The effects of buccal administration of azaperone on the sedation level and biochemical variables of weaned piglets

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Abstract

The aim of the study was to compare the efficacy of buccal and parenteral administration of azaperone in order to achieve pig sedation. The type of study was prospective randomized and double blinded. A total of 40 weaned piglets were divided into 4 groups (10 each) and monitored. Group A was injected intramuscularly (i.m.) with azaperone (Stresnil®, 40 mg/ml inj., Elanco Animal Health) at a dose of 2 mg/kg body weight (b.w.). Group B (control) was given 1 ml of saline buccally. Group C received a dose of 2 mg/kg b.w. of azaperone buccally. Group D was given azaperone buccally at a dose of 4 mg/kg b.w. The response to defined stimulus (a blunt blow of a metal rod into the metal edge of the pen), degree of salivation, movement level, blood plasma azaperone concentration, and the haematological and biochemical variables were included in the study. We found that the buccal administration of azaperone is effective, however, a dose of 4 mg/kg b.w. is required to induce a sedation level comparable to the standard 2 mg/kg b.w. i.m. administration.

Swine, neuroleptic, behaviour, pharmacodynamics

Pigs are very sensitive to stress during handling and transport. This can significantly reduce their welfare (Roldan-Santiago et al. 2013; Martínez-Miró et al. 2016). Stress-related conditions such as weaning, transport, regrouping of pigs, their consequent fighting for a rank in the social hierarchy and behavioural disorders of pigs (cannibalism, puerperal neurosis of sows) necessitate the use of sedative drugs, as they may have important economic consequences (Dantzer 1977). Therefore, the use of neuroleptic drugs in pigs has become widespread since the intensification of pig farming. The sedative most used in pigs is azaperone (Stresnil 40 mg/ml inj.). It is a butyrophenone neuroleptic that is used in pigs to reduce stress and for overall injection anaesthesia (Benson and Thurmon 1979; Porter and Slusser 1985; Lahrmann et al. 2014). It is also used to reduce aggression (Blackshaw 1981). The use of azaperone has been reported to improve the productivity following regrouping of pigs by reducing their aggression (Porter and Slusser 1985; Gonyou et al. 1988). Azaperone treatment can also reduce stress caused by weaning, re-grouping and hierarchical fighting of gilts and sows (Swarz et al. 2018 a,b). According to current manufacturer's recommendations, sedation of pigs is performed by injecting azaperone at a dose of 2 mg/kg body weight (b.w). The disadvantage of this form of application is that azaperone leaves high and persistent concentrations at the injection site (Mestorino et al. 2013). This fact excludes the possibility of its use in the transport of pigs to slaughterhouses. The solution to this problem could be the use of buccal administration of azaperone. Mestorino et al. (2013) found that when administered buccally 6 h before the slaughter at the dose of 4 mg/kg b.w., the azaperone concentration in all analysed tissues did not exceed the EU maximum residual limit. However, that study did not compare

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the efficacy of the injection and buccal form of azaperone. Official recommendations for the use of buccal administration of azaperone are not yet available. Several authors have found that injecting azaperone alone can induce some stress response in the treated pigs. This stress response was manifested by increased blood plasma concentrations of glucose and lactate (Daş et al. 2016). This should be taken into account when azaperone is used, for example, in physiological experiments that evaluate the effect of various factors on the biochemical variables of pigs. It is not yet known whether these negative effects are obvious after the buccal administration of azaperone to pigs. It is clear from the above that the knowledge of some aspects of the use of azaperone in pigs is still inadequate. Therefore, we have decided to conduct this study to compare the efficacy of the injection and buccal forms of azaperone for pig sedation, and to evaluate their effects on the haematological and biochemical variables of piglets.

Materials and Methods

The study was approved by the Ethics Committee of the University of Veterinary and Pharmaceutical Sciences Brno (PP 6-2019, MSMT-27669/2019-16).

The type of study was prospective randomized and double blinded. The piglets were weaned on day 28. The piglets were marked with a plastic ear tag in the right ear. A total of 40 weaned piglets (Danbred) were used in the trial. Twenty piglets were females (gilts) and 20 piglets were castrated males. At weaning, 20 females were assigned randomly to four groups (of five each). Twenty castrated males were also assigned randomly to four groups (of five each). The randomization was achieved by drawing animals using numbered papers. In this way we created four groups (of 10 each). The pigs were then moved into the stables of our clinic to become acclimatized before the beginning of the trial (one week).

