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Efficacy and safety of higher oral doses of azaperone to achieve sedation in pigs

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Abstract: The aim of this study is to evaluate the possibility of achieving more effective and prolonged sedation in pigs by the oral administration of increased doses of azaperone and to evaluate its safety. This was performed through a prospective randomised and double blinded study. A total of 32 weaned piglets were divided into 4 groups (8 in each group). Group A was given 1 ml of saline orally and served as the control group. Group B received azaperone orally at a dose of 4 mg/kg b.w. Group C received azaperone orally at a dose of 8 mg/kg b.w. Group D was given azaperone orally at a dose of 12 mg/kg b.w. The response to the defined stimulus, movement level, degree of salivation, body temperature, respiratory frequency, blood plasma azaperone concentration and biochemical variables were included in the trial. We found that by increasing the dose of the orally administered azaperone, the onset of the sedation is faster, the end of the sedation starts later and the sedation time is longer. However, the use of higher doses of oral azaperone is not suitable for piglets because the doses negatively affect the respiratory rate, body temperature, some biochemical parameters and cause the immobility of the piglets.

Keywords: behaviour; biochemical indicators; pharmacodynamics

The stressful conditions that pigs encounter in intensive farming environments, such as weaning, transport, mixing pigs from different litters and the subsequent fighting, require the use of sedatives (Dantzer 1977; Martinez-Miro et al. 2016). The most commonly used sedative in pigs is azaperone. As a member of the butyrophenone family, azaperone exerts its effects mainly through antagonism toward the G-protein coupled dopamine D2 receptor and its anti-adrenergic properties (Golan 2016). It is used in pigs to reduce stress, but also to reduce aggression (Porter and Slusser

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1985; Schwarz et al. 2018). Under practical conditions, the sedation of pigs is performed by injecting azaperone at a dose of 2 mg/kg b.w. The disadvantage of the i.m. administration is that azaperone leaves high and long-lasting concentrations at the injection site (Mestorino et al. 2013). This fact excludes the possibility of its use in the transport of pigs to the slaughterhouse. A possible solution could be to use an oral administration. Mestorino et al. (2013) showed that after the oral administration of azaperone, the concentration of azaperone in the analysed tissues did not exceed the maximum residue limit set by the EU. In our previous study (Svoboda et al. 2021), we found that the oral administration of azaperone at a dose of 2 mg/kg orally induces sedation in piglets, but to obtain a clinically comparable sedation with an injection (2 mg/kg i.m.), it is necessary to use a dose of 4 mg/kg b.w. We also found that with an increasing orally administered azaperone dose, the onset of the sedation is faster, the end of the sedation occurs later and the sedation time is longer. There are no data available in the literature on how increased doses of azaperone affect piglets. Therefore, we decided to assess the possibility of achieving more effective and prolonged sedation in pigs by the oral administration of increased doses of azaperone and to determine what effect these doses have on the overall condition of the organism.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of the University of Veterinary Sciences Brno. A total of 32 domestic pigs (Danbred) were used after weaning, i.e., at the age of about 28 days. Only gilts were used in the study. The piglets were brought to the stables of our clinic and isolated in quarantine for two weeks, which was sufficient time to acclimatise these animals. The piglets were fed standard granular mixtures. The diets were produced by De Heus a.s. (Marefy, Czech Republic). The piglets were marked with a plastic ear tag in the right ear. After acclimatisation, the experimental piglets were randomly divided into 4 groups of 8 animals. The randomisation was achieved by drawing the animals using numbered papers. We created four groups this way (8 pigs in each group):

- Group A [body weight (b.w.), mean ± standard deviation, 7.12 ± 0.69 kg, control] was given 1 ml of saline orally and served as the control group.
- Group B (7.10 ± 1.10 kg) received azaperone (Stresnil®, 40 mg/ml inj.; Elanco Animal Health, Greenfield, IN, USA) orally at a dose of 4 mg/kg b.w.
- Group C (7.14 ± 0.80 kg) received azaperone orally at a dose of 8 mg/kg b.w.
- Group D (7.14 ± 0.87 kg) was given azaperone orally at a dose of 12 mg/kg b.w.

In all the groups, the level of sedation was monitored based on the response to loud stimulation by a blunt blow of metal onto the metal barrier of the pen. This parameter was monitored every 15 min and evaluated in the following stages: 0 – high degree reaction (jump, escape), 1 – medium degree reaction (no hops, but reaction – step aside, head movement, muscle tremors, raising ears), 2 – no reaction.

