was applied at labeled dosages. The current study determined effects of cyfluthrin and pyrethrin spray products, used in combination with cyfluthrin pour-on and fly tags, on bull sperm motility and serum testosterone concentrations. Angus x Simmental bulls were assigned randomly to one of two treatment groups: 1) pour-on plus fly tags (CONT; n = 12), or 2) pour-on, fly tags, premise spray plus fog spray (EXP; n = 11). Overall and progressive sperm motility and serum testosterone concentrations were measured weekly for nine weeks. There were treatment by week interactions ($P < 0.01$) for overall motility, progressive motility, and testosterone concentrations. At week two, overall motility was greater ($P = 0.05$) for CONT than EXP; however, there were no differences ($P \geq 0.12$) at other times. Progressive sperm motility in EXP tended to be reduced ($P = 0.07$) at week two and increased ($P = 0.08$) at week seven, but did not differ ($P \geq 0.15$) in other weeks. Serum testosterone concentrations were reduced ($P = 0.05$) at week one in EXP; however, concentrations did not differ again until week nine when EXP bulls had a two-fold decrease ($P < 0.01$) in serum testosterone concentrations when compared to CONT bulls. This steep decline suggests a delayed effect of the insecticide applications on blood testosterone concentrations.

Keywords: Bull, fertility, insecticide, pyrethroid, reproduction

Introduction

Pest control is a crucial management strategy for cattle production facilities. Insect control is needed because ectoparasite infestations can cause economic losses in affected cattle. Production losses include poor performance of the animal, reduced value of the hides as a result of irritation, and transmission of a variety of diseases.[1] The severity of these effects is strongly correlated with environmental conditions, with the summer months being the most devastating.[1]

Pyrethroid insecticides have been implicated as a cause for depression of sperm motility in bulls.[2] These claims were based on clinical observations by Volkmann,[3] who reported a correlation between pyrethroid insecticide use and poor semen quality of bulls and rams. Negative effects were evident within a few days after the first exposure to the insecticides, improved approximately two to four weeks after its use was discontinued, and included a variety of applications, such as pour-on, premise sprays, and fogging.[3] Dosage or frequency of application was not reported.[3] Pyrethroid insecticides have been reported to negatively affect semen quality and steroid hormone production *in vivo* in many species such as man,[4,5] dwarf goats,[6] rats,[7] and rabbits.[8] Semen quality and motility were hypothesized to be affected because pyrethroids are antiandrogenic endocrine disruptors *in vitro*.[9-11] More recently, French et al[12]

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found that commercial cyfluthrin (1%) products administered topically to bulls according to label directions (24 mL; or 0.3 mg/kg) had no effect on sperm motility, morphology, or peripheral testosterone concentrations when compared with bulls receiving no insecticide. Similarly, Cain et al[13] found that topical applications of permethrin (1%) at 150% label dose (225 mL; or 3 mg/kg) did not affect the proportion of normal sperm cells in bulls. The contradictory results among these studies[12,13] and other reports[3] could be due to the route, dosage, and/or frequency of administration. Topical/pour-on and fly tag applications at labeled dosages are intended to provide insecticidal properties without allowing absorption of these compounds; whereas in many of the reports in which detrimental results were observed,[7,8] large, oral dosages (≥ 10 mg/kg) of compounds were administered.

Approved routes for direct administration of pyrethroids include pour-on (topicals) and/or fly tags. Approved routes for indirect administration include premise sprays and foggers. Misuse of these products, by more frequent or more concentrated applications than labeled, creates opportunities for inducing detrimental effects. As these insecticides must be diluted with water and then sprayed throughout the barn (premise) or over the entire animal (fogging), administering the correct dosage is reliant upon the accuracy of the applicator. Improper dilutions or over-spraying could lead to inhalation or ingestion of large concentrations of pyrethroid compounds by the animals. These potential misuses of pyrethroid products could account for reported deleterious effects on reproductive parameters in bulls;[3] however, controlled studies on the effects of spray applications have not been performed. Therefore, the objectives of this study were to determine the effects of pyrethroid and pyrethrin sprays used in combination with pour-on and fly tags on bull sperm motility and serum testosterone concentrations. Our hypothesis was that the addition of pyrethrin and pyrethroid spray applications would have a negative effect on bull reproductive parameters.