Group A (body weight, mean ± standard deviation, 7.10 ± 1.10 kg) was injected intramuscularly (i.m.) with azaperone (Stresnil®, 40 mg/ml inj., Elanco Animal Health) at a dose of 2 mg/kg b.w. Group B (7.12 ± 0.69 kg, control) was given 1 ml of saline buccally and served as the control group. Group C (7.14 ± 0.80 kg) received azaperone buccaly at a dose of 2 mg/kg b.w. Group D (7.14 ± 0.87 kg) was given azaperone buccaly at a dose of 4 mg/kg b.w.

The piglets were fed standard granulated feed mixture without any medication (De Heus a.s., Marefy, Czech Republic).

For all groups, the assessment of sedation was based on the reaction to a loud stimulus (a blunt blow of a metal rod into the metal edge of the pen). This variable was monitored during the whole trial at 15-min intervals (from 15 to 120 min). The observations were evaluated as follows: 0 - high grade reaction (jumping, running); 1 - medium grade reaction (no jump, but a reaction: side step, moving the head, muscle ripple, ear prick); 2 - no response (behaviour did not change in response to the stimulus). We used the same system of the assessment of sedation as in the study of Svoboda et al. (2004).

The study also included the assessment of movement. The observations were evaluated as follows: 0 - normal movement; 1- ataxic or less active; 2 - lying down. This was recorded at 0, 30 and 90 min after the administration.

The degree of salivation, the respiratory frequency and rectal temperature were also evaluated. These variables were evaluated in the 30th and 90th min of the experiment.

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).

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

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

The blood from piglets was taken prior to the azaperone administrations (time 0) and at 30-, 90-, and 240-min intervals thereafter. The blood samples were taken from the vena cava cranialis. Heparin was used as an anticoagulant for the determination of the azaperone and biochemical variables in blood plasma. The blood samples were also collected in EDTA (ethylene-diamine-tetraacetic acid) tubes for the determination of haematological variables.

Haematological analysis

The analysis included the red blood cells (RBC), haemoglobin concentration (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution width (RDW).

The Mindway, BC–2800 Vet. device (Guangzhou Medsinglong Medical Equipment Co., Ltd., Guangdong, China) was used for the haematological analysis. The Dia Rinse D, Dia Lyse Diff D-CF, Dia EZ Cleanser D and Probe Cleanser (Medesa, Polička, Czech Republic) were used as flush solutions. The method of colorimetry for

the detection of HGB was used. The method of impedance was used for other variables. The haematological variables were determined at 0, 30, and 240 min after the administration.

Biochemical analysis

The plasma biochemical variables included total protein (TP), albumin, cholesterol, urea, glucose, lactate, creatinine (CREAT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK). They were determined using the biochemical analyser Konelab 20i and commercial kits (Biovendor, Brno, Czech Republic).

The azaperone concentration and pharmacodynamic changes in plasma were determined by the ELISA (Enzyme-Linked Immuno Sorbent Assay) commercial kit (EuroProxima, Arnhem, Netherlands).

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.

Statistical analysis

Statistical analysis was carried out using the statistical software Statistica for Windows 8.0. At first, all data were tested using Shapiro-Wilk test for normality and homogeneity of variance across groups using Levene's test. The assessment of the effect of the test substance application method and sampling time was performed using a two-way analysis of variance with repeated measures (ANOVA). Significance was accepted at $P < 0.05$. Data are expressed as mean ± standard deviation.

Results

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

Assessment of sedation - response to a loud stimulus

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

Table 1. Response to a loud stimulation in weaned piglets after administration of azaperone.

Observation time (min)	Group A (n = 10)			Group B (n = 10)			Group C (n = 10)			Group D (n = 10)		
	0	1	2	0	1	2	0	1	2	0	1	2
15	20%	10%	70%	30%	70%	0%	50%	50%	0%	40%	60%	0%
30	10%	20%	70%	40%	50%	10%	30%	70%	0%	0%	10%	90%
45	0%	10%	90%	30%	60%	10%	10%	10%	80%	0%	0%	100%
60	0%	0%	100%	30%	70%	0%	0%	10%	90%	0%	10%	90%
75	0%	0%	100%	20%	80%	0%	0%	20%	80%	0%	10%	90%
90	0%	0%	100%	50%	50%	0%	0%	30%	70%	0%	0%	100%
105	0%	0%	100%	30%	70%	0%	10%	30%	60%	10%	20%	70%
120	0%	0%	100%	0%	80%	20%	10%	30%	60%	10%	20%	70%

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.

