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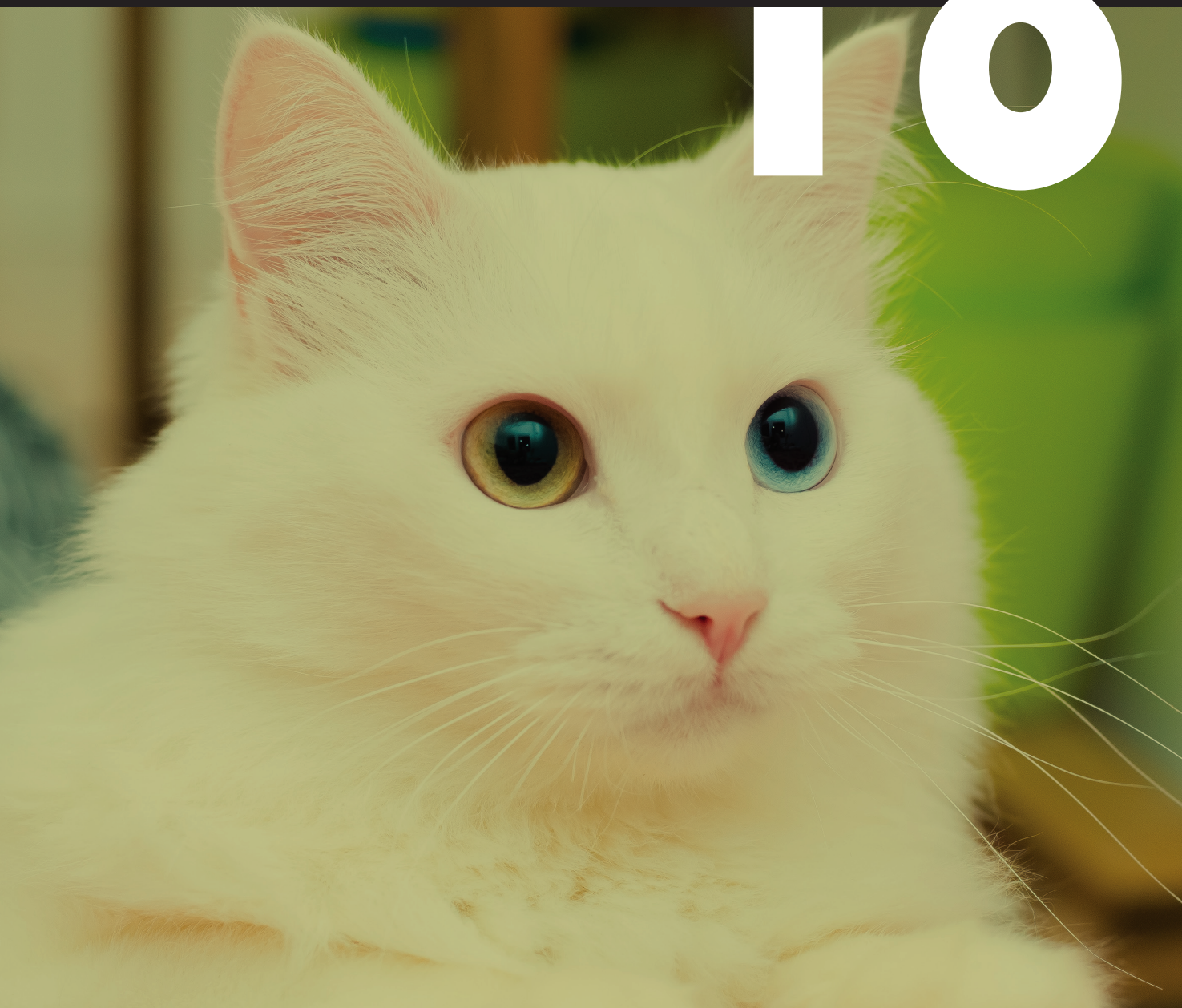

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Acta Veterinaria Eurasia (Acta Vet Eurasia) is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University-Cerrahpaşa Faculty of Veterinary Medicine and published three times a year (January, May and September). The publication language of the journal is English.

Acta Veterinaria Eurasia (Acta Vet Eurasia) aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of veterinary medicine. The journal publishes original articles, reviews, case reports, short communications, and letters to the editor that are prepared in accordance with the ethical guidelines.

The scope of the journal covers all animal species including the topics related to basic and clinical veterinary sciences, livestock breeding and husbandry, veterinary genetics, animal nutrition and nutritional diseases, zooneses, veterinary medicinal products and public health, and food hygiene and technology.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Abstract: An English abstract should be submitted with all submissions except for Letters to the Editor. The Abstract section of all types of articles should be unstructured.

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Original Articles: This is the most important type of article since it provides new information based on original research. The main text of original articles should be structured with Introduction, Materials and Methods, Results, and Discussion subheadings. The results and discussion may be combined into one section, if desired.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489-93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

The Latin scientific names of a species should be written in italics. Apart from the names of species, italicization should be avoided as much as possible.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Chelex-100 (BioRad, California, USA)"

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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While citing publications, preference should be given to the latest, most up-to-date publications. In the main text of the manuscript, references should be cited by author's name and the publication year in parenthesis. In the case of direct citations in the main text, only publication year should be stated in parenthesis after the name of the author. Please see below for examples:

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Reference with multiple authors: (Doyle et al., 2007)

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Contents

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Growth Performance of *Clarias gariepinus* on Diets Fortified with *Lactobacillus plantarum* and *Psidium guajava* Leaf

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Abstract

This study was conducted to assess the effect of dietary inclusions of *Lactobacillus plantarum* (*L. plantarum*) and *Psidium guajava* (*P. guajava*) diets on the growth performance of *Clarias* gariepinus (*C. gariepinus*). Seven treatments were administered to 420 *C. gariepinus* juveniles. They were: Control (Basal diet of 42% crude protein), *L. plantarum* (LPc) (cell/1000 mL) at 40, 60, 80 and *P. guajava* leaf meal (PGLM) (g/1000g) at 40, 60 and 80 per kg of feed. Body Weight Gain (BWG) (g), Specific Growth Rate (SGR) (g/day), Feed Conversion Ratio (FCR), Survival Rate (SR) (%), Protein Efficiency Ratio (PER) and Nitrogen Metabolism (NM) were evaluated. Data was analyzed using descriptive statistics and ANOVA at $p<0.05$. From the results, 40LPc had the highest body weight gain (73.52±2.30) g and Specific Growth Rate (SGR) (1.19±0.02) g/day, while 80 PGLM had the least body weight gain (46.10±0.12) g and SGR (0.99±0.00) g/day. No significant difference ($p>0.05$) was observed in feed conversion ratio across all treatments. There was no significant difference ($p>0.05$) observed in the survival rate between control (93.80±4.98)% and 80 PGLM (96.77±4.09)%. No significance difference ($p>0.05$) was observed in protein efficiency ratio between control (1.01±0.36) and 60 PGLM (0.95±0.36). There was no significant difference ($p>0.05$) observed in nitrogen metabolism across all treatment groups. Hence, the inclusion level of 40 cells/1000 mL of *L. plantarum* fortified diet in cultured juvenile *C. gariepinus* was observed to have a better growth enhancing performance and nutrient utilization than *P. guajava* leaf meal at 40g/1000g.

Keywords: *Clarias gariepinus*, growth, phytobiotics, probiotics

Introduction

Aquaculture is recognized for its rapid growth in the food producing sector globally (Hassan and Ngaski, 2007). The growth of aqua-farming has led to high stock densities of fish, which cause stressful conditions for fish, thus resulting in increased disease incidence and decreased fish productivity (Bondad et al., 2005). Hence, functional feeds are vital in fish nutrition and management. Functional feeds help to reduce fish diseases, boost productivity and enhance profitability. High stocking densities have elicited antibiotic use as growth promoters and immunostimulants. However, restrictions have been imposed regarding the use of antibiotics in fish culturing as they [antibiotics] leave resistant and harmful residues in human and animal flesh (Sayed et al., 2011).

In relation to this, research has been on-going on environmentally-friendly additives that have no residual effects on man and animals. It is worth noting that such additives contain substances that enhance fish growth and stimulate their immune system against diseases. Among the additives used are probiotics such as *Lactobacilli* and phytobiotics such as *P. guajava*. *Lactobacilli* are non-pathogenic facultative organisms that are employed as probiotics in aqua-farming. Lactic acid bacteria (LAB) produce bacteriocins and organic acids that aid in inhibiting the growth/replication of pathogens detrimental

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microorganisms (NRC, 2011). These beneficial properties have made LAB more suitable than other microorganisms used as probiotics (Arimah and Ogunlowo, 2014). *Lactobacillus plantarum* are probiotic bacteria derived from fermented cow milk known as 'wara' in Nigeria (Adegbehingbe, 2013). Even though LAB are currently being used as probiotics, there has been limited information published on the use of LAB isolated from food sources in feed inclusion of African catfish.