Materials and methods

Animals

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Angus x Simmental crossbred bulls (n = 23) ranging from one to six years in age (average initial BW = 796 ± 160 kg) were used in this study and housed at the Dixon Springs Agricultural Center in Simpson, IL. Seventeen of the bulls were owned by the University of Illinois and six of the bulls were privately owned and leased for the purposes of this study. Due to the need to isolate the leased bulls from the university-owned bulls, bulls were blocked by source and assigned randomly to one of two treatment groups: 1) pour-on plus fly tags (CONT, n = 12), or 2) pour-on, fly tags, premise spray plus fog spray (EXP, n = 11). The six leased bulls were housed in one barn (three per treatment group), and the seventeen university-owned bulls were housed in a second barn (eight or nine per treatment group). Within each barn, treatment groups were separated by aisles to avoid cross-contamination of spray products.

Pyrethrin/pyrethroid insecticide treatments

Each bull in both the CONT and EXP groups was treated with 24 mL cyfluthrin 1% solution (average dose 0.3 mg cyfluthrin/kg BW; CyLence®, Bayer Animal Health, Shawnee Mission, KS; active ingredient cyfluthrin; 1%) poured along the topline at weeks zero and four, and each bull received one cyfluthrin-impregnated fly tag in each ear (Cylence® Ultra fly tags, Bayer Animal Health, Shawnee Mission, KS; active ingredients beta-cyfluthrin; 8% and piperonyl butoxide; 20%) at week zero. Additionally, the EXP bulls were fogged daily with a pyrethrin spray (Prozap® LD-44Z, Loveland Industries, Inc., Greeley, CO; active ingredients pyrethrins; 0.5% and piperonyl butoxide; 4%) at approximately 3 seconds per bull, and their stalls were treated with a beta-cyfluthrin premise spray (Tempo®, Bayer Animal Health, Shawnee Mission, KS; active ingredients beta-cyfluthrin, cyano and methyl 3; 11.8%) at 16 mL/1000 sq. ft. once weekly. All products were used according to label directions as approved for use in food-producing animals.

Sample collection

The experiment was performed over nine weeks from May through July. A complete health and reproductive examination was performed before applying treatments at week zero and upon conclusion of the study at week nine. Individual body weights were obtained and each bull was assigned a body condition score (BCS) of one to nine. Scrotal circumference was measured using a scrotal tape. Tone and symmetry were assessed in the testicles and epididymides. Transrectal palpation was performed to assess the accessory sex glands. During semen collection the penis was examined for any abnormalities.

Samples were collected once weekly beginning at week zero (prior to receiving treatments), at similar times of day to account for daily hormonal fluctuations. To obtain semen samples, an electroejaculator was placed rectally and the programmed cycle was allowed to run. At least 3 mL of semen were collected from each bull before the program concluded and the electroejaculator probe removed. Semen was collected into 15 mL conical tubes using collecting handles and sleeves. Immediately after collection, tubes were transported in a warm water (37°C) bath to the laboratory. Semen was diluted 1:60 in warm saline (37°C) and 2 µL of diluted semen was placed into 20 µM chamber slides (Vitrolife, Microcell Counting Chambers, San Diego, CA). Computer-Assisted Sperm Analysis (CASA) equipment (Spermvision, MiniTube of America, Inc., Verona, WI) was used to obtain overall and progressive motility by averaging seven readings from various portions of the chambered slide.

Whole blood, as a source of serum for testosterone analysis, was obtained via the tail vein once per week immediately following electroejaculation. Samples were obtained with 1 to 1.5 inch, 18 to 20 gauge needles into serum separating evacuated tubes. Blood samples were centrifuged for ten minutes at 1200 rpm, serum was removed with a sterile pipette, and the sample was frozen at -20°C. Testosterone concentrations were determined by double-antibody radioimmunoassay (Coat-A-Count®; Diagnostics Products Corporation, Los Angeles, CA) at the Animal Health Diagnostic Center, Cornell University, Ithaca, NY.

