Praca oryginalna *Original paper*

Anesthetic effect of oral administration of α-chloroaldose and dexmedetomidine on Silver Fox*

SHI-MING YU[1], JI-PENG LI[2], LIU-YANG LI[2], DE-ZHANG LU[2], HONG-GANG FAN[1], HONG-BIN WANG[1]

[1]Heilongjiang Key Laboratory for Laboratory Animals and Comparative Medicine, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, P.R. China
[2]Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China

Received 28.09.2022 *Accepted 12.09.2023*

Yu S.-M., Li J.-P., Li L.-Y., Lu D.-Z., Fan H.-G., Wang H.-B.
Anesthetic effect of oral administration of α-chloroaldose and dexmedetomidine on Silver Fox

Summary

In the past, anesthesia was delivered to foxes mostly through intramuscular or subcutaneous injection, which caused substantial stress to the animals, making it necessary to restrain them physically before the procedure. Moreover, research on anesthesia technology for domestic animals has been insufficient. Therefore, in this study, the α-chloroaldose and dexmedetomidine combination was used via oral administration to anesthetize foxes. Ten healthy silver foxes were administered the combination of oral anesthesia, and routine physiological indicators, anesthesia time and effects, and biological reflexes were continuously monitored. The temperature of anesthetized foxes after the administration of oral anesthesia continued to decrease, and by the end of the monitoring, it decreased by 1.3°C.The average SpO_2 was 95.3 ± 2.2% during the whole anesthesia period. Changes in the heart rate were significant, initially showing a decreasing trend and then an increasing trend. The anesthesia period showed characteristics of smooth induction, proper maintenance, and rapid recovery. The sedative, analgesic, and muscle relaxant effects were more balanced and provided a good anesthesia effect time of 30 min: corneal, eyelid, and anal biological reflexes persisted and were inhibited at 20~50 min after oral medication, which shows a suitable depth of anesthesia.

Keywords: anesthesia, oral administration, Silver fox, anesthesia effect, physiological

Anesthesia is the basis of successful clinical treatment and has important supporting effects on surgery, intensive care, and wildlife resource conservation. In clinical veterinary medicine, anesthesia is delivered mainly through infusion and inhalation anesthesia (1, 18). Recently, with the rise in the number of foxes, wolves, and raccoons, the workload and scope of diagnosis and treatment of wildlife have been increasing, with the concomitant increase in demand for better anesthesia security (2, 3). Effective chemical restraint not only ensures the safety of people and animals but also increases animal welfare (3, 8). In the past, anesthesia was administered to foxes via intramuscular injection, and proper physical restraint was needed beforehand, which caused chronic stress to the fox, subsequently leading to the negative effects of anesthesia (6). Compared with other modes, oral anesthesia delivery has the advantages of convenience in administration, traveling with boldness, hence is effective in extremities in the body, and is non-invasive. Moreover, it also has the advantage of effectively reducing stress in poultry and reproduction breeding, animals transported over long distances, captured animals, and so forth. The general anesthetic drug α-chloralose is administered mainly to experimental animals, usually for the chemical restraint of cats when doing blood pressure reduction experiments in the pharmaceutical set-up. It has certain anesthetic effects on dogs, wolves, martens, and other animals and has no obvious toxicity or side effects (11). Alpha-chloralose plays its anesthesia effect by GABA-A receptor activity, and its does not bind to either the benzodiazepine or the barbiturate sites but potentiates the GABA-induced current by increasing the affinity for GABA (7, 13, 16). Dexmedetomidine is an α2-adrenergic receptor agonist, which has good skin and mucous membrane penetration and produces a good sedative effect (17). Balanced anesthesia effects

* This study was supported by the National Key Research and Development Program of China (Grant No. 2016YED0501008) and the Natural Science Basic Research Plan in Shaanxi Province of China (Grant No. 2022JZ-11).

are obtained in various animals when dexmedetomidine is applied as a combination anesthetic (5, 12). Therefore, the researchers compounded α-chloralose with dexmedetomidine, administered them orally to silver foxes, and observed the period of anesthesia, as well as the effect of the compound anesthesia on the main physiological functions. These findings provide experimental evidence and a theoretical basis for further development of compound oral anesthetics for wild canine animals.