To improve aquaculture, the use of natural compounds from plants is highly desired. According to Welker and Lim (2011) phytobiotics are substances derived from plants to enhance fish health and growth. They include *P. guajava*, *Echinacea purpurea* (Guz et et al., 2011) and *Allium sativum* (Shalaby et al., 2006). However, there is on-going research into the use of *P. guajava* leaves as immunomodulators and phytoconstituents in aquaculture industry.

In Nigeria, African catfish (*Clarias gariepinus*) are widely cultured due to their hardiness to stress, high resistance to diseases, spawning ability, fast growth rate and acceptability to consumers' taste (Adewolu et al., 2008).

This study focuses on the comparative evaluation of the efficacy of *L. plantarum* isolated from cow milk, and *P. guajava* leaves on the growth response and utilization of nutrients in *C. gariepinus* juveniles after an 84 day feeding trial.

Materials and Methods

Ethical statement
The experimental procedure followed the International ethical standard of animal use. The ethical report statement was issued by University of Ibadan in 2017 with an ethical statement number of 0028 (UI/ACUREC/App/2017/0028).

Experimental system and design
This study was carried out in the Department of Aquaculture and Fisheries Management wet Laboratory located in the Faculty of Agriculture and Forestry, University of Ibadan, Nigeria.

Experimental procedure
Four hundred and twenty *C. gariepinus* juveniles of the average live weight of 7.79±0.01g were purchased from a known breeding fish farm in Ibadan. They were individually transported inside a bag that was adequately filled with water and sufficiently aerated to the Department of Aquaculture and Fisheries Management Laboratory. Records of the average weights of the fish were taken prior to the experiment. Experimental fish were acclimatized for 14 days and fed with commercial feed. Afterwards, the feeding experiment was carried out for 84 days.

The fish were distributed equally into 7 experimental treatments, which were triplicated. Twenty African catfish juveniles were housed in rectangular fish holding facilities (Neoplastics, Lagos, Nigeria) with each individual facility having the dimensions 50x34x27cm and a 40 liter capacity. Twenty-one fish holding facilities were employed in the experiment with each treatment holding 60 juveniles of African catfish. The fish were weighed individually and collectively in each facility. Weighing was carried out fortnightly. The average weight of fish was determined by dividing the total weight of fish by the number of fish in each facility. Feed was administered to the fish twice daily at 8:00 AM and 6:00 PM (Greenwich Merridian Time-GMT) and was weighed at every feeding period. The fish were fed until satiation. Records of feed consumed and fish weights were kept to calculate feed conversion ratio and feed intake throughout the experiment.

This study followed a Completely Randomized Design (CRD) for 84 days. Water was obtained from the University of Ibadan water depot. Each holding facility was sufficiently oxygenated by using aerators (Cosmos aquarium air pump, double type 3500 50 Hz, 2.5-3 W) as described by Lashkar et al. (2011). Water quality indices were monitored with the use of dissolved oxygen meters (Jenway 3015DO meter, 0.01 accuracy, Genway, Staffordshire, UK), mercury-in-glass thermometers (producer Paragon Scientific Ltd, Birkenhead, Wirral, UK) and pH meters (Jenway 3015pH meter, 0.01 accuracy, Genway, Staffordshire, UK). This was carried out after the standardization of each meter.

Procurement of probiotic candidates
Samples from fermented cow milk (wara) were collected and stored in ice according to Iranmanesh et al. (2014). Homogenised samples of these products were diluted serially by using 10-fold serial dilution in peptone water (Rapids Lab, Essex, England). De Man, Rogosa and Sharpe (MRS) agar (Rapids Lab, Essex, England) and De Man, Rogosa and Sharpe (MRS) broth (Rapids Lab, Essex, England) were prepared and used for bacterial growth according to the manufacturer's recommendations. These were incubated under anaerobic conditions for 24-48 hours at 30°C. The pH of the media was regulated to 5.5 using 0.1 normal sodium hydroxide (N NaOH) and 0.1 normal hydrogen chloride (N HCl). Representative colonies on the MRS agar plates were selected and identification of LAB was presumptively carried out by physiological and biochemical tests. Isolates were re-streaked out on MRS agar medium to collect a pure culture. The culture was maintained on MRS agar plates and in MRS broth and stored at 4°C. These cultures were reactivated on MRS agar for 24 h before experimental use (Mourad and Eddine, 2006; Todorov et al., 2011).

Molecular characterisation
Extraction of Deoxyribonucleic acid (DNA) was carried out by employing the method outlined by Saraniya and Jeevaratnam (2012). Polymerase Chain Reaction (PCR) was carried out with 10 µL of 5x GoTaq colorless reaction buffer, 3 µL of $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5'-AAGGAGGTGATCCAGCC-3' Primers. PCR was carried out in a GeneAmp 9700 PCR Sys-

tem Thermal cycler (Applied Biosystems Life Technologies Corporation, Carlsbad, USA). PCR profile had the first denaturation at 94°C for 5 min for 30 cycles, then at 94°C for 30 sec, 50°C for 60 sec and 72°C for 90 sec. The last extension was at 72°C for 10 min and the extracted DNA was iced at 4°C in gel (Agaliya and Jeevaratnam, 2013).

Imaging

A 1% Agarose gel (Thermo Fisher Scientific, Vigo, Spain) run was used to confirm the integrity of the amplified product of the roughly 1.5 Mb fragment. The process was done with a mixture of 8 µL of amplified product with 4 µL of loading dye. This was run for about an hour on solidified Agarose gel (Thermo Fisher Scientific, Vigo, Spain) at 110 V. Afterwards, quantification of the concentrated product was carried out using a nano drop of model 2000 (Applied Biosystems Life Technologies corporation, Carlsbad, USA) (Agaliya and Jeevaratnam, 2013).

Purification of amplified product

PCR reagents were removed from the DNA extraction with the use of ethanol (Presco-Beam Roanke,Virginia, USA) to purify the amplified fragments. Seven point six microliters of sodium acetate 3M, 240 µL of 95% ethanol and 40 µL PCR amplified product were added to a clean 1.5 µL tube eppendorf. It was vortexed and kept at -20°C for about 30 min. After this, it was centrifuged for 10 min at 13000xg and kept at 4°C. This was followed by the removal of supernatant, washing of the pellet with 150 µL of 70% ethanol (Presco-Beam Roanke, Virginia, and USA). The mixture was centrifuged at 7500 g for 15 min and at 4°C. The supernatant was removed and content was turned-over onto paper tissue and then dried in a fume hood at room temperature for 10-15 min. This was re-suspended in 20 µL of distilled water and kept at -20°C before sequencing. The purified fragment, which was examined on a 1.5% Agarose gel and run at 110 V for about 1 h, confirmed the presence of the purified product (Hata et al., 2010).

Sequencing

A Genetic Analyzer 3130xl sequencer (Applied Biosytems Life Technologies Corporation, Carlsbad, USA) was employed in sequencing the amplified fragments. The kit used was Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems Life Technologies Corporation, Carlsbad, USA). All genetic analyses were carried out using Bio- Edit software and MEGA 6 (Tom hall, North Carolina State University, USA) (Hata et al., 2010).

Plant collection and identification

Guava leaves were purchased from a reputable botanical garden in Akure, Ondo State, Nigeria. These leaves were identified by the Forestry Herbarium of Forestry Research Institute of Nigeria (FRIN) with the identification number 110937 (Sang et al., 2011).

Guava leaf preparation

Guava leaves of 1000 g were thoroughly washed with distilled water and air-dried for three weeks between 20-25°C. Thereafter, these were ground finely using an electric grinder and kept in an air-tight jute bag until used (Sang et al., 2011).

Diet preparation

Fish feed ingredients were obtained from a well-established fish farm in Ibadan, Nigeria. A basal diet of 42% crude protein was prepared by using Pearson Square Method. Guava leaves and single-cell protein of LAB were included in feed ingredients. These were mixed, ground and pelletized using water and a binding medium. A 2mm die was employed in a paste extrusion process, the paste formed was sun-dried and kept in a jute bag for later use. The feed ingredients used for the experiment included: soybean meal, groundnut cake, fishmeal, wheat meal, yellow maize, palm oil, common salt, and a vitamins and minerals premix. There were 7 experimental diets prepared including a control diet. *L. plantarum* and guava leaf meal were added as feed additives at various concentrations as stated below according to Ajani et al., (2011); Owodeinde and Ndimele (2011).