Statistical analysis

All data were analyzed in MIXED procedure of SAS as a randomized complete block. Repeated measures, with compound symmetry covariance structure, were used to test the treatment by week interactions for reproductive parameters. The effect of block (barn) was tested in the model and found not to be significant. Therefore, it was removed from the model. Significance was declared at $P \leq 0.05$ and trends were discussed at $0.05 < P \leq 0.10$.

Results

Physical examination

Bull weight and BCS were not different at the end of the experimental period ($P \geq 0.82$; Table). Bull BCS remained adequate with an average score of 5.4 in both EXP and CONT groups. Subjectively, there were no changes on physical examination, and no differences were observed in initial or final scrotal circumferences between CONT and EXP groups ($P \geq 0.22$; Table).

Reproductive parameters

There were treatment by week interactions ($P < 0.01$) for overall motility (Fig. 1), progressive motility (Fig. 2), and serum testosterone concentrations (Fig. 3). At week two, overall motility was greater ($P = 0.05$) for CONT than EXP; however, there were no differences ($P \geq 0.12$) in overall motility at other times (Fig. 1). Progressive sperm motility in EXP tended to be reduced ($P = 0.07$) at week two and increased ($P = 0.08$) at week seven compared to CONT, but did not differ ($P \geq 0.15$) in other weeks (Fig. 2). Serum testosterone concentrations were decreased ($P = 0.05$) by week one in EXP bulls when compared to CONT; however, concentrations did not differ again until week nine when EXP bulls had a two-fold decrease ($P < 0.01$) in serum testosterone concentrations when compared to CONT bulls (Fig. 3).

Discussion

The use of pyrethroid insecticides on breeding bulls has recently become a controversial topic in the cattle industry due to reports of negative effects on semen quality in other mammals.[4-8] The route of exposure may be a crucial determinant in the manifestation of negative effects.[15] Dermal absorption of pyrethroids across intact skin was reported as very poor,[15,16] and pour-on applications are considered safe for breeding cattle when administered at labeled dosages.[12,13] Concentrations of pyrethroids in body fluids were not directly measured in our previous study,[12] nor were they quantified here; however, no effects were observed on serum testosterone or semen quality in bulls receiving both pour-on and fly tag applications.[12] Therefore, if dermal absorption of pyrethroid compounds did occur, it did not result in deleterious effects on the reproductive parameters measured.[12, 13]

In contrast to transdermal absorption, pyrethroid insecticides are easily absorbed through the gastrointestinal tract and pulmonary membranes,[15] suggesting that routes of administration such as sprays may impart harmful effects on semen quality not observed with dermal applications. In the current study, weekly premise sprays with beta-cyfluthrin were applied while the EXP bulls were housed in the barns. The application likely contaminated feed and surfaces to which bulls were exposed, allowing for potential oral ingestion. The EXP bulls also received a daily pyrethrin fogging application, which presented opportunity for inhalation and oral exposures. Spray applications are consistent with those reported by Volkmann[3] that correlated with the development of poor quality semen in bulls, including increased secondary sperm defects and diminished progressive motility. In one case,[3] Volkmann reported diminished semen quality in numerous ejaculates collected from a bull stud two days after application of a pyrethroid premise spray, and bifenthrin was detected both in serum and semen in two of the exposed bulls.

In the current study, overall motility of both CONT and EXP groups did not decline below 70% and progressive motility did not decline below 50% at any week, both of which exceed the minimum recommendations set by the Society for Theriogenology for classification as satisfactory potential breeders (> 30% for both progressive and overall motility).[14] Although a reduction in overall and progressive motility was observed in EXP bulls at week two in the present study, the percentages were still well above acceptable minimums and did not persist in following weeks, with progressive motility being greater in EXP bulls at week seven. Since this treatment effect was on the threshold of significance ($P = 0.05$), a larger sample size may be warranted to confirm its importance. Additionally, scrotal circumference of CONT and EXP bulls did not differ and were also above the minimum recommended scrotal circumference set by the Society for Theriogenology for satisfactory potential breeders (≥ 34 cm).[14] Information regarding the amount of bifenthrin contained in the premise spray used in the case reported by Volkmann[3] is not available. The potentially higher dosage or the difference between pyrethroid products used (bifenthrin versus cyfluthrin/pyrethrin) in that report[3] may account for contradictory results on semen motility reported here. Further controlled studies would be needed to explore the effects of higher dosages or different pyrethroid products on bull reproductive parameters.