Material and methods

Experimental animals. Ten Finnish foxes, 7-9 months old, weighting 8.53 ± 0.52 kg, 5 males and 5 females, were provided by a Finnish fox breeding base in Jiamusi, Heilongjiang Province. The Experimental Animal Ethics Committee of Northeast Agriculture University approved the experimental design and research content of this study. Physical examination and complete blood count of all foxes determined their good physical condition. Foxes were raised in single cages separately for 14 days and checked again before the experiment. Healthy experimental foxes are selected for experimental studies. Animal use was approved by the Animal Care and Use Committee of the Northeast Agricultural University.

The drugs used for analysis. α-Chloralose API was purchased from Sigma Company, Germany (purity 99.9%) and dexmedetomidine hydrochloride standard was purchased from Shanghai YanmuIndustrial Co., Ltd, China (purity 99.8%). Other drugs included erythromycin ophthalmic ointment, and aquae pro injection (Harbin Pharmaceutical Group, China).

Equipment. For the experimentation, we used Philips Intellivue MP30 circulatory monitor (Philips, Netherlands), SurgiVet V6004 Animal Blood Pressure Monitor (SurgiVet, USA), XH-D Vortex Shaker (Wuxi Jiuping Instrument Co., Ltd., China), and SYST-007 type constant temperature water bath (Liaoning Saias Technology Co., Ltd., China). Other instruments included an electric timer, routine surgical instruments, an electronic thermometer, a single-use syringe, and a venous indwelling needle.

Reagent preparation. α-Chloralose was dissolved in 1.2-propylene glycol to a concentration of 180 mg/mL and stored in the dark. Dexmedetomidine hydrochloride standard was prepared to a concentration of 2 mg/mL using aquae pro injection and stored away from light.

Empirical method. One day before the test, the experimental foxes were weighed accurately and fasted for food for 12 h and water for 6 h. One hour before the experiment, the fox was allowed to enter the trial site. α-Chloralose and dexmedetomidine were mixed, respectively, at 360 mg/kg and 0.6 mg/kg concentrations, in a daily semi-fluid diet for free feeding and one serving per fox. Body temperature (T), heart rate (HR), respiratory rate (RR), pulse oximetry (SpO_2), mean arterial pressure (MAP), and other key physiological indicators were monitored at 10, 20, 30, 40, 50, and 60 min after feeding; moreover, in the anesthetic period, effects and biological reflex activity were monitored, using the methods as shown in Tables 1, 2, and 3. The fox had to be appropriately physically restrained during the experiment to avoid human and animal injuries and damage to the equip-

Tab. 1. Monitoring methods during anesthesia

Anesthesia period	Induction period	Maintenance phase	Awakening period
Starting time-point	From the end of the feeding	Since the righting reflex disappears	Recovery from righting reflex
Ending time-point	The righting reflex disappears	The righting reflex is restored	To stand straight walk

Tab. 2. Scoring criteria for sedation, analgesia, and muscle relaxation

Monitoring project	Score	Scoring criteria
Sedation	0	Normal
	1	Mild sedation (standing or prone, head and neck lowered, eyelids slightly closed)
	2	Moderate sedation (head on the ground or stomach, eyes turned inward)
	3	Deep sedation (smooth lying, large degree of inward eye rotation)
Analgesia	0	Normal
	1	The pain response of the fox is dull when the skin of the abdomen is needled
	2	The pain response of fox abdominal skin was not obvious after acupuncture
	3	After acupuncture on the skin of fox abdomen, the pain response disappeared
Muscle relaxation	0	Normal
	1	Although the fox's upper and lower jaw can be pulled apart, it can feel obvious resistance and weak in walking and running
	2	When the upper and lower jaw were opened, there was no obvious resistance, and it was impossible to walk and run
	3	The upper and lower jaws can be opened smoothly, and the fox lies quietly

Tab. 3. Judgment criteria for biological reflexes

Biological reflex	Stimulation method	Decision criteria	Represents the depth of anesthesia
Corneal reflex	Gently touch the fox's cornea with a sterile gauze Angle	„+" close eyelid quickly „±" slightly dull eyelid closure „–" eyelid closure is obviously dull, or even disappears	„+" means too shallow anesthesia „±" is appropriate for anesthesia „–" means the anesthesia is too deep
Eyelid reflex	Gently touch the fox's third eyelid with a sterile gauze Angle	„+" close eyelid quickly „±" eyelid closure is slow „–" eyelids do not close	Same as above
Anal reflex	Lightly prick the perianal sphincter with the needle of a 1 mL syringe	„+" sphincter contracts more quickly „±" contraction is slower „–" does not shrink	Same as above

ment. Physiologic parameters included MAP, SpO_2 and RT were measured by noninvasive monitor (Philips Intellivue MP30 and SurgiVet V6004 Animal Blood Pressure Monitor). Monitoring of blood pressure was achieved by placing a cuff circumferentially around the left antebrachium of animals, with the cuff width being approximately 40% of the total circumference of the limb. HR was determined by counting heart beats for 1 minute using a stethoscope placed at the lower left lateral thoracic wall and RR was counted from thoracic excursions for 1 minute.