Control- Basal diet of 42% crude protein level

40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed

60 LPc-Basal diet+60 cells/1000 mL *L. plantarum* per kg of feed

80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed

40 PGLM-Basal diet+40 g/1000 g guava leaf meal per kg of feed

60 PGLM-Basal diet+60 g/ 1000 g guava leaf meal per kg of feed

80 PGLM-Basal diet+80 g/1000 g guava leaf meal per kg of feed

Feeding experiment

The feeding trial was conducted for 84 days in a semi-static environment. Fish were fed to satiation two times a day (8:00 am and 6:00 PM GMT). Left-over feed was removed from each facility and the polluted water was replaced with clean water. Water quality indices monitored included dissolved oxygen, pH, and temperature of each aquarium biweekly. Body lengths and weights of the fish were recorded fortnightly as the growth the fish increased.

Biological evaluation

Below are the growth indices taken during the experiment according to Hassan and Ngaski (2007).

Mean weight gain (MWG):

MWG= (W2-W1) g

Where W1= Initial mean weight (g)

W2= Final mean weight (g) (Brown, 1957)

Specific growth rate (SGR)

$$SGR\ (\%) = \frac{\text{Loge W2-logeW1 x100}}{\text{T2-T1}}$$

Where W2=Final weight (g) at time T2 (end of experiment)

W1=Initial weight (g) at time T1 (Beginning of the experiment)

Loge=Natural Logarithm (Brown, 1957)

Percentage weight gain (PWG)

$$PWG\ (\%) = \frac{\text{Mean weight gain} \times 100}{\text{Final mean weight}}$$ (Brown, 1957)

Feed conversion ratio (FCR)

$$FCR = \frac{\text{Dry weight of fish fed (g)}}{\text{Fish weight gain (g)}}$$ (Halver, 1972)

Protein efficiency ratio (PER)

$$PER = \frac{\text{Wet weight Gain}}{\text{Protein fed}}$$

Where protein fed [percentage of protein in diet x total diet consumed/100. (Sandre et al., 2017)

Feed intake (g) = Total Feed consumed by juvenile *C. gariepinus* throughout the 84 day experimental period

Analytical methods

Proximate analyses of fish carcasses and experimental diets were carried out in the Department of Animal Science of University of Ibadan, Nigeria using the methods outlined by the AOAC (1990).

Statistical analysis

Biological indices were subjected to One-way ANOVA using SPSS version 20.0. (International Business Machines, New York, USA). Duncan multiple range tests were used to assess the differences among individual means.

Results and Discussion

Percentage body constituents of *C. gariepinus* after the 84 days feeding trial

Table 1 shows the total composition of the feed formulated. The results in Table 2 show the carcass composition of *C. gariepinus* fed experimental diets for 84 days. With the exception of the control, there was no significant difference ($p>0.05$) in the carcass crude protein content across all treatment groups. There was no significant difference ($p>0.05$) in carcass ash, carcass ether extract, carcass moisture content and carcass crude fiber content across all treatment groups. Control (17.78±0.12) and 80 PGLM (17.73±0.12) had the highest level of carcass nitrogen-free extract without any significant difference ($p>0.05$).

Growth performance and nutrients utilization of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* leaf based diets for 84 days

Table 3 shows the growth performance and nutrients utilization of juvenile *C. gariepinus* fed *L. plantarum* and *P. guajava* leaf based diets for 84 days.

Table 1. Total ingredient constituents (g/1000g diet) of *L. plantarum* and *P. guajava* at various inclusion levels

Feed Ingredients (g)	T1&T2	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM
Fish meal	127.6	127.6	127.6	127.6	127.6	127.6	127.6
Groundnut cake	255.1	255.1	255.1	255.1	255.1	255.1	255.1
Soybean meal	392.7	392.7	392.7	392.7	392.7	392.7	392.7
Yellow maize	34.7	34.7	34.7	34.7	34.7	34.7	34.7
Wheat meal	99.9	99.9	99.9	99.9	99.9	99.9	99.9
Guinea corn	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Starch	5	5	5	5	5	5	5
Palm oil	10	10	10	10	10	10	10
Premix	10	10	10	10	10	10	10
Salt	5	5	5	5	5	5	5
Total	1000	1000	1000	1000	1000	1000	1000
L. plantarum (cells/1000 mL)	0	40	60	80	0	0	0
Guava leaves (g/1000 g)	0	0	0	0	40	60	80

Control- basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40 g/1000 g guava meal per kg of feed, 60 PGLM-Basal diet+60 g/1000 g guava meal per kg of feed, 80 PGLM-Basal diet+80 g/1000 g guava meal per kg of feed.
Premix Composition: Vitamin A=20.500.00 IU, Vitamin B1=20,00.00 mg, Vitamin B2=15,000.00 mg, Vitamin B3=90.000.00 mg, Vitamin B4=4.000.00 mcg, Vitamin B5=40.00 mg, Vitamin B6=20.000.00 mg, Vitamin B=500.00 mcg, Vitamin B12=15.00 mcg, Vitamin C=350.000.00 mg, Vitamin D3=4.250.00.00 IU, Vitamin E=250.00000.00 IU, Vitamin K=8.000.00 mg, Copper Sulphate=4.000.00 mg, Inositol=50.000.00 mcg, Potassium Iodine=2.000.00 mg, Inositol=50.000.00 mg, Methionine=50.000.00 mg, Choline Chloride=600.000.00 mg, Ferrous Sulphate=40.000.00 mg, Manganese oxide=30.000.00 mg, Magnesium=60.000.00 mcg, Molybdenum=100.00 mg, Antioxidant=125.000.00 mg, Lysine=50.000.00 mg, Cobalt=750.00 mg, Sodium Selenite=200.00 mcg, Zinc oxide= 40.000.00 mg

Table 2. Carcass composition of *C. gariepinus* fed *L. plantarum* and guava (*P. guajava*) leaf diets at varying inclusion levels for 84 days

Parameters (%)	Initial	Control	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM	Sig.
Crude Protein	23.13±0.01	50.08±0.01^{b}	53.71±0.01^{a}	52.57±0.01^{a}	52.07±0.01^{a}	53.68±0.01^{a}	52.13±0.01^{a}	52.07±0.01^{a}	0.99
Ash	8.97±0.01	11.31±0.01	10.25±0.04	11.88±0.01	10.39±0.01	10.93±0.01	10.42±0.01	10.39±0.01	0.96
Ether Extract	7.46±0.02	9.55±0.01	9.36±0.01	9.11±0.01	10.59±0.01	9.80±0.01	10.07±0.01	9.80±0.01	0.95
Moisture	6.30±0.01	5.29±0.01	5.86±0.01	5.25±0.01	5.48±0.04	6.25±0.01	5.49±0.01	5.01±0.01	0.97
Crude Fibre	5.06±0.26	6.07±0.12	5.00±0.00	6.00±0.00	5.81±0.02	5.00±0.00	6.00±0.00	5.07±0.12	0.89
NFE	49.87±0.26	17.78±0.12^{a}	16.24±0.02^{b}	15.98±0.01^{c}	15.73±0.04^{c}	15.02±0.13^{c}	16.02±0.01^{b}	17.73±0.12^{a}	0.03

^{a,b,c} Mean values in each row lacking a common superscript differ ($p<0.05$)

NFE: Nitrogen-Free-Extract, Control-Basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40g/1000g guava meal per kg of feed, 60 PGLM-Basal diet+60g/1000g guava meal per kg of feed, 80 PGLM-Basal diet+80g/1000g guava meal per kg of feed.

Table 3. Growth performance and nutrients utilization of *C. gariepinus* juveniles fed *L. plantarum* and *P. guajava* leaf based diets for 84 days

Parameters	CONTROL	40 LPc	60 LPc	80 LPc	40 PGLM	60 PGLM	80 PGLM	Sig.
IMW (g)	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	7.79±0.01	1.00
FMW (g)	67.10±2.06^{cd}	81.31±2.29^{a}	70.02±0.17^{bc}	69.96±2.35^{b}	70.89±0.11^{bc}	65.54±2.87^{d}	53.89±0.10^{e}	0.04
MWG (g)	59.31±2.05^{c}	73.52±2.30^{a}	62.22±0.18^{bc}	62.16±2.35^{bc}	63.10±0.10^{b}	57.74±2.86^{cd}	46.10±0.12^{d}	0.01
%MWG	88.39±2.97^{bc}	90.42±2.94^{a}	88.86±0.32^{b}	88.85±3.34^{b}	89.01±0.03^{b}	88.10±4.35^{bc}	85.54±0.30^{c}	0.03
F I (g)	138.80±0.52^{e}	166.42±2.30^{a}	147.61±0.18^{c}	146.44±0.40^{c}	153.12±0.23^{b}	143.18±2.18^{d}	109.64±0.14^{f}	0.02
F C R	2.34±0.53	2.26±0.81	2.37±0.43	2.36±0.68	2.43±0.53	2.48±0.10	2.38±1.83	0.87
SGR (g/day)	1.11±0.02^{cd}	1.19±0.02^{a}	1.14±0.00^{bc}	1.13±0.02^{bc}	1.14±0.00^{bc}	1.10±0.02^{d}	0.99±0.00^{e}	0.03
SR (%)	93.80±4.98^{c}	98.81±2.69^{a}	97.62±3.40^{a}	96.42±4.51^{ab}	98.33±2.89^{a}	97.62±3.01^{a}	96.77±4.09^{bc}	0.02
PER	1.01±0.36^{a}	1.00±0.38^{ab}	1.00±0.00^{ab}	1.01±0.36^{ab}	0.98±0.00^{ab}	0.95±0.36^{b}	1.00±0.00^{ab}	0.02
NM	1449.27±811.47	1633.81±953.08	1474.91±841.69	1485.24±835.00	1523.97±853.91	1444.18±789.04	1147.41±621.12	0.85