Plasma concentrations of azaperone

The results of determining the concentration of azaperone in the blood plasma for individual experimental groups and the sampling times are given in Table 2.

At 30 and 90 min, the azaperone concentrations in group A were significantly higher than in groups C and D (A30 × C30, $P < 0.001$; A30 × D30, $P < 0.001$; A90 × C90, $P < 0.001$; A90 × D90, $P = 0.002$). At 240 min, the azaperone concentrations in group A were higher than in group C (A240 × C240, $P = 0.005$). No significant differences in the azaperone concentrations between groups C and D were found during the trial.

Table 2. Concentration of azaperone in the blood plasma (ng/ml) in weaned piglets after administration of azaperone.

Blood collection time (min)	Group A	Group B	Group C	Group D
0	n.d.^{x}	n.d.^{x}	n.d.^{x}	n.d.^{x}
30	277.1 ± 56.8^{x}	n.d.^{z}	70.7 ± 32.0^{y}	97.6 ± 58.6^{y}
90	147.2 ± 36.9^{x}	n.d.^{z}	48.1 ± 29.5^{y}	68.2 ± 43.4^{y}
240	98.1 ± 39.2^{x}	n.d.^{z}	23.5 ± 11.2^{y}	46.4±34.4^{xy}

Data are expressed as mean ± standard deviation (n.d., non-detected – azaperone concentration below detection limit – 0.785 ng/ml, in this case half value of detection limit was used for statistical analysis). Means with the same blood collection time and row lacking a common letter of superscript ($^{x, y, z}$) differ significantly ($P < 0.05$).

In all experimental groups (except the control group), the highest concentration of azaperone in the blood plasma was found 30 min after the application, and subsequently, the values decreased relatively quickly.

At 240 min after the application, the concentration of azaperone was approximately one third compared to 30 min (groups A and C), and in the case of group D, approximately half.

The degree of salivation

The results of the evaluation of salivation are presented in Table 3. In groups B, C, and D, the degree of salivation did not increase during the experiment. In group A, 30% of piglets showed an increased level of salivation at 30 min, of which 10% were moderate and 20% were high. At 90 min, 90% of the piglets showed a degree of salivation identical to that before the start of the experiment, and 10% a medium grade.

Table 3. Degree of salivation and movement level in weaned piglets after administration of azaperone.

	Observation time	Degree of salivation			Movement level		
	(min)	0	1	2	0	1	2
Group A n = 10	0	100%	0%	0%	100%	0%	0%
	30	70%	10%	20%	0%	30%	70%
	90	90%	10%	0%	0%	0%	100%
Group B n = 10	0	100%	0%	0%	100%	0%	0%
	30	100%	0%	0%	100%	0%	0%
	90	100%	0%	0%	100%	0%	0%
Group C n = 10	0	100%	0%	0%	100%	0%	0%
	30	100%	0%	0%	20%	80%	0%
	90	100%	0%	0%	0%	0%	100%
Group D n = 10	0	100%	0%	0%	100%	0%	0%
	30	100%	0%	0%	0%	10%	90%
	90	100%	0%	0%	0%	0%	100%

The results are presented as the percentage of piglets in the group belonging to a certain grade. O - 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). O - normal movement, 1- ataxic or less active, 2 - lying down.

Movement level

The results of the evaluation of the movement are presented in Table 3. In group A at 30 min of the experiment, 30% of piglets were ataxic or less active and 70% of piglets were lying down. In group D at 30 min, 10% of piglets were found ataxic, and 90% were lying down. In group C, there were no lying piglets at 30 min, 80% were ataxic, and 20% showed completely normal movement. At 90 min, all piglets in groups A, C and D were lying down. In group B, none of the piglets showed any movement problems throughout the experiment.

Body temperature and respiratory frequency

As part of the monitoring of vital variables, the level of respiration and body temperature were recorded. The results are presented in Table 4. The data are presented as the percentage of piglets in the group, whose respiratory frequency was evaluated as bradypnoea, eupnoea or tachypnoea, or as a mean value and standard deviation (body temperature).

Table 4. Results of vital variables after administration of azaperone in weaned piglets.