The most striking finding in the current study was the significant decline in serum testosterone concentrations of EXP bulls at week nine. This suggests that chronic exposure to cyfluthrin, beta-cyfluthrin, and/or pyrethrin may decrease testosterone production which would be consistent with pyrethroid effects observed in men,[4] rabbits,[8] and rats.[17] While Zhang[11] observed rats treated with oral cyfluthrin and beta-cyfluthrin had serum testosterone levels which did not differ from controls, antiandrogenic properties were demonstrated. More in-depth toxicological studies are needed to explore these findings. The specific compound of pyrethroid, dose, and route of administration may each play a role.

There are several hypotheses regarding how pyrethroid insecticides may affect steroidogenesis. Cholesterol content in the testes, a necessary precursor for testosterone production, was reported to be significantly reduced in cyfluthrin-treated mice,[18] which could inhibit steroidogenesis and cause a drastic decline in peripheral testosterone concentrations. During metabolism of pyrethroids, reactive oxygen species (ROS) are generated, leading to oxidative stress.[19,20] While the body is able to compensate for low-levels of ROS produced naturally, an overproduction could have damaging effects on cell membranes in the testes.[21] Histological examination in mice given oral cyfluthrin showed various structural abnormalities in the testes,[18] indicative of cell damage. Oxidative damage to testosterone-producing Leydig cells in the testes could result in a decline in peripheral testosterone levels. A decline in peripheral blood testosterone could impact steroidal feedback to the hypothalamus and cause increased production and secretion of luteinizing hormone from the anterior pituitary, as was demonstrated in both men[4] and mice.[22] The effects of pyrethroid insecticides on steroidogenesis in beef bulls have not been reported.

We hypothesized that semen motility and testosterone concentrations would be affected by spray applications allowing for potential oral and/or inhalation exposures. However, testosterone concentrations did not decline until week nine and sperm motility was not adversely affected in the EXP bulls. If a toxic level of insecticide were to accumulate over time, the decline in serum testosterone at week nine could be early signs of effects on steroidogenesis. If oxidative damages had occurred, they would likely affect both Leydig and Sertoli cells. Sertoli cells are responsible for supporting developing germ cells; therefore, we would expect sperm motility to deteriorate following a decline in serum testosterone. Zhang et al[22] found that mice treated with oral *cis*-permethrin had decreased testosterone production due to mitochondrial damage to Leydig cells, causing a dose-dependent negative effect on sperm motility. A decline in testosterone concentrations, as seen in the current study, could subsequently alter spermatogenesis and semen motility. Since the current study ended after one spermatogenic cycle, while decreases in testosterone were not detected until the final week, it is not known if sperm quality would be affected at some later time. Additional studies examining sperm motility and morphology at later time points following a decline in testosterone are therefore warranted.

Conclusion

The current study examined the effects of commercially available pyrethroid (cyfluthrin and beta-cyfluthrin) and pyrethrin insecticide applications on bull reproductive parameters over one spermatogenic cycle (~60 days). These results showed no consistent deleterious effects on overall or progressive sperm motility in bulls which received fly tags, pour-on, daily fogging, and weekly premise spray applications (EXP) compared with those which received only fly tags and pour-on products (CONT). Testosterone concentrations were significantly reduced in EXP bulls when compared with CONT bulls at week nine, suggesting a delayed effect of the beta-cyfluthrin and pyrethrin spray applications on testosterone production. While these results conclude that these spray applications do not adversely affect sperm motility in the short-term (nine weeks), additional studies are needed to determine the long-term effects of these application methods on semen quality.

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Table. Effects of cyfluthrin and pyrethrin insecticides on bull BW, BCS, and scrotal circumference.

