THE EFFECT OF A SLOW-RELEASE FORMULATION OF ZUCLOPENTHIXOL ACETATE (ACUNIL®) ON CAPTIVE BLUE WILDEBEEST (*CONNOCHAETES TAURINUS*) BEHAVIOR AND PHYSIOLOGICAL RESPONSE

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THE EFFECT OF A SLOW-RELEASE FORMULATION OF ZUCLOPENTHIXOL ACETATE (ACUNIL®) ON CAPTIVE BLUE WILDEBEEST (*CONNOCHAETES TAURINUS*) BEHAVIOR AND PHYSIOLOGICAL RESPONSE

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Abstract: The study investigated the effect of a slow-release formulation of zuclopenthixol acetate (Acunil®) on blue wildebeest (*Connochaetes taurinus*) in captivity. Two groups of trials were conducted using either Acunil or a placebo (control). Animals (Acunil: $n = 17$; placebo: $n = 12$) were observed for a 12-hr period before the administration of Acunil or the placebo (pretreatment). After 24 hr, animals were administered Acunil (1.5 mg/kg) or a placebo (1.0–3.0 ml of sterile water) and observed again for 12 hr (posttreatment). During both treatments, animals were stimulated every 2 hr for 1 min by a person entering the enclosure (referred to as periods of stimulation). Behavioral observations and continuous heart rate, respiration rate, and motion measurements were taken throughout. Animals treated with Acunil spent more time lying with their heads folded back, eating and standing with their heads down, and less time being vigilant and exploring while walking around. Animals treated with the placebo also spent less time being vigilant and more time lying with heads up. Animals treated with Acunil groomed less while standing and performed less head shaking; no such changes were observed in the control group. Neither Acunil nor the placebo had any effect ($P > 0.05$) on heart rate. However, overall mean respiration rate was lowered ($P = 0.02$) when animals were treated with Acunil (pretreatment: 14.5 ± 0.82 breaths/min; posttreatment: 12.5 ± 0.83 breaths/min). Acunil also caused a lowered ($P < 0.05$) respiration rate during periods when animals were stimulated (pretreatment: 16.2 ± 0.87 breaths/min; posttreatment: 13.7 ± 0.87 breaths/min) and when animals were trotting and being vigilant. No such changes were observed with the placebo. Both placebo- and Acunil-treated animals spent more time being stationary during periods of stimulation. However, Acunil-treated animals also spent less time moving fast when they were stimulated.

Key words: Behavior, blue wildebeest, heart rate, long-acting neuroleptic, respiration rate.

INTRODUCTION

Over 300,000 animals are translocated across South Africa annually.[10] During these procedures, the use of long-acting neuroleptics (LANs) has become increasingly popular since they can induce longer-lasting sedation that can reduce animal anxiety, especially when animals are to be held in captivity for periods exceeding 24 hr.

LANs were initially developed for the treatment of psychoses in noncompliant psychiatric patients.[14] They consist of a fatty acid ester of the active drug ingredient dissolved in vegetable or medicinal oil. Injected intramuscularly, this formulation forms a depot at the injection site. With the slow breakdown of the oil solvent, the ester diffuses out of the depot and once absorbed into the blood, is hydrolyzed and the active ingredient is able to exert its effect.[13,29,33] LANs act primarily as antipsychotics by blocking dopamine receptors in the limbic system and they have no specific pharmacological antidotes.[29]

The long-lasting effect of LANs after a single administration has greatly improved the outcome of wildlife translocations, specifically by decreasing stress-related mortalities.[11,14] Although their contribution to the successful translocation of wildlife is indisputable, their specific effects on physiological and behavioral variables needs further investigation.[14,33] This is particularly true for southern African wildlife species, for which research is limited.

In South Africa, Clopixol-Acuphase® (zuclopenthixol acetate) is one of the most commonly used LANs in wildlife. Its absorption and duration has been extended through esterification with the acetate and its dissolution in vegetable oil.[35] A new LAN, Acunil® (Wildlife Pharmaceutical SA [Pty] Ltd., Rocky's Drift, Mpumalanga, South

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Africa), is, however, currently being developed with the aim of producing a more consistent slow release. This is achieved by the exclusion of the vegetable oil vehicle and instead, the dissolution of the fatty acid ester of zuclopenthixol acetate in a 72-hr slow-release polymer. This polymer has been used in other slow-release drug formulations (for example, buprenorphine), and has been found to result in maximum serum concentrations of the active drug within 6 hr after its administration. In addition, plasma concentrations of the active drug are maintained above therapeutic levels for up to 72 hr and peak drug effects are seen at 4 hr after treatment.[3,6,15,17,31,32] It is expected that a similar release profile will be observed with Acunil. Although the complete pharmacokinetics of Acunil are not described here, further work is underway to determine this. In addition, this study forms part of a larger project in which the clinical effects of Acunil are compared with those of Acuphase. The aim of this study was to investigate clinical effects of Acunil in blue wildebeest (*Connochaetes taurinus*). Concurrently, a placebo study was performed to serve as a control.

MATERIALS AND METHODS

Animal ethics

Ethical approval was received for all aspects of this project from the Research Ethics Committee: Animal Care and Use at the University of Stellenbosch, South Africa (protocol ethical approval number SU-ACUM11-00005).