Furthermore, the physiological functions of the pigs were evaluated (0, 30, 90, 240 and 360 min after application). This included the movement, degree of salivation and respiratory frequency. The observations were evaluated as follows: 0 – normal movement, 1 – ataxic or less active, 2 – lying down.

The degree of salivation was evaluated as follows: 0 – no salivation, 1 – moderate level of salivation (discharge of a small amount of saliva from the corners of the mouth), 2 – high level of salivation (an overflow of saliva from the mouth, drooling).

The respiratory frequency was measured by the chest wall movements per minute. Bradypnoea (< 25), eupnoea (25–40), and tachypnoea (> 40) were distinguished in the evaluation of the results.

The piglets' body temperature was measured with a digital thermometer inserted into the rectum. It was recorded in degrees Celsius (°C).

The piglets were bled before the start of the experiment (before the application of azaperone) and at intervals of 0, 30, 90, 240 and 360 min after the application. A blood sample was taken from the *vena cava cranialis.* Heparin was used as an anticoagulant to determine the azaperone and biochemical indicators in the blood plasma.

Biochemical indicator analysis

The following biochemical indicators were included in the analyses: total protein (TP), albumin (ALB), urea, glucose (GLU), creatinine (CREAT), bilirubin (BIL), alanine aminotransferase (ALT), al-

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kaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH). These indicators were determined using a Konelab 20i Biochemical Analyser and commercial kits (Biovendor, Brno, Czech Republic).

In the plasma, the azaperone concentrations were determined by ELISA (Enzyme-Linked Immunosorbent Assay) with a commercial kit produced by EuroProxima (Arnhem, The Netherlands).

The biochemical variables were determined at 0, 30, 90 and 240 min after the administration. The plasma azaperone concentrations were determined at 0, 30, 90 and 240 min after the administration. The reason for choosing these intervals to determine the concentrations of azaperone in the plasma was information obtained from the study of Heykants et al. (1971) conducted on rats. The maximal blood levels of azaperone in the mentioned study were obtained about half an hour after the i.m. administration which then decline rapidly during the following 4 hours.

Statistical analysis

Standard descriptive statistics was applied in the analysis; the mean supplemented with a standard deviation and the median supplemented with a min-max range. The statistical significance of the differences among the groups was tested using the Kruskal-Wallis test followed by the Mann-Whitney test for between the groups comparison; Friedman's test followed by Wilcoxon's test was applied for the analysis of the statistical significance of the differences among and between the time points. The analysis was computed using SPSS v28.0.1.1 (IBM, Armonk, NY, USA).

RESULTS

The results are presented as the mean ± standard deviation (SD) in the case of the rectal temperature, plasma concentration of azaperone and biochemical indices. In the case of the assessment of the sedation, movement, salivation, and respiratory frequency, the results are presented as the percentage of the piglets in the group belonging to a certain grade.

Evaluation of the sedation level – response to the loud stimulation

The results of the evaluation of the sedation level are presented in Table 1.

Table 1. Response to the loud stimulation

Observation time (min)	Groups											
	A (control; *n* = 8; %)			B (4 mg/kg b.w.; *n* = 8; %)			C (8 mg/kg b.w.; *n* = 8; %)			D (12 mg/kg b.w.; *n* = 8; %)		
	0	1	2	0	1	2	0	1	2	0	1	2
15	87.50	12.50	0	25	62.50	12.50	12.50	37.50	50	0	50	50
30	0	100	0	12.5	50	37.50	0	25	75	0	25	75
45	12.50	75	12.50	0	25	75	0	12.50	87.50	0	25	75
60	12.50	62.50	25	0	12.50	87.50	0	0	100	0	25	75
75	12.50	62.50	25	0	12.50	87.50	0	0	100	0	25	75
90	12.50	62.50	25	0	25	75	0	0	100	0	0	100
105	12.50	62.50	25	0	25	75	0	0	100	0	0	100
120	12.50	50	37.50	0	37.50	62.50	0	50	50	0	12.50	87.50
135	12.50	75	12.50	0	50	50	0	75	25	0	25	75
150	25	50	25	12.50	50	37.50	12.50	50	37.50	0	50	50
165	75	25	0	12.50	50	37.50	25	62.50	12.50	0	75	25
180	62.50	37.50	0	25	37.50	37.50	12.50	87.50	0	25	50	25
195	62.50	37.50	0	25.00	37.50	37.50	12.50	87.50	0	25	75	0
210	75	25	0	37.50	62.50	0	25	75	0	25	75	0
225	50	50	0	37.50	62.50	0	25	75	0	25	75	0
240	37.50	62.50	0	50	50	0	50	50	0	37.50	62.50	0