$^{a, b, c}$ Means within the same row lacking a common superscript differ ($p<0.05$)

IMW: Initial Mean Weight; FMW: Final Mean Weight; MWG: Mean Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; SGR: Specific Growth Rate, PER: Protein Efficiency Ratio; SR: Survival Rate; NM: Nitrogen metabolism. Control- Basal diet of 42% crude protein level, 40 LPc-Basal diet+40 cells/1000 mL *L. plantarum* per kg of feed, 60 LPc-Basal Diet+60 cells/1000 mL *L. plantarum* per kg of feed, 80 LPc-Basal diet+80 cells/1000 mL *L. plantarum* per kg of feed, 40 PGLM-Basal diet+40g/1000g guava meal per kg of feed, 60 PGLM-Basal diet+60g/1000g guava meal per kg of feed, 80 PGLM-Basal diet +80g/1000g guava meal per kg of feed, significance.

There was significant difference ($p<0.05$) in the mean weight gain (g) across all treatments in Table 3. The highest value of final weight gain was 40 LPc (81.31±2.29 g) and least final weight was 80 PGLM (53.89±0.10 g). A significant difference ($p<0.05$) was observed in the final weight among control (87.10±2.06 g), 40 PGLM (70.89±0.11 g) and 60 PGLM (65.54±2.87 g).

The highest percentage mean weight gain was 40 LPc (90.42±2.94%) with a significant difference ($p<0.05$) compared to 60 PGLM (88.10±4.35%) and 80 PGLM (85.54±0.34%). The treatments with least mean weight gain were 60 PGLM (88.10±4.35%) and 80 PGLM (85.54±0.34%) with no significant difference ($p>0.05$). Feed intake was highest in 40 LPc (166.42±2.30 g) and least in 80 PGLM (109.64±0.14 g) with a significant difference ($p<0.05$). No significant difference ($p>0.05$) was obtained in the feed conversion ratio across all treatments. The highest specific growth rate was 40 LPc (1.19±0.02 g/day) with a significant difference ($p<0.05$) from all other treatments. There was no significant difference ($p>0.05$) in survival rate among 40 LPc (98.81±2.69%), 60 LPc (97.62±3.40%), 80 LPc (96.42±4.51%), 40 PGLM (98.33±2.89%), and 60PGLM (97.62±3.01%). There was a significant difference ($p<0.05$) in the survival rate between control (93.80±4.98%) and 80 PGLM (96.77±4.09%). There was significant difference ($p<0.05$) in the protein efficiency ratio of control (1.01±0.36) and 60 PGLM (0.95±0.36). No significant difference ($p>0.05$) was observed in the nitrogen metabolism across all treatment groups.

This research evaluates the impact of *L. plantarum* and *P. guajava* leaf diets on growth response and nutrient utilisation of *C. gariepinus* juveniles. The results of this experiment revealed that *L. plantarum* and *P. guajava* leaf diets enhanced growth performance of fish appreciably. This conforms to the result of El-Haroun (2007) who observed that there was a faster growth

rate in *C. gariepinus* fingerlings fed diets supplemented with commercial feed additive than those fed control diets. This was confirmed further, in the result of Olmedo-Sanchez et al. (2009) who reported a faster growth rate of Shrimps (*Paneaus indicus*) fed feed supplemented with additives.

These results suggest that dietary additions of *L. plantarum* and *P. guajava* leaf diets at varying levels can enhance fish growth. Protein Efficiency Ratio (PER) and gross feed conversion efficiency (GFCE) measure the efficient conversion of protein sources in fish feed into body tissues in fish (DeSilva and Anderson, 1995). However, from this experiment, there was good growth performance across all treatments in relation to their weights, FCR, SGR, PER and FI. This may be due to the minerals present in guava leaves such as calcium, potassium, magnesium and phosphorus. Additionally, the presence of phytochemicals such as terpenoids, steroids, flavonoids and phenols in guava leaf diets may have contributed to the good growth performance of treatments fed guava leaf diets (NRC, 2011).

The low level of weight gain in 80 PGLM (80g/1000g) may have been due to the presence of anti-nutrient substances, which are present in plants such as saponins, tannins and anti-vitamins. They have been proven to disturb the gastro-intestinal-tract (GIT) of animals. These may negatively affect the feeding efficiency and the process of digestion in fish leading to low nutrient utilisation. This culminates to low feed intake which manifests in the slow growth response of fish (NRC, 2011). Probiotics such as *L. plantarum* aid in improvement of digestion in animals which is stimulated by digestive enzyme production. Improvement of digestion could also be through other routes such as the gut environment of the fish. This invariably is transformed into improved growth performance in fish. Hence, a good feed conversion ratio is influenced by feed absorption and utilisation (Welker and Lim, 2011).

A confirmatory report in the study conducted by Lashkar et al. (2011) and Waché et al. (2006) revealed that live yeast addition into fish diet as a probiotic improved the growth performance and food efficiency ratio. This is in agreement with the result of this experiment, which showed a better weight gain in *L. plantarum* diet (40LPc) compared to *P. guajava* and the control diets. This may have been caused by the increased appetite of fish, which led to a better body composition of fish fed dietary *L. plantarum* compared to other additives. Contrary to the results of this study, Albuquerque et al. (2014) and Moura (2011) reported that there were no significant differences in the addition of dietary probiotics on the growth performances of Nile Tilapia fingerlings of GIFT strain. Reports from Nwanna et al. (2012) and Nwanna et al. (2013) showed improved growth performance in fish fed probiotics fortified diet. Further results of Abdel-hamid et al. (2009), Diab et al. (2002) who administered commercial feed additives to fish and reported that the survival of *C. gariepinus* was enhanced by feed additives supplementation confirmed the results of this experiment.

Conclusion

From the findings of this study, it can be deduced that the inclusion of 40 cells/1000 mL of *L. plantarum* in the diet of *C. gariepinus* resulted in enhanced growth when compared to those of *P. guajava* leaf diets.

Ethics Committee Approval: Ethics Committee Approval was received for this study from the ethics committee of University of Ibadan. This was issued in 2017 with ethical approval number 0028.

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The Effect of Uterine Lavage and Oxytocin Administration Before and After Breeding on Fertility in Mares in The First Postpartum Estrus

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Abstract

The objective of this study was to investigate the effects of the combination of uterine lavage and oxytocin administration before and after breeding during post-partum first estrus on the pregnancy rates of mares. Thirty mares whose fetal membranes were released within the first 3 h after parturition were divided into three groups - the control (n=10), pre-breeding (n=10) and post-breeding treatment group (n=10). The uterine lavage was performed 4 h before breeding in the pre-breeding group and 4 h after breeding in the post-breeding group. The oxytocin administration was performed twice in both treatment groups intravenously - immediately after and 12 h after the uterine lavage. A sterile NaCl solution (0.9%) was administrated intravenously in the control group. In the control group there was a longer interval between parturition and first ovulation (14.6 days) compared to treatment groups ($p<0.05$). The pregnancy rates in the control; pre-breeding and post-breeding treatment groups were calculated as 40%, 40%, and 60%, respectively. Although early embryonic loss was not observed in both the pre- and post-breeding treatment groups, this ratio was 25% for the control group. As a conclusion, the administration of a uterine lavage (1 liter of sterile 0.9% NaCl solution +4.000.000 IU crystallized penicillin +4g streptomycin sulfate) and 20 IU oxytocin 4 h before or after breeding mares at their first postpartum ovulation shortens the day interval between parturition and ovulation. It can be assumed that breeding during foal heat can be effective in reducing uterine involution, inflammatory reactions related to breeding and embryonic death.