Animals and study area

The study conducted two sets of trials using blue wildebeest. In the first treatment group animals received Acunil (50 mg/ml; 1.5 mg/kg intramuscular injection), whereas the second treatment group (control) received a placebo consisting of sterile water (2.0–3.5-ml intramuscular injection) (Table 1). Treatments were allocated at random to each group. Animals were sourced from different locations around Mpumalanga, South Africa. Animals were translocated by a professional game capture team from Wildlifevets.com. Thiafentanil oxalate (Thianil®, Wildlife Pharmaceuticals) was used at 0.01–0.02 mg/kg to anesthetize animals for transportation. A short-acting neuroleptic, azaperone tartrate (Wildlife Pharmaceuticals) at up to 0.3 mg/kg body weight, was used during the relocation of animals. All drugs used were administered by a registered veterinarian.

Table 1. Animal numbers and genders within each trial. Animals in trials 1 to 3 received treatment with Acunil®, whereas animals in trials 4 and 5 received treatment with a placebo.

Trial	Treatment administered	Number of animals	Genders
Trial 1	Acunil	6	3♀, 3♂
Trial 2	Acunil	5	3♀, 2♂
Trial 3	Acunil	6	2♀, 4♂
Trial 4	Placebo	6	3♀, 3♂
Trial 5	Placebo	6	3♀, 3♂

The study site was located on Ngongoni Farm (25°31′25.2″S, 31°06′50.8″E), outside Nelspruit, Mpumalanga, South Africa. Once at the study site, animals were off-loaded into an enclosure (6 × 8 m), constructed as per industry guidelines[29] and left for a minimum of 24 hr before the trial commenced. This was done to ensure that there was no residual tranquilization effect from the relocation of the animals. Azaperone is eliminated from the blood within 0.5–2.5 hr because of its rapid and extensive metabolism by the liver and therefore no residual effect was expected after 24 hr.[20] Animals were given Lucerne hay and clean water ad libitum throughout the trials.

Equipment

The enclosure was fitted with two opposing infrared closed-circuit television cameras (Nictec Radio Communications, Nelspruit, South Africa) to record and analyze behavior using Noldus Observer® XT11 behavioral software (Noldus Information Technology, Wageningen, The Netherlands).

Heart rate (beats/min) and respiration rate (breaths/min) as well as motion via a triaxis accelerometer (stationary, moving slowly, or moving fast) were measured using a modified version of the Equivital™ EQ02 (Hidalgo Limited, Unit F, Trinity Court, Buckingway Business Park, Cambridge, United Kingdom) vital sign monitoring system. The modification, fitting, and validation of this system have previously been reported.[20] Biotelemetry recordings were combined with behavioral measurements using the ObserverXT11 software package.

Experimental design

Five trials were conducted in total (Table 1). Trials were limited to six animals per trial because of high equipment costs and limited enclosure space. One Acunil trial had five animals because

an aggressive male animal was removed on account of fighting.

Each trial lasted 48 hr (two consecutive days). On the first trial day, animals were anesthetized in the morning with thiafentanil to fit the biotelemetry belts. The animals were also weighed. At this time, no tranquilizer was administered and anesthesia was reversed with naltrexone (Trexonil®, Wildlife Pharmaceuticals) at 10 times the dose of thiafentanil. Once all the animals were fully awake, reactive, and exhibiting normal behavior, the trial commenced. This occurred within 10 min after the administration of naltrexone. Previous studies have found that thiafentanil is rapidly and completely reversed by naltrexone within less than 2 min, and no residual effects or renarcotizations were observed in the current study.[2,19,36] The first 12 hr thereafter were considered as pretreatment. After 24 hr, animals were anesthetized again in the morning with thiafentanil. This was done to collect blood and fecal samples for a concurrent investigation. At this time, either Acunil or the placebo was administered before the anesthesia was reversed with naltrexone. Once the animals were displaying normal behavior again, the second part of the trial commenced and this was considered as posttreatment. Again, animals were observed for the first 12 hr.

During each 12-hr observation, animals were stimulated for 1 min every 2 hr. This was achieved by a person entering the enclosure and moving around a radius of 1–2 m from the enclosure door. A pipe was used to hit the enclosure wall to make a noise and frighten the animals. Since animals had no other human contact during the trials, it was thought that such stimulations would elicit a stress response, and by doing so, minimize acclimatization to captivity. Each treatment received the same amount of stimulations, performed by the same person each time. No blinding was done by the person performing the stimulations and all the stimulations were performed as consistently as possible in each of the treatment groups. Each treatment and trial started at roughly the same time in the morning and stimulations within a trial occurred at roughly the same time each day.

Behavioral analysis

A behavioral ethogram, constructed for this species using preliminary observations, was used with the ObserverXT11 software package for behavioral analysis. No blinding occurred for the person performing the observations and the same person (the lead researcher) performed all the observations throughout the study to ensure consistency and prevent interobserver variation. Animals were also scored as "stimulated" during periods of stimulation (when a person entered the enclosure) and "not stimulated" during periods between stimulations when no one was present.