The results are presented as the percentage of piglets in the group belonging to a certain grade

0 – high grade reaction (e.g., jumping, running); 1 – medium grade reaction (e.g., moving the head); 2 – no response

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Table 2. Degree of salivation and movement level

Groups	OT (min)	Degree of salivation (%)			Movement level (%)		
		0	1	2	0	1	2
A (n = 8) control	0	100	0	0	100	0	0
	30	100	0	0	100	0	0
	90	100	0	0	100	0	0
	240	100	0	0	100	0	0
	360	100	0	0	100	0	0
B (n = 8) 4 mg/kg b.w.	0	100	0	0	100	0	0
	30	0	100	0	0	100	0
	90	100	0	0	0	100	0
	240	100	0	0	100	0	0
	360	100	0	0	100	0	0
C (n = 8) 8 mg/kg b.w.	0	100	0	0	100	0	0
	30	87.50	12.50	0	0	100	0
	90	100	0	0	0	25	75
	240	100	0	0	50	50	0
	360	100	0	0	100	0	0
D (n = 8) 12 mg/kg b.w.	0	100	0	0	100	0	0
	30	100	0	0	62.50	37.50	0
	90	100	0	0	0	0	100
	240	100	0	0	0	25	75
	360	100	0	0	100	0	0

The results are presented as the percentage of piglets in the group belonging to a certain grade

Salivation score: 0 – no salivation; 1– moderate level of salivation; 2 – high level of salivation; Movement score: 0 – normal movement; 1– ataxic or less active; 2 – lying down

OT = observation time

Table 3. Results of the vital parameters

Groups	OT (min)	Respiratory frequency (%)			Body temperature (°C)
		bradypnoea	eupnoea	tachypnoea	
A (n = 8) control	0	0	100	0	39.3 ± 0.2^{x}
	30	0	100	0	39.4 ± 0.2^{x}
	90	0	100	0	39.5 ± 0.2^{x}
	240	0	100	0	39.4 ± 0.3^{x}
	360	0	100	0	39.3 ± 0.3^{x}
B (n = 8) 4 mg/kg b.w.	0	0	100	0	39.2 ± 0.3^{x}
	30	0	100	0	38.5 ± 0.4^{y}
	90	0	100	0	38.6 ± 0.4^{y}
	240	0	100	0	38.7 ± 0.4^{y}
	360	0	100	0	39.2 ± 0.3^{x}
C (n = 8) 8 mg/kg b.w.	0	0	100	0	39.6 ± 0.3^{x}
	30	0	12.50	87.50	38.2 ± 0.3^{y}
	90	0	12.50	87.50	38.5 ± 0.3^{y}
	240	0	12.50	87.50	39.0 ± 0.2^{z}
	360	0	87.50	12.50	39.4 ± 0.3^{x}
D (n = 8) 12 mg/kg b.w.	0	0	100	0	$39.1 \pm 0.5^{x,z}$
	30	0	12.50	87.50	38.1 ± 0.6^{y}
	90	0	0	100	38.4 ± 0.5^{x}
	240	0	12.50	87.50	38.8 ± 0.4^{x}
	360	0	37.50	62.50	$39.1 \pm 0.3^{x,z}$

$^{x–z}$Means with the same measurement time and column lacking a common superscript letter differ significantly ($P < 0.05$)

The results of the respiratory frequency are presented as the percentage of piglets in the group belonging to a certain grade; bradypnoea (< 25), eupnoea (25–40), tachypnoea (> 40); Body temperatures are presented as the mean and standard deviation

OT = observation time

Table 4. Concentration of azaperone in the blood plasma (ng/ml)

Blood collection time (min)	Groups			
	A (control, n = 8)	B (4 mg/kg b.w., n = 8)	C (8 mg/kg b.w., n = 8)	D (12 mg/kg b.w., n = 8)
30	n.d.	168.7 ± 66.1^{x}	198.1 ± 9.2^{x}	260.1 ± 110.6^{x}
90	n.d.	106.3 ± 46.5^{x}	117.3 ± 67.4^{x}	188.8 ± 90.4^{x}
240	n.d.	44.1 ± 13.4^{x}	56.9 ± 31.7^{x}	93.2 ± 55.9^{x}
360	n.d.	22.0 ± 7.3^{y}	38.6 ± 20.5^{x}	36.3 ± 16.8^{x}