Keywords: Foal heat, lavage, ovulation period, oxytocin, pregnancy rates

Introduction

Fertility problems lead to profitability problems in horse breeding. Racehorse breeding, especially, is a very expensive and time consuming profession due to financial considerations such as the commercial need to have foals regularly. In the racehorse industry, it is crucial to breed the mare in the post-partum first estrus right after foaling in order to gain competitive advantage in the races. This period, which is also called the foal heat, is characterized by physiological follicular development and ovulation of the mare. Mares should become pregnant within one month postpartum to continue producing foals each year. Breeding the mares in the first post-partum estrus is one of the methods used to improve the chance of maintaining yearly foal production. Successful pregnancy rates for breeding during foal heat has been indicated in various studies (Gündüz et al., 2008; Le Blanc, 2003).

One important factor in successful early postpartum breeding is uterine fluid load after foaling. Mares with a clinically normal uterus should not have a significant volume of fluid in the uterus. The presence of a moderate or large volume of fluid in the uterus visible on ultrasound suggests the presence of an active infection, a prolonged non-infectious inflammatory condition, inadequate uterine clearance mechanism or failure of normal cervical function (Dadarwala et al., 2004; Katila and Reilas, 2001;

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McKinnon et al., 1988; Sertich and Watson, 1992). The incidence of intrauterine fluid retention in mares was indicated as 11-39% (Reilas et al., 1997; Watson, 2000). The visible amount of intrauterine fluid in the estrus cycle may refer to endometritis and may decrease sperm mobility, which may cause failure of pregnancy (Pycock and Newcombe, 1996). The goal of oxytocin therapy is to stimulate uterine contractions that will expel the fluid out of the uterus through the cervix. Oxytocin administration may be recommended for a fluid volume more than 1 cm radius. If the radius is 2 cm or more, moderate examinations should be performed for diagnosis of uterine inflamation. In general, according to the findings of the examination, intrauterine lavage and administration of antibiotics are performed (Pycock, 2001).

Administration of intrauterine lavage, antibiotics, oxytocin and human Chorionic Gonadotropin (hCG) after breeding has shown to be positively effective on fertility (Azawi, 2008; Kılıçarslan, 2002; Kılıçarslan, 2013; Kılıçarslan et al., 1996).

The aim of the study was to investigate the possible effects of uterine lavage and oxytocin administration during foal heat on fertility parameters such as length of ovulation periods, pregnancy rates and rates of early embryonic death. The expected hypothesis was that the combination of uterine lavage and oxytocin administration performed 4 h before and 4 h after breeding in foal heat would shorten the interval between parturition and first ovulation, increase pregnancy rates and decrease early embryonic death rates.

Materials and Method

The study was conducted on 30 Thoroughbred mares in good body condition (6/9), weighing approximately 500 kg, with an average age of 13±2. All breeding activities were held between the years 2011 and 2012. All mares had normal clinical and gynecological characteristics according to history findings, general clinical examinations and gyneacological ultrasound examinations before entering the study. Breeding criteria at foal heat were accepted as physiological parturition process without abnormality, correct timing of placenta expulsion, no visible trauma at the vagina and perineum, no trace of infection, acceptable ultrasound and cytological examination results (Le Blanc, 2009). In the present study, all criteria except cytological examination were considered as animal selection criteria. Additionally, uterine biopsy sampling was not performed due to the absence of any suspicious findings such as endometrial cysts or intrauterin fluid accumulation with a diameter ≥2 cm at ultrasound examination. All mares used in the study delivered without any complication and released their fetal membrane within the first three h after parturition.

Mares were randomized into 3 groups-control (n=10), pre-breeding (n=10) and post-breeding treatment (n=10) groups.

Twenty IU intravenous oxytocin (Oksitosin, Vetaş, İstanbul, Turkey) and uterine lavage using a combination of 1 liter of sterile 0.9% NaCl solution (Izotonik Sodyum Klorür Solusyonu, Eczacıbası-Baxter, İstanbul, Turkey) at 40˚C; 4.000.000 IU crystallized penicillin and 4 g streptomycin sulfate (Clemipen-Strep, Topkim, İstanbul, Turkey) was administrated 4 h before breeding for the pre-breeding treatment group and four hours after mating for the post-breeding treatment group.

Oxytocin administration was performed intravenously immediately after the uterine lavage. Injections were done twice during the same day at 12 h intervals. Two ml of 0.9% NaCl solution was injected intravenously in the control group 12 h before and 12 h after breeding.

All mares were examined with the same ultrasound device (ALOKA SSD500, Mindray, Shenzhen, China) used by the same operator in the same room. The ultrasound examinations were performed daily from detection of a preovulatory follicle with a diameter ≥35 mm until the day of ovulation. The diameter of the preovulatory follicle, edema of endometrial folds and intrauterin fluid accumulation were evaluated ultrasonographically. The mean preovulatory follicle at foal heat was recorded as 48±2 mm while the volume of intrauterin fluid accumulation was below 1 cm in diameter for all mares.

Twelve stallions with known reproductive performance with progressive sperm motility of 40-60% were used during the study. Mares in foal heat were mated with stallions with an interval of 48 h. The mean breeding time was recorded as 1.8 per mare. Ovulation periods after last breeding were investigated with ultrasonography every two days. Pregnancy controls were done at day 16, 30 and 42, respectively. Neither any multiple ovulation nor twin pregnancy was detected in this study.

Statistical analysis

Statistical analysis were calculated using the The Statistical Package for the Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical evaluation of the first ovulation periods after parturition in the control, pre-breeding and post-breeding treatment groups was performed using the "One-way ANOVA test" and the "Duncan test". Pregnancy rates and early embryonic death ratios were evaluated using the "Chi-square test". The statistically significant rate was set as $p<0.05$.

Results

The ovulation periods of all mares were determined ultrasonographically every two days. According to ultrasound inspections performed once every two days, the days interval from parturition to first ovulation - or foal heat as it is known - in the control, pre-breeding and post-breeding treatment groups were measured as 14.6 days, 12 days and 11.1 days, respectively (Table 1), which started on the postpartum 7.6th day. The days interval from parturation to first ovulation in the control groups

Table 1. Mean values and standard deviations for ovulation times after foaling

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
The days interval from parturation to first ovulation	$14.60^{a}\pm1.176$	$12.00^{b}\pm0.471$	$11.10^{b}\pm0.674$	*

*: $p<0.05$

[a,b]: Difference between mean values with different letters in the same line is significant.

Table 2. Pregnancy rates at first postpartum mating

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
Pregnancy rates	4 /10 40%	4 /10 40%	6 / 10 60%	NS

NS: Not significant.

Table 3. Early embryonic loss rates at first postpartum mating

Parameter	Groups			Significance
	Control Group (n=10)	Pre-breeding Treatment Group (n=10)	Post-breeding Treatment Group (n=10)	
Early embryonic loss rates	1/4 25%	0/4 0	0/6 0	NS

NS: Not significant.

was statistically higher ($p<0.05$) than both pre- and post-treatment groups, however there was no statistically significant difference between the two treatment groups.

The pregnancy rates calculated for the three groups are shown in Table 2. Although the pregnancy rate for the post-breeding treatment group was found to be slightly higher than the other two groups, no statistically significant difference was detected ($p>0.05$).

Early embryonic loss was not observed in either pre- or post-breeding treatment groups, however it was recorded as 25% in the control group ($p>0.05$) as shown in Table 3.

Discussion

Given the ideal requirement of producing one foal per year from each Thoroughbred mare, tracking reproductive efficiency is critical for stud managers. The mare is a seasonally polyestrous breeder. They have multiple estrus cycles during one part of the year, which is called the ovulatory season; and is followed by an anestrous period for the rest of the year. In early spring, the mare enters a transitional period between the anovulatory season and the first ovulation of the year. As the foals are accepted to be born in the first day of January, it is crucial for the stud manager to arrange the parturitions as early as possible, to allow foals maximum growth during the year in order to get an advantage in races, in the Thoroughbred breeding industry (Gündüz et al., 2008; Kılıçarslan and Uçar, 2015).

Pregnancy is a long period in mares. Therefore, it is also important to breed the mare in the closest date after parturition. Gündüz et al. (2008) reported that ovulation during the first postpartum estrus was seen at 6 to 12 days post partum. Another study performed by Keskintepe et al. (1988) showed that ovulation was seen between 8 to 42 days after foaling. When all study groups were assessed together, the first postpartum estrus was detected to start at 7.6 days after parturition in our study.

Keskintepe et al. (1988) stated that first ovulation at postpartum occurred between the 13th and 64th day. In another study the first ovulation was detected at approximately 12.2 days after foaling (Katilla et al., 1988). Loy (1980) observed the first ovulation at an average of 14.6 days. In another study, it was found that the first ovulation occurred earlier at postpartum during the year from spring to summer (Nagy et al., 2000). Le Blanc (2009) stated that the timing of first ovulation at postpartum tends to occur gradually closer to the parturition date from January to May. In the present study, findings indicated that the first ovulation in the control group occurs significantly later ($p<0.05$) than the ovulation in the pre-breeding treatment (12 days) and the post-breeding treatment (11.1 days) groups. The lack of significancy between the first postpartum ovulation

date of pre- and post-treatment groups suggests that the use of uterine lavage and oxytocin administration combination may not be effective in ovulation induction. However, the period between foaling and first ovulation of the season was found physiological for all study groups.