The scoring criteria of anesthesia according to the standard are presented in Table 2. Sedative effects were mainly assessed by observing the head and neck and the inversion of the eyeball. Analgesic effects were assessed by observing the response of the fox when its left hindfoot pad was pricked with the needle of a disposable 10 mL syringe. Muscle relaxation effects were assessed by observing the jaw tension and limb movements of the fox.

Statistical analyses. Microsoft Excel 2018 was used to collect the trial data, and SPSS 23.0 was used to analyze and process the data. Data were expressed as mean ± standard deviation (SD), using one-way analysis of variance, posthoc tests, multiple comparison tests, the least significant difference, and the S-N-K method. $P < 0.05$ was considered significant.

Results and discussion

The primary physiological indicators. There was a gradual decrease in T the monitoring period for anesthesia. At 40-60 min and 10-20 min durations after oral anesthesia administration, T decreased to a minimum at 60 min after the completion of feeding, with a decrease of 1.3°C compared to pre-anesthesia and the difference was significant ($P < 0.05$). HR tended to drop and then recovered after the experimental foxes were fed a normal diet. HR dropped to a minimum at 40 min of dosing and the values at 10 min and 50-60 min after dosing differed significantly ($P < 0.05$). The trend in RR was similar to that of HR, but the minimum RR occurred 30 min after dosing, after which there was a gradual recovery, with no significant difference between the time points compared ($P > 0.05$). SpO_2 showed a brief decrease at the beginning of anesthesia administration, followed by a gradual recovery; the lowest value of 95.32% was observed 20 minutes after the completion of feeding and the average SpO_2 was 96.31% during the whole monitoring period. MAP changes followed a similar trend as HR and did not differ significantly at any time point ($P > 0.05$). The specific monitoring results of primary physiological indicators are presented in Table 4.

Monitoring induction, maintenance, and awakening in the anesthesia period. After oral anesthesia administration, all experimental foxes showed a gradual decrease in activity, unresponsiveness, and slowness, followed by ataxia, the inability to stand, and finally, slumped to the ground with a loss of the righting reflex. No abnormal excitement or vomiting was observed during the induction period, the shortest induction period was 8 min and the average was 12.8 ± 2.9 min. During the anesthesia maintenance period, foxes showed a loss of consciousness, smooth supination, and general relaxation, with a minimum maintenance time of 62 min and a maximum of 74 min (mean 69.0 ± 3.4 min). The awakening period was relatively smooth and rapid, with no re-sleeping or other neurological abnormalities, and the mean awakening time was 38.5 ± 4.5 min. A comprehensive analysis of the trial results showed that the oral anesthesia was smoothly induced, maintained for a suitable time, and resulted in a relatively rapid awakening. The specific monitoring results are shown in Table 5.

Monitoring the effects of anesthesia. The results of the monitoring showed rapid sedation and inotropic effects after oral administration, and good effects were achieved at 10 min post-dosing. The analgesia also entered a good state 20 min post-dosing, after which this good and balanced anesthetic effect lasted for about 30 min (20-50 min post-dosing), and the difference was significant ($P < 0.05$) between this time period and other time points. Sedation, analgesia, muscle relaxation then showed recovery, with

Tab. 4. Monitoring results of basic physiological indicators ($\bar{x}$ ± SD, n = 10)