Statistical analysis

Data within a treatment group (Acunil or control) were pooled and means between pretreatment and posttreatment within a specific treatment group were statistically compared. Each animal within a treatment group served as its own control (pre- and posttreatment). Means between the Acunil and control (placebo) groups were not statistically compared because the control group served to establish whether statistically significant changes occurred in the absence of Acunil and not to what extent such changes occurred.

A mixed-effect model was used and the data were analyzed using a restricted maximum likelihood estimation that included treatment, gender, animal weight, and trial as fixed effects. Animal was included as a random effect. For the analysis of vital sign data with behavior data, behavior and stimulation were included as fixed effects. Behavior within periods of stimulation could not be analyzed since not all behaviors occurred within a period of stimulation. However, changes in biotelemetry data were analyzed within stimulations. Data analysis was performed using the variance estimation and precision module of Statistica (version 12) statistical software (StatSoft Inc., 2013). All results were considered significant if $P < 0.05$ and are expressed as least-squared means ± SEM.

RESULTS

Behavior

No extrapyramidal symptoms or side effects were observed in any of the animals during any of the trials. The percentage of time spent on each behavior, before and after treatment with Acunil or the placebo, is given in Table 2. Behaviors classified as "other" were those behaviors that did not occur frequently enough to warrant independent classification.

Two-point behaviors (without a measurable duration) were observed, namely grooming (subcategorized into grooming while lying down and grooming while standing up) and agitation (subcategorized into head shaking and feet stomping). It was found that animals groomed less ($P = 0.03$) while standing after the administration of Acunil

Table 2. Time spent (least-squared means ± SEM) per behavior by blue wildebeest within a treatment in descending order, expressed as a percentage. Values in each row between treatments (pretreatment [PreT] and posttreatment [PostT]) within a group (Acunil® or control) with different uppercase alphabetical superscripts are significantly different ($P < 0.05$). No statistical comparison was made between groups.

Behavior	Acunil		Control	
	PreT	PostT	PreT	PostT
Lying with head up	$33.4^{A} \pm 2.60$	$33.8^{A} \pm 2.74$	$14.0^{A} \pm 2.46$	$24.5^{B} \pm 2.20$
Being vigilant	$25.3^{A} \pm 2.23$	$13.9^{B} \pm 1.55$	$42.7^{A} \pm 2.62$	$29.0^{B} \pm 3.36$
Eating	$12.1^{A} \pm 1.57$	$15.9^{B} \pm 1.40$	$14.4^{A} \pm 2.21$	$16.4^{A} \pm 2.53$
Standing with head up, ruminating	$10.4^{A} \pm 1.17$	$9.8^{A} \pm 1.04$	$16.4^{A} \pm 2.55$	$19.1^{A} \pm 3.88$
Walking	$6.5^{A} \pm 0.67$	$8.9^{A} \pm 1.50$	$7.4^{A} \pm 0.78$	$4.9^{A} \pm 0.67$
Standing with head down	$2.5^{A} \pm 0.95$	$5.6^{B} \pm 1.54$	$1.3^{A} \pm 0.76$	$0.5^{A} \pm 0.44$
Lying with head folded back	$2.7^{A} \pm 0.45$	$5.6^{B} \pm 1.03$	$1.0^{A} \pm 0.24$	$2.5^{A} \pm 0.77$
Other	$1.7^{A} \pm 0.19$	$1.7^{A} \pm 0.21$	$0.2^{A} \pm 0.04$	$0.2^{A} \pm 0.09$
Exploring (walking and sniffing surfaces)	$1.2^{A} \pm 0.32$	$0.6^{B} \pm 0.14$	$0.9^{A} \pm 0.14$	$0.6^{A} \pm 0.16$
Fighting	$1.1^{A} \pm 0.21$	$1.0^{A} \pm 0.16$	$0.9^{A} \pm 0.25$	$0.8^{A} \pm 0.24$
Exploring (standing and sniffing surfaces)	$0.8^{A} \pm 0.10$	$0.7^{A} \pm 0.08$	$0.5^{A} \pm 0.11$	$0.5^{A} \pm 0.11$
Defending	$0.7^{A} \pm 0.15$	$0.7^{A} \pm 0.13$	$0.7^{A} \pm 0.20$	$0.7^{A} \pm 0.17$
Rubbing against another animal	$0.4^{A} \pm 0.15$	$0.6^{A} \pm 0.16$	$0.2^{A} \pm 0.05$	$0.3^{A} \pm 0.11$
Drinking	$0.5^{A} \pm 0.05$	$0.7^{A} \pm 0.10$	$0.2^{A} \pm 0.03$	$0.2^{A} \pm 0.03$
Being rubbed by another animal	$0.3^{A} \pm 0.09$	$0.6^{A} \pm 0.13$	$0.2^{A} \pm 0.08$	$0.3^{A} \pm 0.07$
Trotting	$0.3^{A} \pm 0.07$	$0.2^{A} \pm 0.04$	$0.2^{A} \pm 0.01$	$0.1^{A} \pm 0.01$

(19.2% ± 4.25%) than before (27.1% ± 4.14%). In addition, animals also shook their heads less often ($P = 0.001$) after the administration of Acunil (27.7% ± 3.30%) than before (46.4% ± 4.23%). No such changes were observed in the control group (grooming while standing: pretreatment = 28.7% ± 8.37%; posttreatment = 27.6% ± 7.44%; head shaking: pretreatment = 81.6% ± 12.31%; posttreatment: 65.9% ± 7.95%).