Uterine lavage is recommended in order to assist the uterus to physically clear the normal inflammatory byproducts, which occur as a response to breeding, and to increase uterus muscle tone (Brinsko, 2001). Brinsko et al. (1991) assumed that the timing of uterine lavage could have an effect on pregnancy rates. Intrauterine treatments that were performed just before breeding (Vanderwall and Woods, 2003) and also performed after 4 h to 4 days following breeding do not have a negative influence on fertility (Brinsko et al. 1990; Brinsko et al. 1991; Knutti et al. 2000). Additionally, intrauterine treatments including uterine lavage and antibiotic administration which are combined with oxytocin and hCG injections are reported to increase fertility (Azawi 2008; Kılıçarslan et al. 1996; Kılıçarslan 2002; Kılıçarslan 2013). Therefore, treatment protocols of uterine lavage and oxytocin administration were tested 4 h before and 4 h after breeding in the present study. However, no statistically significant difference was observed between the pre-breeding treatment and post-breeding treatment groups similar to previous studies (Brinsko, 2001; Malschitzky et al., 2002).

Oxytocin is widely accepted as an effective therapy in aiding mechanical clearance mechanisms and improving fertility. The endometrium shows irregularities because of incomplete involution during foal heat. Myometrial contractions help bacterial elimination and stimulate mucosal rejeneration (Katila and Reilas 2001), and oxytocin injections promote uterine involution especially when administered during estrus (Nikolakopoulos and Watson, 1999). Cadario et al. (1999) stated that mares weighing 450 kg could be given 10 IU or 20 IU of oxytocin. In another study, it was emphasized that 25 IU oxytocin applications 72 h after breeding does increase pregnancy rates by 7% (Pycock and Newcombe, 1996). In the present study, the results indicate that 20 IU oxytocin given after breeding was positively effective on pregnancy rates by showing a slightly higher pregnancy rate in the post-breeding treatment group, however, this difference was not significant.

It is known that the use of oxytocin and antibiotic combination is effective on fluid elimination and increasing pregnancy rates in mares (Pycock and Newcombe, 1996). The findings of the present study indicate that the combination of oxytocin and antibiotics could have an effect on increasing pregnancy rates when used after breeding.

Embryonic death rate for fertile mares was approximately calculated as 5-24% and the highest rate was seen at 10 to 14 days after detection of pregnancy via ultrasound (Ball, 1993). It was advocated that the embryonic death rate was higher in the mares bred at foal heat (Blanchard and Varner, 1993; Lowis and Hyland, 1991; McKinnon et al., 1988). In the present study, one embryonic death was noticed at the 30th day among the 4 mares in the control group.

Conclusion

Administration of uterine lavage (1 liter of sterile 0.9% NaCl solution + 4.000.000 IU crystallized penicillin + 4 g streptomycin sulfate) and 20 IU oxytocin 4 h before or after breeding mares at their first postpartum ovulation shortens the day interval between parturition and ovulation. It can be assumed that breeding in foal heat could be effective in reducing uterine involution, inflammatory reactions related to breeding and embryonic death.

Ethics Committee Approval: The study was approved by Istanbul University Local Committee on Animal Research Ethics (no: 2011/122) (29/09/2011).

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Ion Transfer as a Co-Adjuvant to Acupuncture for Treatment of Inflammatory Injuries in Horses

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Abstract

Equine spinal lesions are a common occurrence. These lesions are often caused by excessive use of certain spinal regions resulting in tissue injury that releases K+, Na+, and Ca++ ions, in addition to allogenic substances that are present during inflammation. Several therapies are available for treating spinal lesions, including acupuncture which has been highlighted as a safe and positive technique. Of the techniques developed in Japan, ionic pumping may be a suitable co-adjuvant to the dry needling technique, offering the benefit of being less invasive. The purpose of this study was to evaluate the action of dry acupuncture and ionic pumping by diode wire in horses with vertebral column inflammation. Twenty-three sports horses with inflammatory changes in the thoracic spine region were evaluated. This study confirmed that both the ionic pumping technique by diode wire as well as dry needling were adequate in regulating the homeostasis of the studied region. After one week of dry acupuncture ($p=0.0006$) and ionic pumping, the local temperature of the injury reduced significantly, allowing the inflammatory state to subside from moderate to mild to absent ($p=0.001$).

Keywords: Acupuncture, athletic horse, bioelectricity, inflammation

Introduction

The study on safe therapies that promote recovery in a fast and satisfactory way allowing the patient better quality of life is fundamental for the advancement of medical practice related to horses. As for equine sport, this research is even more important, since the healthy animal is able to maximize its athletic potential.

In horses, conditions that often and directly influence their quality of life and performance are those related to the spine. In a study by Martin et al. (2016), it was reported that the pressure applied by the saddle and rider on the horses' spine was considerable and can be directly related to spinal injuries. For Merriam (1997), horses of various riding modalities, especially for the purposes of training, often work with back pain. These cases of back pain are due to training with repetitive movements, contributing to the development of inflammatory lesions such as defects, arthritis, myositis and other disorders of the spine.

In these cases, it is hoped that therapies will be developed in addition to nonsteroidal anti-inflammatory drugs, which will cure or improve the degree of injury, since physical activity may be the cause of the disease. Among the forms of therapy, acupuncture has been highlighted as a safe and successful technique, the use of which is allowed in large competitions (Xie et al., 1996; Xie et al., 2001). Many studies at peripheral, medullary and supraspinal levels using animal models of inflammatory pain have already been conducted evaluating the effect and mechanism of action of acupuncture (Cantwell, 2010; Habacher et al., 2006; Schweinitz, 1998; Shmalberg et al., 2014; Su et al., 2012).

Although Traditional Chinese medicine (TCM) is the most explored, Traditional Japanese Medicine (TJM) has been attracting

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a great deal of attention by presenting itself more pleasantly, with superficial stimuli, and use of fewer acupuncture needles in addition to the association of other techniques results. Over many years, these characteristics have been based on TCM and have been influenced by culture, interpretation and the search for better results and better acceptance by Japanese and Western society (Fratkin, 1999).

Among the techniques developed in Japan, ionic pumping was first described by a Japanese acupuncturist physician named Yoshio Manaka (Manaka et al., 1995). Based on the observation of human patients with burns during World War II, Manaka suggested that potassium (K^+) ion concentration in the lesion region due to cell destruction was one of the factors responsible for pain and delayed healing. Based on this idea, a simple technique was developed using a wire capable of transferring ions in one direction. This wire, variously called semiconductor wire or diode wire, is made of copper or silver. One end is attached to a small piece of diode, a material that allows the unidirectional flow of electric current forming part of a circuit (Manaka et al., 1995).

The purpose of this therapy applied by Manaka, is to transfer electrons from one area of normal tissue to another where it will have excess of potassium ion for the balance of charges to occur. Later, Manaka adapted the technique for the use associated with acupuncture points, known to have greater electrical conductivity, as well as application in other injuries caused by trauma or injury, with release of sodium ions (Na^+), calcium (Ca^{++}) and other algogenic substances in addition to potassium ion, responsible for nociceptive stimuli (Manaka et al., 1995).

Since then, there have been few scientific studies which have attempted to look into these techniques. With the aim of raising awareness of less invasive techniques that improve the forms of treatment of inflammatory lesions affecting sports horses, the objective of this study was to evaluate the use of acupuncture associated with ionic pumping and acupuncture by dry needling in inflammatory changes in the thoracic spine of horses.

Materials and Methods

Experimental design
This study was approved by the Ethics Committee on Animal Use (CEUA) of the Institute of Biological Sciences of the University of Brasília (protocol 160100/2013).

In the first stage, 23 athletic horses presenting clinical signs (palpation pain and positive for dorsiflexion test) and thermographic signs (relevant local temperature increase) of inflammation along the thoracic spine were selected from jumping and dressage disciplines. The images that presented heterogeneous heat distribution were considered relevant, in these cases, the central temperature of the hot spot (SP1) was measured and then compared with the temperature of the caudal region of the inflamed area (neutral point-SP2) (Figure 1). As proposed by (Basile et al., 2010; Çetinkaya and Demirutku, 2012; Schweinitz, 1999), a temperature difference greater than 1°C was characterized as relevant alteration.

Exclusion criteria were scars in the dorsal region, use of systemic and topical medications and presence of tricotomy in the dorsal region. No animals were exercised during the 3 h prior to the thermographic evaluation. During the clinical examination, it was observed that the internal body temperature was within the normal range for equine species, with an average of 37.05±0.9°C. The clinical examination was performed at room temperature from 19 to 29°C as suggested by Turner (2007).