	Observation time (min)	Respiratory frequency			Body temperature
		Bradypnoea	Eupnoea	Tachypnoea	Mean ± SD (°C)
Group A n = 10	0	0%	100%	0%	39.2 ± 0.4^{x}
	30	50%	50%	0%	37.9 ± 0.5^{x}
	90	20%	80%	0%	37.8 ± 0.5^{x}
Group B n = 10	0	0%	100%	0%	38.4 ± 0.7^{x}
	30	0%	100%	0%	38.9 ± 0.4^{x}
	90	0%	100%	0%	38.7 ± 0.4^{x}
Group C n = 10	0	0%	100%	0%	38.9 ± 0.8^{x}
	30	0%	100%	0%	39.1 ± 0.3^{x}
	90	0%	100%	0%	38.7 ± 0.6^{x}
Group D n = 10	0	0%	100%	0%	39.1 ± 0.5^{x}
	30	0%	100%	0%	38.8 ± 0.4^{x}
	90	0%	100%	0%	38.6 ± 0.7^{x}

The results of 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 expressed as mean and standard deviation. Means with the same blood collection time and column lacking a common letter of superscript (^{x,y}) differ significantly ($P < 0.05$).

Piglets in groups B, C, and D had a normal, calm, regular breathing throughout the trial. In group A, 50% of the piglets had a reduced respiratory frequency at 30 min and 20% of the piglets had a reduced respiratory frequency at 90 min. No increased respiratory frequency was observed in any piglet throughout the experiment.

In group A, there was a slight decrease of the average body temperature at 30 and 90 min compared to the beginning of the experiment. The body temperature fell below the physiological value (39.3 ± 0.3 °C) in all of piglets at 30 min and in all of piglets at 90 min of the experiment in the group A.

Haematological variables

The results are shown in Table 5.

In group A, there was a decrease of the red blood cell count between time 0 and 30 min. No other significant differences in the red blood cell variables were found during our study.

At 240 min, the lymphocyte count in group A (control group) was lower than in group D ($P < 0.042$). In group A, there was an increase of the granulocyte count from 30 to 240 min. No other significant differences were found for these variables.

Biochemical variables

The results are shown in Tables 6 and 7. At all sampling times, the cholesterol concentration in group A was lower than in group C (A0 × C0, $P = 0.006$; A30 × C30, $P = 0.001$; A90 × C90, $P = 0.001$; A240 × C240, $P = 0.012$). At all sampling times, the cholesterol concentration in group A was lower than in group D (A0 × D0, $P = 0.002$; A30 × D30, $P = 0.001$; A90 × D90, $P = 0.001$; A240 × D240, $P = 0.010$).

Table 5. Results of red and white blood cell variables in weaned piglets after administration of azaperone.

Group	Blood collection time (min)	Red blood cell count (10^{12}/l)	Haemoglobin concentration (g/l)	Haematocrit (%)	White blood cell count (10^9/l)	Lymphocytes (10^9/l)	Monocytes (10^9/l)	Granulocytes (10^9/l)
A	0	6.77 ± 1.53^{x}	98.40 ± 22.38^{x}	32.51 ± 7.40^{x}	13.47 ± 4.46^{x}	6.58 ± 2.42^{x}	0.41 ± 0.15^{x}	6.48 ± 2.3^{x}
	30	6.59 ± 0.46^{x}	94.90 ± 6.45^{x}	31.89 ± 2.04^{x}	14.25 ± 2.28^{x}	6.33 ± 0.83^{x}	0.42 ± 0.09^{x}	7.50 ± 1.64^{x}
	240	5.85 ± 1.04^{x}	84.80 ± 14.37^{x}	27.96 ± 4.68^{x}	15.72 ± 4.5^{x}	5.00 ± 1.09^{y}	0.51 ± 0.17^{x}	10.21 ± 3.40^{x}
B	0	6.87 ± 1.21^{x}	98.60 ± 19.06^{x}	32.60 ± 6.05^{x}	14.74 ± 4.33^{x}	7.48 ± 2.22^{x}	0.44 ± 0.24^{x}	6.82 ± 2.27^{x}
	30	7.01 ± 0.52^{x}	100.40 ± 10.86^{x}	33.54 ± 3.29^{x}	14.70 ± 3.00^{x}	7.06 ± 1.23^{x}	0.45 ± 0.26^{x}	7.19 ± 2.11^{x}
	240	6.55 ± 0.49^{x}	96.40 ± 9.99^{x}	31.08 ± 3.26^{x}	13.63 ± 1.96^{x}	6.10 ± 1.01^{xy}	0.37 ± 0.13^{x}	7.16 ± 1.50^{x}
C	0	6.81 ± 0.58^{x}	101.30 ± 5.81^{x}	33.37 ± 2.73^{x}	14.99 ± 3.40^{x}	8.64 ± 1.46^{x}	0.41 ± 0.12^{x}	5.94 ± 2.66^{x}
	30	6.83 ± 0.38^{x}	97.60 ± 6.72^{x}	33.21 ± 2.26^{x}	14.32 ± 3.21^{x}	7.59 ± 1.34^{x}	0.40 ± 0.12^{x}	6.33 ± 2.62^{x}
	240	6.41 ± 0.49^{x}	94.80 ± 6.03^{x}	31.23 ± 2.16^{x}	15.43 ± 3.79^{x}	7.26 ± 1.53^{xy}	0.40 ± 0.12^{x}	7.77 ± 3.03^{x}
D	0	7.21 ± 0.45^{x}	106.40 ± 6.08^{x}	36.02 ± 1.76^{x}	15.42 ± 2.93^{x}	8.76 ± 1.79^{x}	0.43 ± 0.07^{x}	6.23 ± 1.49^{x}
	30	6.88 ± 0.48^{x}	99.90 ± 6.49^{x}	34.29 ± 1.92^{x}	15.50 ± 3.65^{x}	8.56 ± 2.06^{x}	0.42 ± 0.11^{x}	6.52 ± 2.08^{x}
	240	6.53 ± 0.34^{x}	97.70 ± 4.69^{x}	32.24 ± 1.41^{x}	16.13 ± 4.55^{x}	7.54 ± 1.94^{x}	0.42 ± 0.13^{x}	8.17 ± 3.93^{x}