Heart rate and respiration rate per behavior

Neither the administration of Acunil nor the placebo had any effect ($P > 0.05$) on mean heart rate per behavior (Table 3). Respiration rate for certain behaviors changed after both administration of Acunil and the placebo (Table 4).

Motion

Motion was measured every 15 sec in terms of the categories stationary, moving slowly, and moving fast. Acunil-treated animals spent more time ($P < 0.05$) being stationary (pretreatment: 75.1% ± 1.70%; posttreatment: 82.8% ± 1.70%) and less time moving fast (pretreatment: 10.2% ± 1.72%; posttreatment: 4.8% ± 1.80%). In the control group, animals also spent more time being stationary (pretreatment: 75.8% ± 1.98%; posttreatment: 86.8% ± 2.08%) and less time moving slowly (pretreatment: 15.7% ± 1.69%; posttreatment: 10.7% ± 1.78%) after treatment with the placebo. Differences ($P < 0.05$) were also observed in both the Acunil and control groups when motion was analyzed between periods of stimulation and periods of no stimulation (Table 5).

Mean heart rate per motion category was unaffected ($P > 0.05$) by treatment with Acunil. However, mean respiration rate was lower ($P = 0.04$) in Acunil-treated animals when they were stationary (pretreatment: 12.3 ± 1.29 breaths/min; posttreatment: 10.3 ± 1.31 breaths/min). No changes in heart or respiration rate were observed within a motion category in the control group.

Heart rate and respiration rate between periods of stimulation and periods of no stimulation

Mean heart rates (beats/min) and respiration rates (breaths/minute) for periods of stimulation and periods of no stimulation in the Acunil and control group are given in Table 6. Treatment with Acunil had no effect ($P = 0.72$) on overall mean heart rate (pretreatment: 69.7 ± 4.06 beats/min; posttreatment: 68.5 ± 4.05 beats/min); however, overall mean respiration rate was higher ($P = 0.02$) before treatment (14.5 ± 0.82 breaths/min) compared with after (12.5 ± 0.83 breaths/min). In the control group, both overall heart rate (pretreatment: 84.48 ± 6.28 beats/min; posttreatment: 86.57 ± 6.45 beats/min) and respiration rate (pretreatment: 16.42 ± 1.04 breaths/min; posttreatment: 16.75 ± 1.08 breaths/min) were unaffected by treatment.

Table 3. Heart rates (beats/min) (least-squared means ± SEM) of blue wildebeest per behavior between treatments (pretreatment [PreT] and posttreatment [PostT]) within a group (Acunil® and control). No significant differences ($P > 0.05$) were found between treatments within any of the two groups. No statistical comparison was made between groups.

	Acunil		Control	
Behavior	PreT	PostT	PreT	PostT
Trotting	92.7 ± 6.16	85.9 ± 6.27	93.1 ± 6.56	84.3 ± 7.50
Fighting	79.9 ± 6.44	89.0 ± 6.48	82.4 ± 7.10	75.8 ± 6.87
Other	80.7 ± 6.64	86.9 ± 6.53	81.8 ± 7.96	79.1 ± 1.97
Defending	79.5 ± 6.44	85.1 ± 6.38	78.0 ± 6.76	73.8 ± 7.29
Drinking	74.9 ± 6.57	78.4 ± 6.57	77.0 ± 7.20	80.6 ± 7.41
Walking	73.7 ± 6.10	78.7 ± 6.04	84.9 ± 6.10	77.5 ± 6.33
Rubbing against another animal	73.0 ± 6.65	73.1 ± 6.75	65.2 ± 7.20	75.2 ± 7.50
Exploring (walking and sniffing surfaces)	77.4 ± 6.49	68.2 ± 6.59	72.5 ± 7.20	70.2 ± 7.12
Eating	72.8 ± 6.57	70.8 ± 6.49	76.9 ± 7.00	73.0 ± 7.22
Being rubbed by another animal	69.8 ± 6.57	71.6 ± 6.65	79.6 ± 7.46	72.7 ± 7.41
Exploring (standing and sniffing surfaces)	72.1 ± 6.49	69.9 ± 6.57	73.3 ± 7.20	74.9 ± 7.22
Being vigilant	70.6 ± 6.02	69.2 ± 6.00	78.2 ± 6.06	75.8 ± 6.21
Standing with head down	62.9 ± 6.61	66.1 ± 6.62	71.1 ± 7.90	72.5 ± 8.88
Standing with head up, ruminating	65.2 ± 6.57	63.4 ± 6.22	65.5 ± 7.00	69.7 ± 6.93
Lying with head folded back	61.7 ± 6.65	60.4 ± 6.44	71.9 ± 7.90	69.1 ± 8.33
Lying with head up	61.2 ± 6.51	59.5 ± 6.28	71.7 ± 7.00	67.4 ± 7.22