^{x,y}Means with the same blood collection time and row lacking a common superscript letter differ significantly ($P < 0.05$)

The results are presented as the mean ± standard deviation

n.d. = non-detected – azaperone concentration below the detection limit (0.785 ng/ml)

In our experiment, after the oral azaperone administration at a dose of 4 mg/kg b.w., satisfactory sedation was achieved approximately after 45 min and the duration of the sedation was about 135 minutes. With a dose of 8 mg/kg, satisfactory sedation started already in the 30th minute after the application. This condition lasted until the 135th minute. With a dose of 12 mg/kg, satisfactory sedation started already in the 15th minute after the application and lasted up to 165 minutes.

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Table 5. Results of the biochemical parameters

Parameters	OT (min)	Groups			
		A (control, *n* = 8)	B (4 mg/kg b.w., *n* = 8)	C (8 mg/kg b.w., *n* = 8)	D (12 mg/kg b.w., *n* = 8)
TP (g/l)	0	48.3 ± 2.3^{x}	49.7 ± 3.8^{x}	48.8 ± 2.8^{x}	49.3 ± 2.9^{x}
	30	46.5 ± 1.2^{x}	46.9 ± 3.5^{x}	45.3 ± 2.1^{x}	45.8 ± 1.6^{x}
	90	46.5 ± 2.9^{x}	45.5 ± 3.6^{x}	44.8 ± 3.4^{x}	44.8 ± 0.9^{x}
	240	44.6 ± 4.3^{x}	46.7 ± 4.3^{x}	45.0 ± 3.7^{x}	45.2 ± 2.4^{x}
	360	45.8 ± 2.9^{x}	46.3 ± 2.8^{x}	45.2 ± 3.3^{x}	45.4 ± 5.1^{x}
ALB (g/l)	0	32.0 ± 2.0^{x}	31.7 ± 1.9^{x}	32.0 ± 2.3^{x}	32.7 ± 1.7^{x}
	30	32.4 ± 2.4^{x}	31.1 ± 2.3^{x}	29.9 ± 2.3^{x}	29.9 ± 1.0^{x}
	90	32.3 ± 2.0^{x}	31.2 ± 2.4^{x}	30.7 ± 2.5^{x}	30.2 ± 1.0^{x}
	240	29.7 ± 4.2^{x}	31.0 ± 2.5^{x}	30.2 ± 4.9^{x}	29.9 ± 2.0^{x}
	360	31.3 ± 2.1^{x}	31.6 ± 1.7^{x}	31.0 ± 2.7^{x}	29.3 ± 1.9^{x}
UREA (mmol/l)	0	1.5 ± 0.3^{x}	1.6 ± 0.5^{x}	1.5 ± 0.4^{x}	1.3 ± 0.2^{x}
	30	1.4 ± 0.3^{x}	1.9 ± 0.7^{x}	1.6 ± 0.3^{x}	1.3 ± 0.3^{x}
	90	1.5 ± 0.4^{x}	1.5 ± 0.4^{y}	1.7 ± 0.3^{x}	1.3 ± 0.3^{x,z}
	240	1.6 ± 0.4^{x}	2.3 ± 0.3^{y}	2.0 ± 0.4^{x}	1.7 ± 0.4^{x,z}
	360	1.6 ± 0.3^{x}	2.4 ± 0.3^{y}	2.1 ± 0.5^{y,z}	1.7 ± 0.4^{x,z}