		Treatment			
Body Weight (kg)		CONT[a]	EXP[b]	SEM	***P*-value**
	Week 0	886	870	33	0.72
	Week 9	881	872	34	0.84
BCS[c]					
	Week 9	5.4	5.4	0.17	0.82
Scrotal Circumference (cm)					
	Week 0	41.11	42.28	0.71	0.22
	Week 9	41.71	41.61	0.73	0.92

[a]CONT: pour-on, fly tags
[b]EXP: pour-on, fly tags, weekly premise spray, daily fogging
[c]BCS scores ranged from 1 to 9

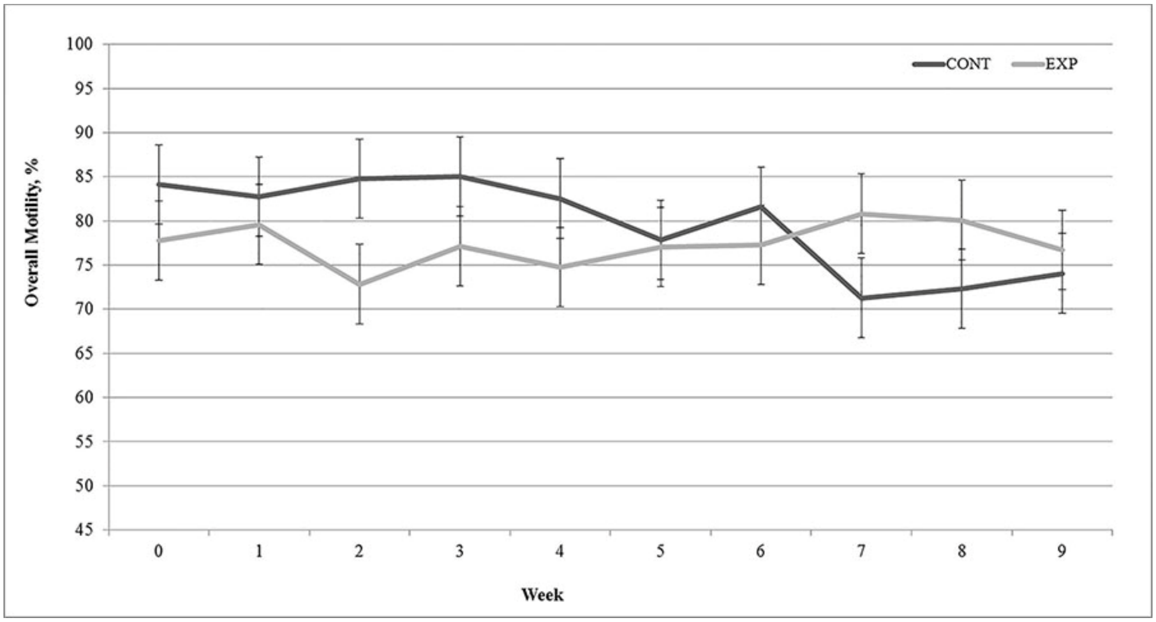

Figure 1. Effects of cyfluthrin and pyrethrin insecticides on overall sperm motility (%) throughout nine weeks (one spermatogenic cycle) in bulls. CONT=pour-on, fly tags; EXP=pour-on, fly tags, weekly premise spray, daily fogging