The 23 equines were separated into 3 groups:

Acupuncture group by dry needling (GA): Animals treated with acupuncture by dry needling (n=6);

Ionic pumping group (GB): Animals treated with acupuncture associated with the ionic pumping technique (n=11);

Control group (GC): Animals with inflammatory disorder, but not treated (n=6).

In order to observe the inflammation prior to the proposed treatments, a thermographic examination was used to indicate the increase in surface temperature, a pathognomonic characteristic finding of an acute inflammation (Schweinitz, 1998). The evaluations took place in two stages, as follows:

M0 – initial stage before any therapeutic intervention;

MF – second stage after one week of the dry acupuncture session or that associated with the ionic pumping technique.

The animals of the GC group were evaluated in both the M0 stage and in the stage that corresponded to the MF.

Thermographic evaluation
For thermographic evaluation, the animals were kept in their stalls, exposed to normal resting conditions and protected from the sun, rain or drafts. To obtain the images, the thermographic apparatus (Flir® model E49001/E40; FLIR Systems Australia Pty Ltd, Melbourne, Australia) was positioned at a distance of one and a half meters from the animal in the upper position, so that the field of view was approximately 45 degrees and emissivity equal to 0.98. Such characteristics were adopted in all evaluations. To obtain the data, the central temperature of the pre-diagnosed abnormal focus (Sp1 region) was checked three times to obtain a mean (ΔTa) (Figure 1). In the same way, the temperature of the region of the caudal vertebral column was measured at the abnormal focus (Sp2) with absence of thermographic abnormality, obtaining the value used as self-control (ΔTn) (Figure 1).

In this way, we neutralized the influence of the variation of the ambient temperature in the evaluated moments, since the ef-

fects of the external environment were equal for the two foci. After obtaining these data, the thermal differential (ΔT: ΔTa-ΔTn), which is the temperature difference between the mean of the abnormal focus (ΔTa) and the mean of the normal focus (ΔTn), was calculated. The ΔT was the final data to be evaluated. This methodology was similar to that performed by Um et al. (2005).

To evaluate the clinical significance of the thermographic findings, the inflammation was characterized according to the degree of temperature recorded by the thermographic analysis before (M0) and after (MF) treatment, namely, absent, mild, moderate and severe. These parameters were determined in accordance with several authors (Çetinkaya and Demirutku, 2012; Holmes et al., 2003; Schweinitz, 1999; Turner, 2007). Thus, the following criteria were adopted:

ΔT up to 0.5°C were considered normal, therefore, they were absent from inflammatory alterations;

ΔT between 0.5 and 1.5°C were considered as mild inflammatory grade;

ΔT between 1.5 and 2.5°C were considered as moderate inflammatory grade;

ΔT above 2.5°C were considered as severe inflammatory grade.

Acupuncture treatment

After the thermographic evaluation in M0 of all the animals, treatment with acupuncture by dry needling (GA group) and acupuncture using the ionic pumping technique (GB group) was started.

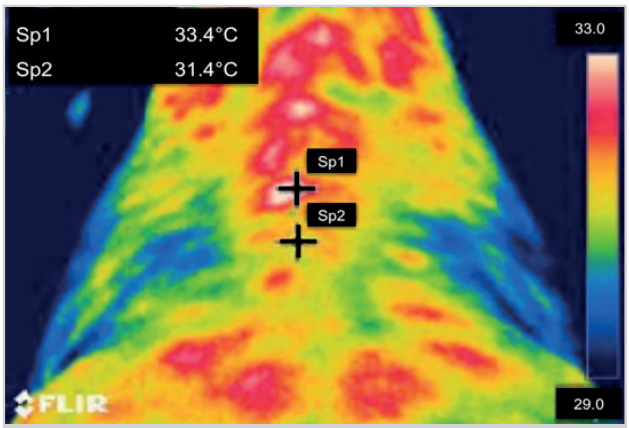

Figure 1. Thermographic image of the thoracolumbar spine of an equine in sports training, in relation to the initial evaluation (M0). Thermographic image of the initial moment with intersection (Sp1) indicating a point temperature of 33.4°C in an infrared light with significant change and intersection (Sp2) at a point temperature of 31.4°C in an unchanged infrared light.

In the GA group, the selection of acupuncture points was based on the technique of Lun Huan Chiao Ti Fa. This technique, almost always referred to as "closing the dragon," consists of circling an area using points around the perimeter of the inflammation, including bladder meridian (paravertebral) and governing vessel (dorsal midline in intervertebral spaces). Other points such as special and mastery points related to spinal injuries were used, namely: B-40, B-60, VG-20, VB-27, BP13, B-23, VG-3, VG-4, B-52 (Altman, 2006). All points were stimulated using only 0.25mm x 30mm acupuncture needles inserted into the subcutaneous tissue and held for 20 min. A total of 15 needles were used in the treatment of GA.

In the technique used in the GB group only three acupuncture needles were used. The first one was inserted guided by thermography into the center of the inflammation, and at that point a acupuncture needle of size 0,18mm x 25mm was inserted superficially. Two other needles were inserted into the bilateral paravertebral region at the time of the third lumbar vertebra (corresponding to acupuncture point B-23). Diode wire was attached to all needles using the positive pole in the inflamed region and the negative pole in the lumbar region. In this way, the transfer of ions occurs from the inflamed region to the neutral region. This technique was applied for 20 min.

At the end of treatment, the number of animals (%) that presented homeostasis (the thermal equilibrium between the inflamed and non-inflamed area) was quantified.

Statistical analysis

At the end of the data collection, the results obtained were statistically analyzed using GraphPad Prism® software version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). All data were submitted to descriptive analysis to obtain the mean and standard deviation of the mean. Then, the Kolmogorov-Smirnov normality test was applied to then submit the data to Student's t-paired test between the two corresponding moments (M0xMF) of each group (GA, GB, GC) and one-way ANOVA among GA, GB, GC groups. The $p \leq 0.05$ was adopted for all treatments as statistically significant.

Result and Discussion

The GA group showed a 48% decrease ($p=0.0006$) in the temperature variation in the focus of the lesion measured in M0 (1.68±0.39) compared to MF (0.81±0.4°C) (Figure 2). The GB group showed a 53% ($p=0.0001$) decrease in the temperature variation in the focus of the lesion measured in M0 (1.09±0.08) compared to MF (0.58±0.1°C). In the GC group, no change in temperature ($p>0.05$) was observed between the times M0 (2.03±0.9°C) and MF (2.05±0.91°C) (Table 1).

In GA, it was observed that, after treatment with dry needling, in five of the six individuals, the degree of inflammation went

from moderate to absent, which was considered to be clinically irrelevant. In GB, clinical recovery was also obtained in nine of eleven individuals. In contrast to what was observed in the GA and GC groups, in the GC group the degree of inflammation was not altered, remaining moderate in MF (Table 1) (Figures 1 and 2).

The observed effect was evident in the GA and GB groups, where ΔT was considerably reduced. The data obtained in this study contributed to other findings of authors who also affirmed the action of acupuncture versus the sympathetic division of the autonomic nervous system, promoting an active action on vasomotor tone concomitant to the inflammatory response (Schweinitz, 1998; Schweinitz, 1999; Scognamillo-Szabó and Bechara, 2001).

More precisely, Huang et al. (2013) reported that acupoint stimulation acted locally on receptors in complex structures such as nerves, blood vessels, and lymphatics. Acupuncture exerts a stimulus that triggers the production of neuropeptides, processing and integration of cytokines and causes the nerve impulses to be sent through the peripheral and autonomic nervous system, thus generating a precise and complete regulation of the neuropeptide chain, for example the cytokines, which acted directly in the process of inflammation (Fu et al., 2007; Huang et al., 2013).

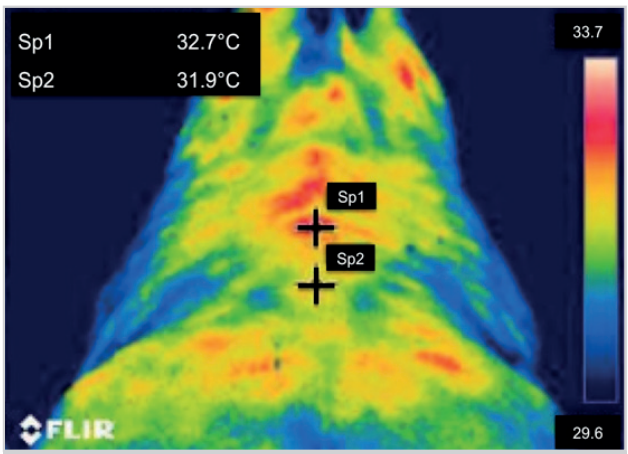

Figure 2. Thermographic images of the thoracolumbar spine of an equine in sports training, after the acupuncture session in association with ionic pumping (MF). Thermographic image with intersections indicating the focal point temperature initially with a relevant change (Sp1) of 32.7°C and that of unchanged focus (Sp2) of 31.9°C after one week of the associated acupuncture session to ionic pumping.