DISCUSSION

To our knowledge, this is the first reported data on the behavior, heart rate, respiration, and motion of ambulatory blue wildebeest in captivity. Only the first 12 hr of each trial day were used since peak effects were expected to be observed around 4–6 hr and maximum serum concentrations of zuclopenthixol acetate were expected within 6 hr after treatment with Acunil. As a result, no increase in sedation was expected after 12 hr, although levels of zuclopenthixol acetate were anticipated to be maintained above therapeutic levels for up to 72 hr.[3,6,15,17,31,32] Moreover, the battery life of the Equivital system does not exceed 38 hr and longer behavioral observations

Table 4. Respiration rates (beats/min) (least-squared means ± SEM) of blue wildebeest per behavior between treatments (pretreatment [PreT] and posttreatment [PostT]) within a group (Acunil® and control). Values in each row between treatments (PreT and PostT) within a group (Acunil or control) with different uppercase alphabetical superscripts are significantly different ($P < 0.05$). No statistical comparison was made between groups.

	Acunil		Control	
Behavior	PreT	PostT	PreT	PostT
Other	20.4^{A} ± 1.26	20.2^{A} ± 1.24	16.4^{A} ± 1.05	15.2^{A} ± 1.05
Trotting	19.1^{A} ± 1.04	16.5^{B} ± 1.12	17.8^{A} ± 1.21	16.3^{A} ± 1.39
Defending from other animal	17.1^{A} ± 1.17	18.7^{A} ± 1.17	16.5^{A} ± 1.25	13.9^{A} ± 1.35
Fighting with other animal	17.0^{A} ± 1.17	17.6^{A} ± 1.24	16.6^{A} ± 1.32	14.6^{A} ± 1.28
Walking	17.0^{A} ± 1.02	17.1^{A} ± 1.02	17.8^{A} ± 1.13	14.4^{B} ± 1.17
Drinking	17.1^{A} ± 1.23	16.6^{A} ± 1.26	14.9^{A} ± 1.33	13.7^{A} ± 1.37
Rubbing against other animal	16.1^{A} ± 1.26	16.5^{A} ± 1.30	14.9^{A} ± 1.33	14.3^{A} ± 1.39
Exploring (standing and sniffing surfaces)	16.5^{A} ± 1.20	14.5^{A} ± 1.27	14.3^{A} ± 1.33	15.9^{A} ± 1.34
Exploring (walking and sniffing surfaces)	17.0^{A} ± 1.20	15.6^{A} ± 1.26	15.9^{A} ± 1.33	13.7^{A} ± 1.32
Eating	16.1^{A} ± 1.23	14.3^{A} ± 1.23	16.9^{A} ± 1.29	14.5^{A} ± 1.34
Being rubbed by other animal	12.7^{A} ± 1.23	15.6^{A} ± 1.30	13.1^{A} ± 1.38	12.1^{A} ± 1.37
Being vigilant	15.1^{A} ± 0.98	12.8^{B} ± 1.00	16.3^{A} ± 1.12	14.7^{A} ± 1.15
Standing head down	13.9^{A} ± 1.24	10.7^{B} ± 1.27	13.7^{A} ± 1.47	12.3^{A} ± 1.66
Standing head up ruminating	13.7^{A} ± 1.23	10.8^{B} ± 1.11	13.1^{A} ± 1.29	12.3^{A} ± 1.28
Lying with head up	9.9^{A} ± 1.20	9.2^{A} ± 1.14	9.9^{A} ± 1.29	13.5^{B} ± 1.34
Lying with head folded back	7.9^{A} ± 1.26	7.5^{A} ± 1.20	9.6^{A} ± 1.55	11.4^{A} ± 1.55

Table 5. Frequency at which each motion was measured (least-squared means ± SEM) in blue wildebeest within periods of stimulation and periods of no stimulation between treatments (pretreatment [PreT] and posttreatment [PostT]) within groups (Acunil® and ontrol), expressed as a percentage of total motion measured within a period. Values in each column within a group (Acunil or control) with different uppercase alphabetical superscripts are significantly different ($P < 0.05$). No statistical comparison was made between groups.

		Acunil			Control		
Treatment	Period	Stationary	Moving slowly	Moving fast	Stationary	Moving slowly	Moving fast
PreT	NS[a]	$87.3^{A} \pm 0.96$	$11.3^{A} \pm 0.79$	$1.4^{A} \pm 0.24$	$86.8^{A} \pm 0.73$	$12.2^{A} \pm 0.69$	$1.0^{A} \pm 0.10$
	S	$63.0^{B} \pm 2.43$	$18.9^{B} \pm 2.94$	$19.2^{B} \pm 2.94$	$74.8^{B} \pm 4.59$	$19.2^{B} \pm 3.42$	$6.0^{B} \pm 1.61$
PostT	NS	$86.3^{A} \pm 1.83$	$11.9^{A,B} \pm 1.62$	$1.3^{A} \pm 1.33$	$90.9^{A} \pm 1.53$	$8.4^{A} \pm 1.35$	$0.6^{A} \pm 0.21$
	S	$78.8^{A} \pm 2.70$	$15.0^{A,B} \pm 1.24$	$8.4^{C} \pm 174$	$82.7^{A} \pm 3.98$	$13.0^{B} \pm 3.74$	$4.4^{B} \pm 0.43$

[a] NS indicates not stimulated; S, stimulated.

increased the risk of observational errors due to the difficulty of observing and identifying individual animals at night.