Monitoring project Time (min)	T (°C)	HR (b/min)	RR (b/min)	SPO_2 (%)	MAP (mmHg)
10	39.3 ± 0.3^{A}	97.5 ± 3.8^{A}	22.3 ± 2.5	95.6 ± 2.1	137.4 ± 10.5
20	39.1 ± 0.4^{A}	94.4 ± 4.5^{AB}	19.5 ± 3.7	95.3 ± 2.2	134.5 ± 11.8
30	38.7 ± 0.3^{AB}	93.2 ± 6.3^{AB}	18.7 ± 3.4	96.3 ± 1.8	128.2 ± 12.4
40	38.5 ± 0.5^{B}	82.8 ± 7.2^{B}	20.2 ± 3.3	96.4 ± 2.0	128.0 ± 14.7
50	38.3 ± 0.5^{B}	95.4 ± 5.6^{A}	22.5 ± 2.1	97.2 ± 1.7	132.2 ± 13.0
60	38.0 ± 0.5^{B}	102.7 ± 4.2^{A}	24.9 ± 1.8	97.0 ± 1.8	133.1 ± 12.5

Explanations: When comparing the same monitoring project at different time points, the letters on the shoulders are different, indicating significant differences between the groups ($P < 0.05$). HR – heart rate; RR – respiratory rate; SPO_2 – pulse oximetry; MAP – minimal arterial pressure; T – temperature

Tab. 5. Anesthesia time monitoring results ($\bar{x}$ ± SD, n = 10, min)

Anesthesia period	Test fox number										
	1	2	3	4	5	6	7	8	9	10	
Induction period	14	15	18	13	14	8	13	12	12	9	12.8 ± 2.9
Maintenance period	74	68	67	73	72	68	68	62	72	66	69.0 ± 3.4
Awakening period	36	36	50	41	38	37	33	37	35	42	38.5 ± 4.8

Tab. 6. Monitoring results of anesthesia effect ($\bar{x} \pm SD$, n = 10)

Time-points	Sedation	Analgesia	Muscle relaxation
10 min	3.0^{A}	2.2 ± 0.8^{B}	3.0^{A}
20 min	3.0^{A}	3.0^{A}	3.0^{A}
30 min	3.0^{A}	3.0^{A}	3.0^{A}
40 min	3.0^{A}	3.0^{A}	3.0^{A}
50 min	3.0^{A}	3.0^{A}	3.0^{A}
60 min	2.8 ± 0.4^{B}	2.2 ± 0.8^{B}	2.8 ± 0.4^{B}

Explanation: When comparing the same monitoring project at different time points, the letters on the shoulders are different, indicating significant differences between the groups ($P < 0.05$)

a more significant recovery of an analgesic effect than others. The specific monitoring findings are mentioned in Table 6.

Monitoring of biological reflex activity. Ten minutes after oral administration of anesthesia, the corneal and eyelid reflexes were blunted in 60% of the experimental foxes, and the anal reflexes were blunted in all experimental foxes. Specifically, each of these three biological reflexes were significantly inhibited in the 20-50 min post-dose period, resulting in a more appropriate depth of anesthesia. The three biological reflexes began to recover to varying degrees 60 min after administration, and the most significant recovery was of the corneal reflex. The specific monitoring results are shown in Table 7.

Tab. 7. Monitoring results of biological reflection activities (n = 10)

Point in time	Corneal reflex			Eyelid reflex			Anal reflex		
	+	±	−	+	±	−	+	±	−
10 min	4	6	0	4	6	0	0	10	0
20 min	3	7	0	0	10	0	0	10	0
30 min	0	10	0	0	10	0	0	10	0
40 min	0	10	0	0	10	0	0	10	0
50 min	1	9	0	1	9	0	0	10	0
60 min	7	3	0	3	7	0	2	8	0

Explanation: The data in the table represents the basic situation of biological reflection monitoring of 10 experimental foxes

Monitoring the circulatory and respiratory system during the clinical administration of anesthesia is an important aspect of evaluating the safety of anesthesia and can objectively and scientifically evaluate the effects of anesthetic drugs and delivery methods on the normal physiological functions of the body (14, 20). If on-time measures are not taken to correct the abnormal changes in physiological parameters during anesthesia administration, this can lead to significant anesthetic complications in the peri-anesthetic period and may even lead to anesthesia failure (10, 19). The significant temperature changes (T) seen in this trial were more closely related to α-chloralose, and similar changes in T were seen during anesthesia in dogs and wolves using this drug, which was associated with delayed receipt of the signal for significant inhibition by the brain thermoregulatory center; prompt warming measures are recommended during anesthesia to prevent the occurrence of postoperative chills (9, 21). Previous pre-anesthesia tests showed that HR was also significantly altered, mainly concerning the effect of dexmedetomidine, and the degree of HR decrease was closely related to the dose of the drug used (6).