Results are expressed as mean ± standard deviation. Means with the same blood collection time and column lacking a common letter of superscript ($^{x-y}$) differ significantly ($P < 0.05$).

In groups A, C, D, there was an increase of glucose concentration from time 0 to 30 min. Total protein, urea, and creatinine showed a similar pattern of variations between sampling times within the same group in all groups including control group B, no differences between groups at the same time were found. No other significant differences were found for these variables.

At times 0 and 240 min, ALT in group B was lower than in group C (B0 × CO, $P = 0.041$; B240 × C240, $P = 0.006$).

There was a decrease of ALP in groups B, C, D from time 0 to 240 min. There was an increase of AST in group A from time 0 to 240 min. No other significant differences were found for these variables.

Discussion

In general, azaperone can be characterized by a fast but short action. In our experiment, satisfactory sedation was achieved with the intramuscular administration of 2 mg/kg b.w. in about 15 min; the duration of sedation was more than 2 h.

Our results are in agreement with other authors. Symoens and Van Den Brande (1969) found that an i.m. injection of azaperone provided an effective sedation within 10–15 min and the effect lasted for approximately 2 h. Jones (1972) reported that azaperone (i.m.) reached its peak effect after 15 min in young pigs and after 30 min in adult pigs, with the duration of action being within 2–4 h.

Svoboda et al. (2004) compared in their study the onset of action and the duration of effective sedation with buccal administration of different doses. The limitation of that study was that it used only one variable, i.e. the response to a loud stimulus, and did not include other physiological variables, haematological and biochemical analysis and the measurement of plasma azaperone concentrations.

In the study, the onset of action always occurred within 15 min after the buccal

Table 6. Results of biochemical variables in weaned piglets after administration of azaperone – part 1.