It must be mentioned that no observer blinding occurred during the study but that bias was avoided as much as possible and that results are reported as observed. Behaviors were also well defined and clear so that observer error was minimized.

Animals treated with Acunil spent significantly more time eating ($P < 0.001$), but no such results were observed in the control group. It can therefore be inferred that the stress of captivity may partly cause a reduction in feed intake so that a reduction in this stress due to treatment with Acunil may cause an increase in time spent eating.

Animals treated with Acunil spent more time lying with their heads folded back and standing with their heads down. Animals in the control group also spent more time lying with their heads up after receiving the placebo, indicating that, to some extent, some of the increase in resting was due to habituation. Nonetheless, some of the increase in resting behavior was at least partially due to the drug effect of Acunil since animals in the control group showed no increase in time spent lying with their heads folded back (i.e., sleeping). Similar reductions in activity were reported by Read et al.[30] in wapiti (*Cervus canadensis*) treated with Clopixol-Acuphase. In contrast, Fick et al.[13,14] found that Clopixol-Acuphase had no significant effect on activity in goats and blue wildebeest, respectively. Interestingly, Fick et al.[13] reported that boma housing had a greater effect on reducing the activity of blue wildebeest than treatment with Clopixol-Acuphase. The authors concluded that habituation to captivity resulted in the greatest decrease in activity and that Clopixol-Acuphase had no additive effect on this decrease. Neither Read et al.[30] nor Fick et al.[14] found any significant changes in activity in animals treated with a placebo.

Animals treated with Acunil spent almost 50% less time being vigilant, but a similar change in vigilance was observed in the control group. According to Lind,[21] vigilant behavior is related to the gathering of information about an animal's environment. Such behavior is usually brought on by an environmental stimulus. It is possible that after the first day, animals became more accustomed to captivity and human stimulation so that they were less reactive. However, animals treated

Table 6. Heart rate (beats/min) and respiration rate (breaths/min) (least-squared means ± SEM) of blue wildebeest between treatments (pretreatment [PreT] and posttreatment] PostT) and between periods where animals were stimulated and not stimulated within a group (Acunil® and control). Values in each column within a group (Acunil or control) with different uppercase alphabetical superscripts are significantly different ($P < 0.05$). No statistical comparison was made between groups.

		Acunil		Control	
Treatment	Period	Heart rate	Respiration rate	Heart rate	Respiration rate
PreT	NS[a]	$60.1^{A} \pm 4.44$	$12.9^{A} \pm 0.80$	$73.2^{A} \pm 1.76$	$14.7^{A} \pm 0.39$
	S	$79.4^{B} \pm 3.24$	$16.2^{B} \pm 0.94$	$96.6^{B} \pm 6.27$	$19.1^{B} \pm 0.87$
PostT	NS	$64.4^{A} \pm 3.25$	$11.3^{A} \pm 0.73$	$72.6^{A} \pm 1.84$	$13.4^{A} \pm 0.41$
	S	$72.5^{B} \pm 4.60$	$13.7^{C} \pm 0.98$	$81.4^{B} \pm 4.06$	$17.3^{B} \pm 0.95$

[a] NS indicates not simulated; S, stimulated.

with Acunil also spent 50% less time on explorative behavior, which indicates an increased disinterest in their surroundings.

Animals groomed less while standing after treatment with Acunil. According to Estes,[12] this behavior forms part of displacement activities (maintenance activities performed under stressful situations). In addition, there was a significant decrease in head shaking in Acunil-treated animals. This head shaking was observed to occur in response to stressful stimuli.

Heart rate was unaffected by both the administration of Acunil and the placebo. Read et al.[30] reported that Clopixol-Acuphase had no significant effect on the heart rate of wapiti. However, these authors also reported no significant effect on respiration rate[30] Diverio et al.,[8] on the other hand, reported that red deer (*Cervus elaphus*) treated with Clopixol-Acuphase showed a greater increase in heart rate when a stressor was applied than untreated animals. They attributed this to reflex tachycardia, which is commonly seen after administration of a LAN because of a lowered peripheral resistance resulting in hypotension. This may partially explain the lack of significant effect ($P > 0.05$) that Acunil had on heart rate since an increase in heart rate due to treatment with Acunil would counterbalance any reduction in heart rate due its sedative effect. Similar findings have been reported with the use of a variety of neuroleptics in wildlife, with heart rate becoming elevated after the administration of the neuroleptic.[9,22,24,25,27] Alternatively, it may be that Acunil, at the dose given, had no appreciable effect on blood pressure and as a result no changes in heart rate were observed.