GLU (mmol/l)	0	6.4 ± 1.4^{x}	7.4 ± 1.1^{x}	6.3 ± 1.4^{x}	6.4 ± 0.8^{x}
	30	7.0 ± 0.5^{x}	6.1 ± 1.1^{x}	6.2 ± 0.9^{x}	6.3 ± 0.8^{x}
	90	7.0 ± 0.6^{x}	7.9 ± 1.1^{x}	6.8 ± 1.1^{x}	7.1 ± 0.6^{x}
	240	6.7 ± 0.7^{x}	7.4 ± 1.2^{x}	6.5 ± 0.8^{x}	7.4 ± 0.4^{x}
	360	5.9 ± 0.6^{x}	5.9 ± 0.6^{x,z}	6.8 ± 1.1^{y,z}	7.4 ± 0.7^{y}
CREAT (µmol/l)	0	124.0 ± 29.4^{x}	109.1 ± 20.7^{x}	107.3 ± 19.0^{x}	96.6 ± 5.2^{x}
	30	99.0 ± 12.2^{x}	99.5 ± 19.7^{x}	99.2 ± 19.7^{x}	94.2 ± 10.7^{x}
	90	96.5 ± 12.7^{x}	93.4 ± 13.0^{x}	94.6 ± 10.5^{x}	86.1 ± 12.4^{x}
	240	92.2 ± 22.1^{x}	84.4 ± 12.7^{x}	82.0 ± 9.1^{x}	116.7 ± 22.7^{y}
	360	89.5 ± 19.6^{x}	86.5 ± 24.6^{x}	111.2 ± 22.8^{x}	118.4 ± 27.2^{x}
BIL (µmol/l)	0	2.1 ± 0.4^{x}	2.2 ± 1.6^{x}	1.7 ± 0.4^{x}	1.9 ± 0.6^{x}
	30	1.8 ± 0.2^{x}	2.7 ± 1.0^{y}	2.6 ± 0.8^{y}	2.9 ± 0.6^{y}
	90	1.9 ± 0.3^{x}	2.8 ± 0.8^{y}	2.7 ± 0.9^{y}	2.5 ± 0.5^{y}
	240	1.3 ± 0.5^{x}	1.7 ± 0.5^{x,z}	2.3 ± 0.8^{y,z}	1.9 ± 0.3^{y,z}
	360	2.1 ± 0.3^{x}	2.2 ± 0.2^{x}	2.3 ± 0.4^{x}	2.0 ± 0.3^{x}
ALT (µkat/l)	0	0.8 ± 0.1^{x}	0.9 ± 0.2^{x}	1.0 ± 0.2^{x}	1.0 ± 0.1^{x}
	30	1.0 ± 0.1^{x}	1.1 ± 0.1^{x}	1.1 ± 0.3^{x}	1.1 ± 0.2^{x}
	90	1.1 ± 0.2^{x}	1.0 ± 0.1^{x}	1.0 ± 0.2^{x}	1.0 ± 0.2^{x}
	240	1.0 ± 0.2^{x}	1.0 ± 0.1^{x}	1.0 ± 0.3^{x}	1.0 ± 0.1^{x}
	360	1.1 ± 0.2^{x}	1.0 ± 0.1^{x}	1.1 ± 0.3^{x}	1.1 ± 0.3^{x}
ALP (µkat/l)	0	9.0 ± 1.4^{x}	8.6 ± 1.6^{x}	8.3 ± 1.5^{x}	8.8 ± 1.1^{x}
	30	8.9 ± 1.4^{x}	8.4 ± 1.6^{x}	7.9 ± 1.7^{x}	8.2 ± 1.0^{x}
	90	8.8 ± 1.5^{x}	8.3 ± 1.4^{x}	8.0 ± 1.9^{x}	7.9 ± 1.1^{x}
	240	8.5 ± 1.5^{x}	8.3 ± 1.5^{x}	7.3 ± 1.2^{x}	8.0 ± 0.9^{x}
	360	8.6 ± 1.3^{x}	8.5 ± 1.7^{x}	7.7 ± 1.4^{x}	8.1 ± 0.7^{x}
GGT (µkat/l)	0	0.8 ± 0.1^{x}	0.8 ± 0.1^{x}	0.8 ± 0.1^{x}	0.8 ± 0.1^{x}
	30	0.7 ± 0.1^{x,z}	0.7 ± 0.1^{x}	0.8 ± 0.1^{y,z}	0.8 ± 0.1^{y}
	90	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}	0.8 ± 0.1^{x}	0.8 ± 0.1^{x}
	240	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}
	360	0.8 ± 0.1^{x}	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}	0.7 ± 0.1^{x}