Group	Blood collection time (min)	Total protein (g/l)	Albumin (g/l)	Cholesterol (mmol/l)	Lactase (mmol/l)	Glukose (mmol/l)	Urea [mmol/l)	Creatinine (μmol/l)
A	0	51.90 ± 2.97 ^{x}	34.22 ± 2.56 ^{x}	1.74 ± 0.22 ^{y}	5.82 ± 3.67 ^{x}	4.20 ± 0.68 ^{x}	2.82 ± 1.36 ^{x}	124.73 ± 42.46 ^{x}
	30	46.60 ± 2.56 ^{x}	30.71 ± 2.64 ^{x}	1.54 ± 0.15 ^{y}	6.42 ± 2.56 ^{x}	4.61 ± 0.43 ^{x}	3.07 ± 1.38 ^{x}	180.42 ± 12.70 ^{x}
	240	44.90 ± 2.72 ^{x}	30.66 ± 3.87 ^{x}	1.60 ± 0.46 ^{y}	5.19 ± 2.34 ^{x}	5.55 ± 0.60 ^{x}	3.05 ± 1.42 ^{x}	248.11 ± 49.30 ^{x}
B	0	52.25 ± 3.33 ^{x}	34.27 ± 2.49 ^{x}	2.13 ± 0.49 ^{xy}	4.57 ± 2.19 ^{x}	4.79 ± 0.79 ^{x}	2.66 ± 1.93 ^{x}	106.89 ± 19.94 ^{x}
	30	50.95 ± 2.06 ^{x}	32.01 ± 3.72 ^{x}	2.07 ± 0.40 ^{xy}	5.01 ± 1.84 ^{x}	5.31 ± 0.65 ^{x}	2.86 ± 2.09 ^{x}	167.27 ± 44.39 ^{x}
	90	49.47 ± 3.26 ^{x}	30.59 ± 2.02 ^{x}	1.99 ± 0.43 ^{xy}	3.40 ± 1.06 ^{y}	5.45 ± 0.76 ^{x}	2.09 ± 1.97 ^{x}	108.79 ± 15.22 ^{x}
	240	46.53 ± 3.09 ^{x}	31.35 ± 2.97 ^{x}	1.92 ± 0.46 ^{xy}	3.42 ± 0.15 ^{x}	5.52 ± 0.53 ^{x}	2.57 ± 1.86 ^{x}	195.09 ± 82.68 ^{x}
C	0	51.67 ± 3.35 ^{x}	31.92 ± 3.68 ^{x}	2.41 ± 0.36 ^{x}	5.80 ± 1.67 ^{x}	4.15 ± 0.72 ^{x}	3.81 ± 1.54 ^{x}	111.02 ± 29.91 ^{x}
	30	49.86 ± 2.85 ^{x}	30.57 ± 2.33 ^{x}	2.34 ± 0.27 ^{x}	4.99 ± 2.72 ^{x}	5.22 ± 0.94 ^{x}	4.36 ± 1.07 ^{x}	166.71 ± 18.83 ^{x}
	90	47.30 ± 3.61 ^{x}	30.98 ± 3.03 ^{x}	2.27 ± 0.28 ^{x}	4.35 ± 1.75 ^{xy}	6.11 ± 0.97 ^{x}	3.51 ± 1.30 ^{x}	108.24 ± 12.66 ^{x}
	240	47.78 ± 7.95 ^{x}	28.73 ± 7.80 ^{x}	2.24 ± 0.45 ^{x}	4.76 ± 2.68 ^{x}	5.92 ± 1.75 ^{x}	3.73 ± 1.08 ^{x}	201.39 ± 89.16 ^{x}
D	0	53.76 ± 2.78 ^{x}	34.79 ± 1.29 ^{x}	2.48 ± 0.31 ^{x}	4.80 ± 1.29 ^{x}	4.67 ± 0.68 ^{x}	4.68 ± 1.25 ^{x}	118.52 ± 42.11 ^{x}
	30	51.11 ± 2.80 ^{x}	31.58 ± 3.00 ^{x}	2.32 ± 0.29 ^{x}	4.17 ± 1.82 ^{x}	5.52 ± 1.28 ^{x}	5.04 ± 1.05 ^{x}	163.89 ± 28.23 ^{x}
	90	49.93 ± 3.12 ^{x}	31.71 ± 3.03 ^{x}	2.28 ± 0.28 ^{x}	4.05 ± 1.52 ^{y}	6.26 ± 1.30 ^{x}	4.14 ± 1.09 ^{x}	107.32 ± 16.16 ^{x}
	240	48.89 ± 2.70 ^{x}	31.25 ± 3.74 ^{x}	2.25 ± 0.34 ^{x}	3.52 ± 0.81 ^{x}	5.38 ± 1.39 ^{x}	4.28 ± 1.10 ^{x}	229.75 ± 87.21 ^{x}

Results are expressed as mean ± standard deviation. Means with the same blood collection time and column lacking a common letter of superscript (^{x,y}) differ significantly ($P < 0.05$).
Group A – 2 mg/kg i.m.; group B – 1 ml of saline p.o.; group C – 2 mg/kg p.o.; group D – 4 mg/kg p.o.
Means with the same blood collection time and column lacking a common letter of superscript (^{x,y}) differ significantly ($P < 0.05$).

administration (for both doses, i.e. 2 mg/kgb.w. and of 4 mg/kgb.w.). In our present experiment, a satisfactory sedation was achieved approximately 45 min after the buccal administration of azaperone at the amount of 2 mg/kg b.w. and approximately 30 min with the buccal administration of 4 mg/kg b.w.