Treatment with Acunil resulted in a lowered ($P = 0.02$) overall mean respiration rate (pretreatment: 14.5 ± 0.82 breaths/min vs. posttreatment: 12.5 ± 0.83 breaths/min) and this is possibly the result of its sedative effect. Mean heart and respiration rates fell within acceptable ranges reported for this and other African ungulate species, although no published data are available for untreated animals.[1,4,5,7,18,26,28]

Neither treatment with Acunil nor the placebo resulted in any significant changes in heart rate during specific behaviors. However, animals treated with Acunil had lower respiration rates during several behaviors. Two of these behaviors included trotting and being vigilant, which are considered alarm behaviors, suggesting a subdued state in treated animals. Animals in the control group also exhibited lower respiration rates after treatment while they were walking. However, respiration rate in this group was higher while animals were lying with their heads up after treatment, alluding to a slight increase in alertness while resting.

Animals treated with Acunil had lower respiration rates at times when they were stationary (pretreatment: 12.3 ± 1.29 breaths/min vs. posttreatment: 10.3 ± 1.31 breaths/min). No such results were found in the control group. Similar results were reflected in the analysis of respiration rate per behavior, with tranquilization causing a lowered respiration for certain stationary behaviors.

It is not surprising that animals spent more time moving fast during periods of stimulation, since stimulations would elicit the "fight-or-flight" response.[34] Both treatment with Acunil and the placebo resulted in an increase in the time spent being stationary during periods of stimulation, suggesting that animals were becoming accustomed to the stimulations. However, treatment with Acunil also caused a decrease in the time spent moving fast during periods of stimulation. In addition, tranquilized animals spent the same amount of time being stationary and moving slowly regardless of whether they were being stimulated. These results suggest that Acunil may have resulted in a decreased flight response. However, some bias may also have been present because of the lack of blinding during stimulations, so care must be taken when interpreting these results.

CONCLUSION

The use of Acunil was found to be safe (no adverse effects seen) and effective (reduced response to stimulus and increased resting behaviors). To some extent the amount of time animals spent being vigilant and lying down was affected by habituation, as illustrated in the control group. Although treatment with Acunil had no significant effect on heart rate, it caused a lowered respiration rate overall, during specific behaviors as well as during periods of stimulation compared with untreated animals. In addition, treated animals spent more time eating than before being tranquilized. Similarly, the animals spent less time moving fast when stimulated, suggesting a reduction in stress response. It must be borne in mind, though, that no blinding occurred during stimulations, which may have partly affected the results observed during these stimulations. Overall, it does appear as if Acunil produced a suitable sedative effect in the animals with no observable side effects. Future investigations should focus on

its use in other species and species in noncaptive environments. In addition, the use of blinding in similar studies will remove any bias that may have been present in the current investigation.

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LITERATURE CITED

1. Ancrenaz M, Ostrowski S, Anagariyah S, Delhomme A. Long-duration anesthesia in arabian oryx (*Oryx leucoryx*) using a medetomidine:etorphine combination. J Zoo Wildl Med. 1996;27(2):209–216.

2. Arnemo JM, Kreeger TJ, Soveri T. Chemical immobilization of free-ranging moose. Alces. 2003;39: 243–253.

3. Carbone ET, Lindstrom KE, Diep S, Carbone L. Duration of action of sustained-release buprenorphine in 2 strains of mice. J Am Assoc Lab Anim Sci. 2012; 51(6):815–819.

4. Chittick E, Horne W, Wolfe B, Sladky K, Loomis M. Cardiopulmonary assessment of medetomidine, ketamine and butorphanol anaesthesia in captive Thomson's gazelles (*Gazella thomsoni*). J Zoo Wildl Med. 2001;32(2):168–175.

5. Citino SB, Bush M, Grobler D, Lance W. Anesthesia of boma-captured Lichtenstein's hartebeest (*Sigmoceros lichtensteinii*) with a combination of thiafentanil, medetomidine, and ketamine. J Wildl Dis. 2002;38(2):457–462.

6. Clark TS, Clark DD, Hoyt RF. Pharmacokinetic comparison of sustained-release and standard buprenorphine in mice. J Am Assoc Lab Anim Sci. 2014; 53(4):387–391.

7. Dittberner M. Accelerated induction of etorphine immobilization in blue wildebeest (*Connochaetes taurinus*) by the addition of hyaluronidase. MS Thesis, 2011. Univ. Pretoria, South Africa.

8. Diverio S, Goddard PJ, Gordon IJ. Use of long-acting neuroleptics to reduce the stress response to management practices in red deer. Appl Anim Behav Sci. 1996;49(1):83–88.

9. Diverio S, Goddard PJ, Gordon IJ, Elston DA. The effect of management practices on stress in farmed red deer (*Cervus elaphus*) and its modulation by long-acting neuroleptics: behavioural responses. Appl Anim Behav Sci. 1993;36(4):363–376.

10. Dugmore H. Jan Oelofse: the man who revolutionized game capture. Farmer's Weekly. 2013; April 19:1–3.

11. Ebedes H. The use of long-acting tranquilizers in captive wild animals. In: McKenzie AA (ed.). The capture and care manual. Pretoria (South Africa): Lynnwood Ridge: Wildlife Decision Support Services; 1993. p. 71–98.

12. Estes RD. The behavior guide to African mammals. 1st ed. Johannesburg (South Africa): Russel Friedman Books CC, Halfway House; 1991.

13. Fick LG, Matthee A, Mitchell D, Fuller A. The effect of boma-housing and long-acting tranquillizers on body temperature, physical activity and food intake of blue wildebeest (*Connochaetes taurinus*). J Therm Biol. 2006;31(1–2):159–167.