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Table 5 to be continued

Parameters	OT (min)	Groups			
		A (control, *n* = 8)	B (4 mg/kg b.w., *n* = 8)	C (8 mg/kg b.w., *n* = 8)	D (12 mg/kg b.w., *n* = 8)
LDH (μkat/l)	0	12.5 ± 1.9^{x}	11.9 ± 1.9^{x}	13.4 ± 2.1^{x}	13.9 ± 2.1^{x}
	30	14.3 ± 1.9^{x}	13.2 ± 1.9^{x}	15.1 ± 3.2^{x}	16.7 ± 2.8^{x}
	90	14.8 ± 3.0^{x}	13.8 ± 2.0^{x}	17.2 ± 4.2^{x}	17.5 ± 4.2^{x}
	240	14.2 ± 1.5^{x}	13.6 ± 1.6^{x}	15.7 ± 5.4^{x}	16.3 ± 3.1^{x}
	360	14.6 ± 1.5^{x}	13.7 ± 1.8^{x}	14.6 ± 4.6^{x}	15.0 ± 3.3^{x}

The results are presented as the mean and standard deviation

$^{x–z}$Means with the same blood collection time and row lacking a common superscript letter differ significantly ($P < 0.05$)

ALB = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; BIL = bilirubin; CREAT = creatinine; GGT = gamma-glutamyl transferase; GLU = glucose; LDH = lactate dehydrogenase; OT = observation time; TP = total protein

Degree of salivation

The results of the evaluation of the salivation are presented in Table 2.

In all the groups, the degree of salivation did not increase during the experiment.

Movement level

The results of the evaluation of the movement are presented in Table 2.

Normal movement was observed in the control group throughout the experiment. Lying and ataxic piglets were observed in the time interval from the 90th minute to the 240th minute in groups C and D.

Vital parameters

As part of the monitoring of the vital parameters, the level of respiration and body temperature were recorded. The results are given in Table 3.

Eupnoea was observed in the control group A and group B throughout the experiment. In control group A and group B, the monitored temperature only showed small deviations. In groups C and D, we observed tachypnoea which occurred between 30 and 360 minutes. A significant drop in temperature was observed in both groups from the 30th minute.

Plasma concentrations of azaperone

The results of the concentration of azaperone in the blood plasma are presented in Table 4.

No plasma azaperone concentrations were detected in the control group.

In all the experimental groups, the maximal azaperone levels in the blood plasma were detected 30 min after administration (time to maximum plasma concentration, T_{max}). Thereafter, the values decreased relatively quickly.

The maximum plasma concentrations (C_{max}) in groups B, C, and D reached values of 270.94, 354.85, 475.91 ng/ml, respectively.

At the 360th minute, the azaperone concentrations in group C and D were significantly higher than in group B. No statistically significant differences in the azaperone concentrations between the experimental groups were found for the other times during the trial.

Biochemical indicators

The results are shown in Table 5.

The bilirubin concentrations in the blood plasma were significantly higher in groups B, C and D compared to control group A 30, 90 and 240 min after administration.

The GGT enzyme activities in the blood plasma were significantly higher in group D compared to control group A 30 min after application.

The plasma glucose concentrations in groups C and D were significantly higher compared to control group A 30 min after administration.

The plasma creatinine levels were significantly higher in group D compared to control group A 240 min after administration.

The plasma urea concentrations in group B were significantly higher than in control group A at 90,

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240 and 360 min after administration. In group C, the plasma urea concentrations were higher 360 min after administration.

No other significant differences in the biochemical profile were found during the experiment in comparison to each of the groups.

DISCUSSION

It is well known that increased stress situations can have a negative impact on the productivity of pigs. Stress in breeding sows can negatively affect the reproduction (Etim et al. 2013). Stress can also affect the boar's reproductive functions (Kamanova et al. 2021). Weaned piglets' stress can be a significant predisposition to diarrhoea (Lin et al. 2022). The use of sedatives can reduce the negative effects of these factors (Dantzer 1977).

The disadvantage of the i.m. administration of azaperone is the short duration of action of the active substance. Jones (1972) states that azaperone (i.m.) reaches its maximum effect after 15 min in young pigs, after 30 min in adult pigs and the duration of action is from 2 h to 4 hours. Any repeated injection is laborious and stressful for pigs. The short-term effect may be limiting, for example, during prolonged transport or when used to reduce aggression and fighting between piglets after weaning (Tan and Shackleton 1990). Based on our previous study (Svoboda et al. 2021), we hypothesised the possibility of achieving more effective and prolonged sedation in pigs by the oral administration of increased doses of azaperone.