Period monitoring during anesthesia administration is a fundamental part of anesthesia assessment and involves recording the duration of induction, maintenance, and awakening (20). The length of the maintenance period of general anesthesia, in combination with its effect, determines the basic elements of the treatment and is an important reference for the adjustment of drug dose and the subsequent addition of anesthetic drugs. The length of the recovery period can guide post-treatment supervision and is of great significance for large domestic animals as well as wild animals (17). The ideal period of anesthesia should be one of smooth and rapid induction and awakening, with appropriate and effective maintenance of anesthesia (6). This study shows that the induction of oral anesthesia was smooth, with no common complications such as abnormal excitement, salivation, and vomiting. In addition, the anesthesia maintenance time was appropriate and adequate for the clinical treatment of foxes; the awakening was smooth, with no common complications such as re-sleeping and chills, which helped to improve the controllability and safety of the anesthesia.

Monitoring the sedative, analgesic, and inotropic effects of the anesthetic state is the main indicator for evaluating the suitability of an anesthetic drug or the method for clinical use (1, 6). α-Chloralose significantly enhances the affinity of the gamma-aminobutyric acid (GABA) type A receptor for GABA in compound oral anesthesia methods and has been found to activate this receptor (15). Thus, α-chloralose has a more definite anesthetic effect. Dexmedetomidine has a sedative, inotropic, and analgesic effect, in addition to its pre-anesthetic administration, and has been used in clinical studies involving compound anesthesia in a variety of animals (5, 6, 12). The combination of the two drugs, on the one hand, reduces the dose of both drugs and thus also reduces the toxic effects of both; nonetheless, the primary objective is to achieve a good balance of anesthetic effects (4). In this study, the sedative, analgesic, and inotropic effects of the two drugs were well balanced after 20-50 min of oral administration, giving full play to the complementary anesthetic effects. In addition, the routine physiological indicators showed transient and tolerable effects of the combination of the two drugs on the body, all of which reflected the advantages of the combination of anesthesia and effectively improved its safety.

The biological reflexes of the eyelids, cornea, and anus are important indicators of the depth of anesthesia (6). Besides indicating the state of consciousness, the biological reflexes of the eyelids, cornea, and anus are also important indicators of the depth of anesthesia. Reflex monitoring is practical, convenient, non-invasive, and effective, and is used in the peri-anesthetic monitoring of humans and animals (19). These three biological reflexes were apparent throughout this experiment. The finding that the inhibition of the three reflexes diminished over time indicates that there was no over-anesthesia and that the inhibition of the three biological reflexes during the 20-50 min period was appropriate to produce a good depth of anesthesia. Complementing the results of this finding with the effects of sedation, analgesia, and inotropic effects provides further evidence that this combination of oral anesthesia produces good anesthesia at an appropriate depth and balanced effect for approximately 30 min.

In conclusion, α-chloralose and dexmedetomidine were evenly mixed into the feed at 360 mg/kg and 0.6 mg/kg, respectively, to induce oral anesthesia in foxes, resulting in smooth anesthesia induction, a good anesthetic effect for about 30 min with a balanced effect, at the right depth, and a relatively smooth awakening, which has a relatively mild and brief effect on the main physiological parameters.