14. Fick LG, Mitchell D, Fuller A. Long-acting neuroleptics used in wildlife management do not impair thermoregulation or physical activity in goats (*Capra hircus*). Comp Biochem Physiol A. 2007;147(2): 445–452.

15. Foley PL, Liang H, Crichlow AR. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. J Am Assoc Lab Anim Sci. 2011; 50(2):198–204.

16. Food and Drug Administration of the United Nations. Residues of some veterinary drugs in animals and foods. 38th Meeting of the Joint FAO/WHO Expert Committee on Food Additives [Internet]. Food and Drug Administration of the United Nations; 1991. p. 1–143. Available from: http://www.fao.org/docrep/014/T0500E/T0500E.pdf

17. Healy JR, Tonkin JL, Kamarec SR, Saludes MA, Ibrahim SY, Matsumoto RR, Wimsatt JH. Evaluation of an improved sustained-release buprenorphine formulation for use in mice. Am J Vet Res. 2014;75(7): 619–624.

18. Howard LL, Kearns KS, Clippinger TL, Larsen RS, Morris PJ. Chemical immobilization of rhebok (*Pelea capreolus*) with carfentanil–xylazine or etorphine–xylazine. J Zoo Wildl Med. 2004;35(3):312–319.

19. Kreeger TJ, Cook WE, Piché CA, Smith T. Anesthesia of pronghorns using thiafentanil or thiafentanil plus xylazine. J Wildl Manage. 2001;65(1):25–28.

20. Laubscher LL, Hoffman LC, Pitts NI, Raath JP. Validating a human biotelemetry system for use in captive blue wildebeest (*Connochaetes taurinus*). Zoo Biol. 2015;34:321–327.

21. Lind J. Vigilance and models of behavior. In: Moore MJ, Breed MD (eds.). Encyclopedia of animal behaviour. London (United Kingdom): Elsevier,; 2010. p. 506–510.

22. Marco I, Lavín S, Mentaberre G, Lopez-Olvera R, Casas-Díaz E. Haloperidol and azaperone in drive-net captured southern chamois (*Rupicapra pyrenaica*). J Wildl Dis. 2010;46(3):923–928.

23. McKenzie AA. The capture and care manual: capture, care, accommodation, and transportation of wild African animals. Lynnwood Ridge (South Africa): Wildlife Decision Support Services; 1993.

24. Mentaberre G, López-Olvera JR, Casas-Díaz E, Bach-Raich E, Marco I, Lavín S. Use of haloperidol and azaperone for stress control in roe deer (*Capreolus capreolus*) captured by means of drive-nets. Res Vet Sci. 2010;88(3):531–535.

25. Mentaberre G, López-Olvera JR, Casas-Díaz E, Fernández-Sirera L, Marco I, Lavín S. Effects of azaperone and haloperidol on the stress response of drive-net captured Iberian ibexes (*Capra pyrenaica*). Eur J Wildl Res. 2010;56(5):757–764.

26. Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D, Fuller A. Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. S Afr Vet Assoc. 2008;79(3):121–129.

27. Montané J, Marco I, Manteca X. Transport stress in roe deer (*Capreolus Capreolus*): effect of a short-acting antipsychotic. Anim Welf. 2002;11:405–417.

28. Mortola JP, Lanthier C. Breathing frequency in ruminants: a comparative analysis with non-ruminant mammals. Respir Physiol Neurobiol. 2005;145(2–3): 265–277.

29. Read M. Long acting neuroleptic drugs. In: Heard DJ (ed.). Zoological restraint and anaesthesia. Ithaca (New York): International Veterinary Information Service; 2002. p. 4–10.

30. Read M, Caulkett NA, McCallister M. Evaluation of zuclopenthixol acetate to decrease handling stress in wapiti. J Wildl Dis. 2000;36(3):450–459.

31. SR Veterinary Technologies. Clinical research bulletin September 2011: pharmacokinetic properties of novel sustained release buprenorphine and meloxicam formulations in rats. Windsor (Colorado): SR Veterinary Technologies; 2011. p. 1–4.

32. SR Veterinary Technologies. Clinical research bulletin September 2012: pharmacokinetic properties of a novel sustained release meloxicam formulation in dogs. Windsor (Colorado): SR Veterinary Technologies; 2012. p. 1–2.

33. Swan GE. Drugs used for the immobilization, capture, and translocation of wild animals. In: McKenzie AA (ed.). The capture and care manual. Pretoria (South Africa): The South African Veterinary Foundation; 1993. p. 3–59.

34. Uetake K, Morita S, Hoshiba S, Tanaka T. Flight distance of dairy cows and its relationship to daily routine management procedures and productivity. Anim Sci J. 2002;73:279–285.

35. West G, Heard DJ, Caulkett NA. Zoo animal and wildlife immobilization and anaesthesia. 1st ed. Ames (Iowa): Blackwell Publishing; 2007.

36. Wolfe LL, Lance WR, Miller MW. Immobilization of mule deer with thiafentanil (A-3080) or thiafentanil plus xylazine. J Wildl Dis. 2004;40(2): 282–287.

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