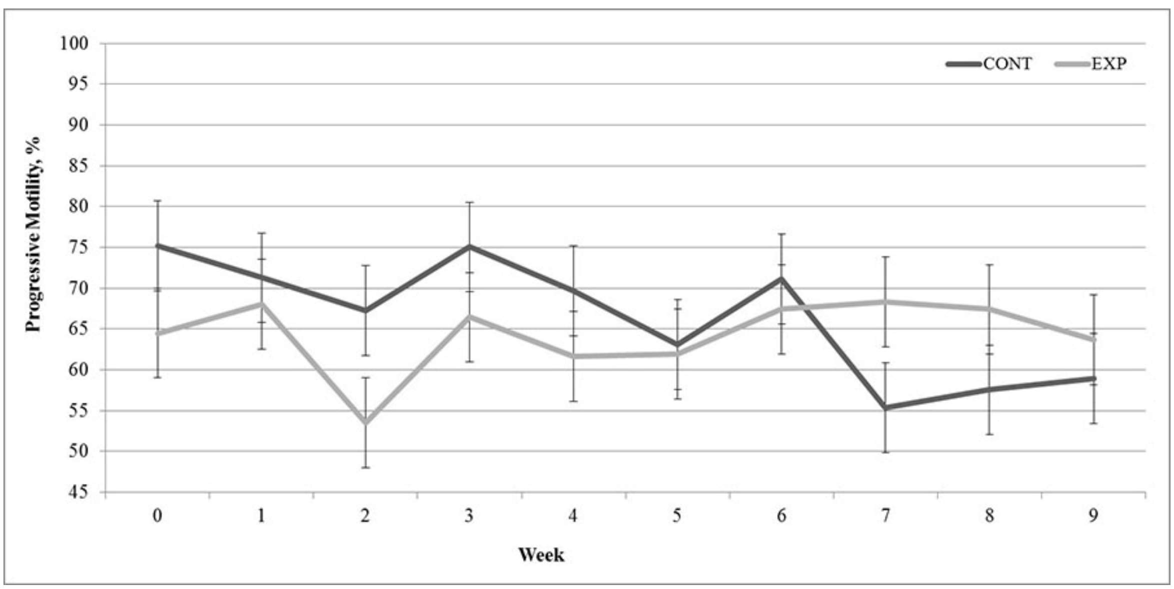

Figure 2. Effects of cyfluthrin and pyrethrin insecticides on progressive sperm motility (%) throughout nine weeks (one spermatogenic cycle) in bulls. CONT=pour-on, fly tags; EXP=pour-on, fly tags, weekly premise spray, daily fogging

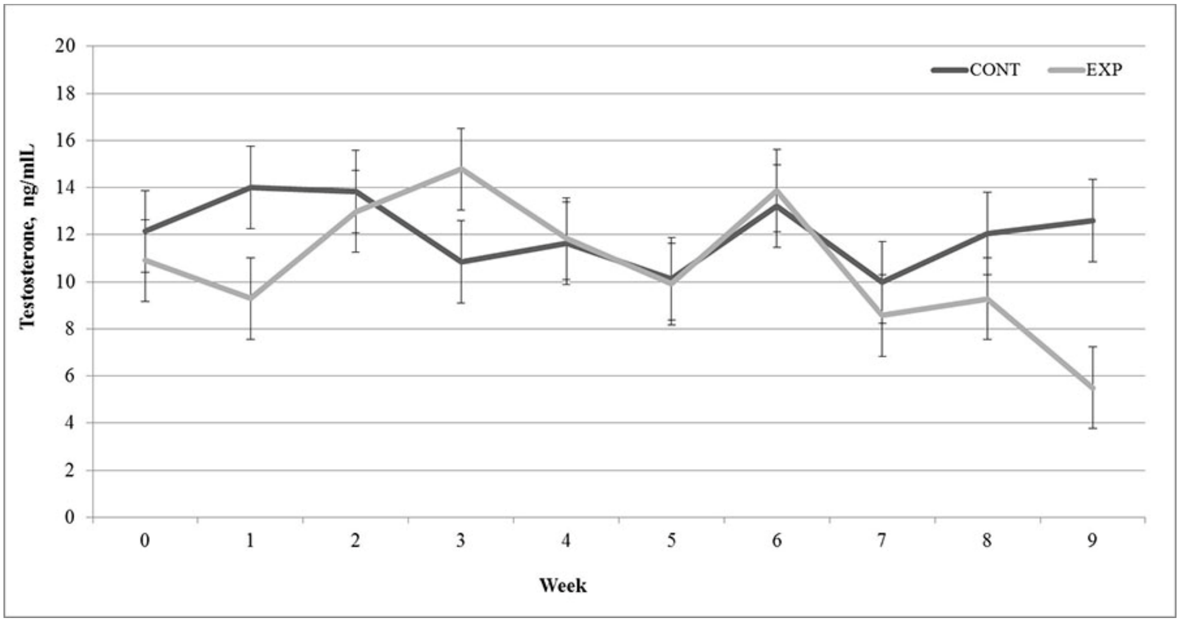

Figure 3. Effects of cyfluthrin and pyrethrin insecticides on serum testosterone concentrations (ng/mL) throughout nine weeks (one spermatogenic cycle) in bulls. CONT=pour-on, fly tags; EXP=pour-on, fly tags, weekly premise spray, daily fogging