References

1. *Ansah O. B., Raekallio M., Vainio O.*: Comparison of three doses of dexmedetomidine with medetomidine in cats following intramuscular administration. J. Vet. Pharmacol. Ther. 1998, 21 (5), 380-387.
2. *Baldwin J. R., Winstead J. B., Hayden-Wing L. D., Kreeger T. J., Dzialak M. R.*: Field sedation of coyotes, red foxes, and raccoons with medetomidine and atipamezole. J. Wildlife. Manag. 2008, 72 (5), 1267-1271.
3. *Beam D. M., Neto-Neves E. M., Stubblefield W. B., Alves N. J., Tune J. D., Kline J. A.*: Comparison of isoflurane and α-chloralose in an anesthetized swine model of acute pulmonary embolism producing right ventricular dysfunction. Comparative Med. 2015, 65 (1), 54-61.
4. *Boorman S., Stefanovski D., Southwood L. L.*: Clinical findings associated with development of postoperative reflux and short-term survival after small intestinal surgery in geriatric and mature nongeriatric horses. Vet. Surg. 2019, 48 (5), 795-802.
5. *Bunke L. G., Sladky K. K., Johnson S. M.*: Antinociceptive efficacy and respiratory effects of dexmedetomidine in ball pythons (Python regius). Am. J. Vet. Res. 2018, 79 (7), 718-726.
6. *Diao H. X., Zhang S., Hu X. Y., Guan W., Luan L., Liu H. Y., Fan H. G.*: Behavioral and cardiopulmonary effects of dexmedetomidine–midazolam and dexmedetomidine–midazolam–butorphanol in the silver fox (Vulpes vulpes). Vet. Anaesth. Analg. 2017, 44 (1), 114-120.
7. *Garrett K. M., Gan J.*: Enhancement of γ-aminobutyric acidA receptor activity by α-chloralose. J. Pharmacol. Exp. Ther. 1998, 285 (2), 680-686.
8. *Holzgrefe H. H., Everitt J. M., Wright E. M.*: Alpha-chloralose as a canine anesthetic. Lab. Anim. Sci. 1987, 37 (5), 587-595.
9. *Jie Z. Z., Chao T. Q., Wei G., Biao M., Gang F. H., Bin W. H.*: Observation on the anesthetic effect of oral administration of dex-medetomidine and α-chloralose on wolves. Heilongjiang Anim. Sci. Vet. Med. 2021, 23, 83-87 (in Chinese).
10. *Jones J. L., Rinehart J., Spiegel J. J., Englar R. E., Sidaway B. K., Rowles J.*: Teaching tip: development of veterinary anesthesia simulations for pre-clinical training: design, implementation, and evaluation based on student perspectives. J. Vet. Med. Educ. 2018, 45 (2), 232-240.
11. *Kaneko I., Takeuchi Y., Fukumori Y., Fukuda T.*: Effect of alpha-chloralose on disposition and pharmacological action of orally administered chlorzoxazone in rats. J. Pharmacobiodyn. 1991, 14 (8), 429-434.
12. *Khenissi L., Nikolayenkova-Topie O., Broussaud S., Touzot-Jourde G.*: Comparison of intramuscular alfaxalone and ketamine combined with dexmedetomidine and butorphanol for castration in cats. J. Feline. Med. Surg. 2017, 19 (8), 791-797.
13. *Kumamoto E., Murata Y.*: Enhancement by lanthanide of general anesthetic-induced GABAA-receptor current in rat septal cholinergic neurons in culture. J. Neurophysiol. 1996, 75 (6), 2294-2299.
14. *Lee C., Jones T. A.*: Effects of ketamine compared with urethane anesthesia on vestibular sensory evoked potentials and systemic physiology in mice. J. Am. Assoc. Lab. Anim. Sci. 2018, 57 (3), 268-277.
15. *Lees P.*: Pharmacology and toxicology of alpha chloralose: a review. Vet. Rec. 1972, 91 (14), 330-333.
16. *Luckl J., Keating J., Greenberg J. H.*: Alpha-chloralose is a suitable anesthetic for chronic focal cerebral ischemia studies in the rat: a comparative study. Brain Res. 2008, 1191, 157-167.
17. *Neudeck S., Kästner S. B. R., Wittenberg-Voges L., Rohn K., Hopster K.*: Comparison of desflurane and propofol at equipotent doses in combination with a constant rate infusion of dexmedetomidine on global and peripheral perfusion and oxygenation in horses. Am. J. Vet. Res. 2018, 79 (5), 487-495.
18. *Pypendop B. H., Barter L. S., Stanley S. D., Ilkiw J. E.*: Hemodynamic effects of dexmedetomidine in isoflurane-anesthetized cats. Vet. Anaesth. Analg. 2011, 38 (6), 555-567.
19. *Raamsdonk L. W. D. van, Prins T. W., Meijer N., Scholtens I. M. J., Bremer M. G. E. G., de Jong J.*: Bridging legal requirements and analytical methods: a review of monitoring opportunities of animal proteins in feed. Food Addit. Contam. A 2019, 36 (1), 46-73.
20. *Schmeling W. T., Farber N. E.*: Anesthetic actions on cardiovascular control mechanisms in the central nervous system[M]. Adv. Pharmacol. 1994, 31, 617-642.
21. *St-Georges D., Matthews C., Nattel S.*: Continuous maintenance infusion technique for stable anesthesia in the dog, using α-chloralose. Lab. Anim. Sci. 1997, 36 (2), 62-65.

Corresponding author: Hong-Bin Wang, Heilongjiang Key Laboratory for Laboratory Animals and Comparative Medicine, College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, P. R. China; e-mail: hbwang1940@neau.edu.cn