In the study of Svoboda et al. (2004), a reliable sedation persisted for approximately 30–40 min in the group of buccal administration of 2 mg/kg b.w., whereas in the group of buccal administration of 4 mg/kg b.w. the sedation time of approximately 2 h was achieved. In our present experiment, the duration of sedation was as follows: with the buccal administration of 2 mg/kg b.w., about 45 min; and with the buccal administration of 4 mg/kg b.w., for approximately 90 min.

The differing results could be caused by different conditions in our experiment, i.e. the piglets were exposed to more handling, especially the blood sampling associated with fixation.

According to the European Agency for the Evaluation of Medicinal Products (Anonymous 1997), after a single intramuscular administration to pigs at a dose of 1 mg/kg b.w., the plasma levels of azaperone peaked within 30 min. This is in agreement with our study. We measured the maximum blood concentration of azaperone 30 min after the administration in all

Table 7. Results of biochemical variables in weaned piglets after administration of azaperone – part 2.

Group	Blood collection time (min)	ALP (µkat/l)	ALT (µkat/l)	AST (µkat/l)	GGT (µkat/l)	Creatine kinasa (µkat/l)	Lactate dehydrogenase (µkat/l)
A	0	6.90 ± 1.37 ^{x}	1.05 ± 0.27 ^{xy}	0.85 ± 0.20 ^{x}	1.09 ± 0.25 ^{x}	11.79 ± 3.20 ^{x}	13.89 ± 2.05^{x}
	30	6.61 ± 1.25 ^{x}	1.01 ± 0.20 ^{x}	0.98 ± 0.20 ^{x}	1.22 ± 0.43 ^{x}	19.77 ± 9.58 ^{x}	12.96 ± 3.18 ^{x}
	90	6.67 ± 1.41 ^{x}	1.10 ± 0.20 ^{x}	1.14 ± 0.35 ^{x}	1.06 ± 0.24 ^{x}	21.54 ± 10.81 ^{x}	13.48 ± 2.13 ^{x}
	240	6.53 ± 1.41 ^{x}	1.01 ± 0.18 ^{xy}	1.14 ± 0.31 ^{x}	0.91 ± 0.19 ^{x}	20.61 ± 12.58 ^{x}	13.17 ± 2.94 ^{x}
B	0	6.83 ± 1.59 ^{x}	0.89 ± 0.15 ^{y}	0.76 ± 0.13 ^{x}	1.32 ± 0.38 ^{x}	9.82 ± 3.43 ^{x}	14.92 ± 2.26 ^{x}
	30	6.67 ± 1.26 ^{x}	0.94 ± 0.19 ^{x}	0.81 ± 0.15 ^{x}	1.26 ± 0.32 ^{x}	13.38 ± 4.45 ^{x}	15.47 ± 1.79 ^{x}
	90	6.41 ± 1.19 ^{x}	0.89 ± 0.16 ^{x}	0.81 ± 0.30 ^{x}	1.10 ± 0.25 ^{x}	14.47 ± 8.49 ^{x}	14.39 ± 2.93 ^{x}
	240	5.65 ± 1.75 ^{x}	0.80 ± 0.31 ^{y}	0.79 ± 0.18 ^{x}	1.13 ± 0.43 ^{x}	10.14 ± 2.64 ^{x}	14.03 ± 2.07 ^{x}
C	0	9.04 ± 1.50 ^{x}	1.39 ± 0.47 ^{x}	1.07 ± 0.30 ^{x}	1.40 ± 0.67 ^{x}	20.93 ± 10.52 ^{x}	15.85 ± 4.13 ^{x}
	30	7.94 ± 1.71 ^{x}	1.37 ± 0.48 ^{x}	1.04 ± 0.30 ^{x}	1.07 ± 0.18 ^{x}	28.63 ± 25.31 ^{x}	15.98 ± 3.63 ^{x}
	90	8.07 ± 1.68 ^{x}	1.34 ± 0.45 ^{x}	1.07 ± 0.34 ^{x}	1.06 ± 0.24 ^{x}	20.74 ± 14.23 ^{x}	16.90 ± 3.98 ^{x}
	240	7.46 ± 1.56 ^{x}	1.35 ± 0.43 ^{x}	1.02 ± 0.30 ^{x}	1.02 ± 0.17 ^{x}	19.69 ± 14.07 ^{x}	16.01 ± 4.00 ^{x}
D	0	8.61 ± 1.87 ^{x}	1.23 ± 0.20 ^{xy}	1.03 ± 0.19 ^{x}	1.17 ± 0.51 ^{x}	13.14 ± 3.68 ^{x}	14.17 ± 1.57 ^{x}
	30	7.93 ± 1.70 ^{x}	1.20 ± 0.20 ^{x}	1.08 ± .022 ^{x}	1.07 ± 0.52 ^{x}	13.67 ± 0.32 ^{x}	13.60 ± 1.84 ^{x}
	90	7.56 ± 1.34 ^{x}	1.19 ± 0.19 ^{x}	1.05 ± 0.28 ^{x}	0.94 ± 0.29 ^{x}	16.25 ± 15.52 ^{x}	14.40 ± 1.34 ^{x}
	240	7.20 ± 1.74 ^{x}	1.17 ± 0.21 ^{xy}	0.97 ± 0.16 ^{x}	0.94 ± 0.41 ^{x}	14.31 ± 10.74 ^{x}	13.56 ± 2.46 ^{x}