The advantage of an oral application of azaperone from the point of view of welfare is the possibility of its mass use in drinking water or feed without the need to restrain the pigs. However, with this route of administration, there is a risk that some pigs will take higher doses of the active substance. There are no data available in the literature on how increased doses of oral azaperone affect piglets. Only data from acute toxicity tests in laboratory animals are available (oral administration, mouse LD50 385 mg/kg, rat LD50 245 mg/kg, guinea pig LD50 202 mg/kg (Niemegeers et al. 1974). Therefore, an evaluation of the effect of high oral doses on the overall condition of the organism was included in the study.

It is obvious, from our results, that by increasing the dose of orally administered azaperone, the onset of the sedation is faster, the end of the sedation starts later and the sedation time is longer.

Other physiological indicators were included in the study (degree of salivation, motoric activity, respiratory rate, rectal temperature). In the available literature, there are no data about the influence of oral azaperone on these indicators.

Increased salivation after azaperone administration was noted by Nishimura et al. (1993) after an intramuscular administration at a dose of 8 mg/kg b.w. In our experiment, no change in the salivation was observed with the oral azaperone even after the administration of very high doses (8 mg/kg, 12 mg/kg b.w.).

As found by Holzchuh and Cremonesi (1991), the muscle tone can be diminished after azaperone administration. This was also confirmed in our study. With a dose of 8 mg/kg, 75% of the piglets were lying down at 90 minutes. With a dose of 12 mg/kg, 100% of the piglets were lying down at 90 min and 75% of the piglets were still lying down at 240 minutes. The immobility of the piglets would exclude the use of such high azaperone doses under practical conditions (loading piglets before transport).

Lang (1970) noticed an increased respiratory rate after the intramuscular administration at a dose of 5–6 mg/kg b.w. This is in agreement with our findings since, after the oral azaperone administration at very high doses (8 mg/kg, 12 mg/kg b.w.), tachypnoea was present in these groups throughout the experiment.

Marsboom and Symoens (1968) reported that the rectal temperature decreased by 1–2 °C during the 4 h after a parenteral administration (5 mg/kg b.w. i.m.). This is in agreement with our findings since, after the oral azaperone administration at very high doses (8 mg/kg, 12 mg/kg b.w.), a significant decrease in the temperature occurred in these groups from the 30th minute to the 240th minute.

Heykants et al. (1971) found, in rats, that the maximal blood levels of azaperone are obtained about half an hour after the i.m. administration and then decline rapidly during the following 4 hours.

According to the European Agency for the Evaluation of Medicinal Products (1997), after a single intramuscular administration to pigs at a dose of 1 mg/kg b.w., the plasma concentrations of azaperone peaked within 30 minutes.

In the case of the oral azaperone administration in our experiment, the results were similar, i.e., we also measured the maximum blood concentra-

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tion of azaperone 30 minutes after administration (T_{max}) in all the experimental groups.

Heykants et al. (1971) found, in rats, that the blood levels declined to only 5% of the maximum value after 4 h after the i.m. administration. A significant decline in the azaperone concentrations was also found in all the experimental groups of our study.

The concentrations of azaperone in the blood correspond to its concentrations in the brain. According to Heykants et al. (1971), the uptake and elimination patterns of azaperone in the blood and brain are similar with the brain concentrations at several times higher than in the blood. In our experiment, as expected, the highest plasma concentrations of azaperone were found in the group of piglets that received the highest dose. Given that the highest degree of sedation was achieved in this group, it can be assumed that the highest concentrations of azaperone were also reached in the brain.

Biochemical values were included in the analysis for the purpose of a comprehensive assessment of the overall state of the organism. Plasma bilirubin levels, which were found to be higher in groups with the elevated azaperone doses, can indicate an impaired liver function. The higher GGT activities in groups with high azaperone doses may be a sign of liver damage. Elevated creatinine concentrations in the groups with high azaperone doses may indicate an impaired renal function.

It can be concluded that by increasing the dose of orally administered azaperone, the onset of sedation is faster, the end of sedation starts later and the sedation time is longer. However, the use of higher doses of oral azaperone (groups C and D) is not suitable for piglets as the doses negatively affect some physiological parameters, i.e., they cause a significant increase in the respiratory rate and a decrease in the rectal temperature.

In the case of higher azaperone doses, the results of the biochemical examination also suggest a possible negative effect on the liver and kidney function. Moreover, the immobility of the piglets caused by higher doses of oral azaperone would exclude its use under practical conditions (loading piglets before transport).

Conflict of interest

The authors declare no conflict of interest.

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