Results are expressed as mean ± standard deviation. ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase. Means with the same blood collection time and column lacking a common letter of superscript (^{x,y}) differ significantly ($P < 0.05$).

experimental groups, with the highest values being measured in piglets after the injection. A significant decline in the azaperone concentrations was found thereafter. This is also in agreement with Heykants et al. (1971) who found that in rats, the maximum blood and brain levels of azaperone were obtained about 0.5 h after the i.m. administration and then declined rapidly over the following 4 h.

The azaperone injection resulted in significantly higher plasma concentrations of azaperone compared to the buccally treated groups. No significant differences in the azaperone concentrations between the buccally treated groups were found during the trial.

Lang (1970) noticed increased salivation after the azaperone administration at a dose of 5–6 mg/kg b.w. i.m. Increased salivation after the azaperone administration was also noted by Nishimura et al. (1993). Azaperone in their study was administered i.m. at a dose of 8 mg/kg b.w. In our experiment, salivation increased only in piglets to which azaperone was administered intramuscularly. No change in salivation was observed with the buccal administration.

Holzchuh and Cremonesi (1991) reported that there was an intense vasodilatation and a moderate increase in the respiratory frequency when a dose of 2 mg/kg b.w. i.m. was used. Lang (1970) noticed increased respiratory frequency at the dose of

5–6 mg/kg b.w. i.m. This is contradictory to our study where no piglets increased their respiratory frequency during the experiment.

Marsboom and Symoens (1968) also reported that the rectal temperature decreased by 1–2 °C within 4 h after the parenteral administration (at least 5 mg/kg b.w. i.m.). This is in agreement with our findings, since after injective azaperone administration body temperatures of all parenterally treated piglets fell below the physiological values.

Holzchuh and Cremonesi (1991) found that the muscle tone was slightly diminished at the dose of 2 mg/kg bw i.m. Azaperone also decreases the motor activity of pigs (Plumb 2002). This is in agreement with our study, where the motor activity of piglets decreased after 30 min in all experimental groups.

Haematological and biochemical variables were included in the analysis for the purpose of a comprehensive assessment of the overall state of the organism. Variables indicating liver (total protein, albumin, ALP, AST, GGT, LDH) and kidney functions (urea, creatinine) did not differ significantly between the control group and the groups that were given azaperone. This indicates a good safety of the used preparation.

Adetunji and Osunbunmi (2000) found that the azaperone sedation in pigs caused a decrease in the mean PCV, Hb and RBC values during a 1-hour period after the administration of the drug at a dose of 8 mg/kg b.w. i.m. In our present study, there was also a decrease of RBC between time 0 and 30 min in the i.m. treated group. According to Adetunji and Osunbunmi (2000), this could be explained by the adrenolytic properties of azaperone that reversed the effects of stress on the spleen, leading to its relaxation and re-uptake of red blood cells.

In the study of Daş et al. (2016), pigs (females aged 8.5 months) were sedated with a single administration of azaperone (0.8 mg/kg bw i.v.). This resulted in the elevation of plasma glucose and lactate. According to the authors, it indicates that azaperone elicits a stress response in pigs. In agreement with the mentioned study, we found a significant increase in glucose concentrations in all azaperone treated groups.

Our results demonstrate that buccally administered azaperone is absorbed and causes the sedation of piglets. It is evident from our results that by increasing the dose of buccally administered azaperone, the onset of sedation is faster, and the duration of sedation is longer. It can be concluded that the buccal administration of azaperone at a dose of 4 mg/kg b.w. is required to induce a comparable sedation level to the standard 2 mg/kg b.w. i.m. administration.

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