According to the degree of change in heat distribution, the final result of GA and GB showed complete resolution of the initial vasomotor change. These results suggest restoration of the process of inflammation, since the ΔT was below 0.5°C in several animals, being considered clinically irrelevant (Basile et al., 2010; Schweinitz, 1999). For M2, the effects of acupuncture were sufficient to reduce the change from moderate to mild which may represent an important clinical improvement. In contrast to the treated groups, GC did not show any significant results at the end of the experiment, and ΔT was not observed below 1°C, which led to the belief that the disorder was maintained.

As cited by Parmen et al. (2014), local acupuncture applied to spinal diseases may decrease local edema, inflammation, vasodilation or vasoconstriction, release of histamine or kinins. These signs were stronger in severe and moderate changes, which may have made it possible to more clearly visualize the local repair effects of acupuncture. On the other hand, mild alterations, for the most part, may be those with chronic characteristics caused by the high physical requirements and functionality of the spine in performing repetitive exercises. In addition, the mechanical action of the rider, or rider and mount equipment, regardless of discipline, must be considered, since these act continuously along the thoracic and lumbar vertebral column. According to Schweinitz (1998), many of these cases can become incurable unless the animals end their athletic life, as the cause of the injury will constantly be present. However, the results of our study suggest that acupuncture is effective in restoring spinal vasomotor alterations in horses with training routine and with active participation in competitions, since it is able to significantly reduce or eliminate the degree of the alteration observed in the study.

Table 1. Representation (mean and standard deviation) of the results obtained in the thermographic evaluation of acupuncture groups by dry needling (GA) and ionic pumping (GB), and control group (GC)

	GA (n=6)	GB (n=11)	GC (n=6)
M0	1.68±0.39	1.09±0.08	2.03±0.9
MF	0.81±0.4	0.58±0.1	2.05±0.91
M0-MF	0.87±0.01[a]	0.51±0.02[b]	0.02±0.03[c]
Homeostasis (%)	83.3	81.8	0.0
Inflammatory grade in M0 / MF	Moderate / absent	Light / absent	Moderate / Moderate

M0: Initial moment; MF: Final moment, M0-MF: Thermal difference between MF and M0; Homeostasis (%): percentage of cases where homeostasis was achieved.
[a, b, c] Different superscripts within the same line indicate significant difference among groups p≤0.05.

Conclusion

Acupuncture actively influences the disorders that lead to vasomotor alteration of the spine of horses. This treatment significantly reduced the problems in the present study. Both acupuncture by dry needling and acupuncture associated with the ionic pump clinically reestablished the homeostasis of the studied region, something that was not observed in the control group. The thermographic examination is adequate to detect vasomotor alterations in the thoracolumbar region of the spine and to monitor the response obtained by acupuncture treatment.

Ethics Committee Approval: Ethics Committee Approval was received for this study from the Ethics Committee of the Institute of Biological Sciences of the University of Brasília (protocol 160100/2013).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.A.O.B.; Design - C.A.O.B., A.R.C.B.V., E.M.M.L.; Supervision - E.M.M.L.; Resources - E.M.M.L.; Materials - C.A.O.B., A.R.C.B.V., E.M.M.L.; Data Collection and/or Processing - CA.O.B., K.N., A.C.S.; Analysis and/or Interpretation - C.A.O.B., K.N., A.C.L., A.R.C.B.V., E.M.M.L.; Literature Search - C.A.O.B., A.C.S.; Writing Manuscript - C.A.O.B., K.N., A.C.L., A.R.C.B.V., E.M.M.L.; Critical Review - A.R.C.B.V., E.M.M.L.

Conflict of Interest: The authors have no conflict of interest to declare.

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Effects of Diazepam/Propofol and Diazepam/Remifentanil Induction Protocols on the Coagulation in Dogs

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Abstract

Studying the effect of general anaesthesia on blood parameters is extremely important both in terms of patient safety and determining protocol suitability for the patient. There is no study on the assessment of the effects of Diazepam/Propofol and Diazepam/Remifentanil combination administered to dogs on clotting time, thrombin time (TT), prothrombin time (PT), active partial thromboplastin time (aPTT) and buccal mucosa bleeding time (BMBT). The purpose of the study presented is to investigate the effects of Diazepam/Propofol and Diazepam/Remifentanil combinations on coagulation parameters in dogs aged 5 years and older, requiring surgery for various reasons. Prior to anaesthesia (T0), it was found that there was no difference between the two groups in terms of PT, TT, aPTT and BMBT (p=0.426 p=0.091, p=0.166, p=0.686, p=0.209, respectively). Following anaesthesia (T1), it was found that the buccal mucosal bleeding time in dogs in the Diazepam/Remifentanil group had a tendency to be shorter (p=0.084) than those in the Diazepam/Propofol group. Also, PT in the Diazepam/Remifentanil group was longer (p=0.031) compared to the Diazepam/Propofol group. No significant difference was found between the groups with respect to clotting time, TT or aPTT (p=0.191, p=0.467, p=0.972). While it is stated that neuroleptanalgesia produces reliable anaesthesia induction in unwell patients, based on the data obtained at the end of the study, it was determined that Diazepam/Propofol combination is more reliable in the anaesthesia of patients requiring surgical intervention.

Keywords: Coagulation, diazepam, dog, remifentanil, propofol

Introduction

Hemostasis is a complex process arising as a result of the dynamic relationships between the circulatory system, thrombocytes and coagulation proteins (Chohan et al., 2011; Kamal and Kamal, 2008). The hemostatic process consists of three major phases. These are: vasoconstriction, primary hemostasis including platelet formation and secondary hemostasis comprising coagulation and fibrinolysis (Forsythe and Willis, 1989; Kamal and Kamal, 2008).

Bleeding related disorders occur in relation to vascular integrity, thrombocyte function, thrombocytopenia and von Willebrand disease. These disorders emerge with findings such as petechiae, surgical bleeding, haematomas and recurrent bleeding following formation of the first blood clot (Forsythe and Willis, 1989; Smith et al., 2005).

Thrombocyte count, clotting time, prothrombin time (PT), thrombin time (TT), active partial thromboplastin time (aPTT) and buccal mucosa bleeding time (BMBT) are the most frequently used parameters in determining coagulation disorders (Forsythe and Willis, 1989; Ogurtan et al., 2002; Smith et al., 2005).

Prothrombin time is one of the extrinsic blood coagulation tests and is used to assess extrinsic factor VII and III (Chohan

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et al., 2011; Mischke, 2011; Smith et al., 2005) PT may be prolonged in extensive intravascular coagulation, hepatic diseases or patients with Vitamin (Vit) K deficiency (Ogurtan et al., 2002).

Thrombin time is used to evaluate the conversion of fibrinogen to fibrin. Prolongation of thrombin time indicates either fibrinogen deficiency or thrombin inhibition (Smith et al., 2005).

Active partial thromboplastin time is used in the assessment of intrinsic factor XII, XI, IX and VIII. In the event of any one of these factors being less than 35% of the normal value, the aPTT will lengthen and clinical bleeding problems may be encountered. This parameter is not affected by thrombocyte count deficiency (Smith et al., 2005).

Buccal mucosa bleeding time is the best assessment method for thrombocyte function and clot formation (Chohan et al., 2011; Fresno et al., 2005). It includes the time from the incision to the first moment the bleeding stops. In healthy dogs, normal BMBT is 1.7-4.2 minutes (Forsythe and Willis, 1989; Jandrey 2012; Smith et al., 2005).

In dogs, hepatic diseases in particular affect the coagulation mechanism and cause prolongation of aPTT and PT. Normal aPTT is 8.4-14.8 seconds and PT is 6.4-8.2 seconds in dogs (Chohan et al., 2011; Ogurtan et al., 2002).

Studying the effect of general anaesthesia on blood parameters is extremely important both in terms of patient safety and determining protocol suitability for the patient (Arca and Sarıtaş, 2017; Binici et al., 2015).

Diazepam is a tranquilizer belonging to the benzodiazepine group of drugs and has no suppressive effect on heart rate, myocardial contractility or arterial blood pressure. It possesses muscle relaxant and vasodilatation producing properties. When used in combination with opioids, it produces safe sedation, particularly in elderly dogs (Guzel et al., 2018; Kürüm et al., 2013).

Remifentanil is an ultra-short-acting synthetic μ opiod agonist (Gimenes et al., 2011). In dogs, it causes a decrease in heart rate and cardiac output, bradycardia and hypotension. However, the fact that it enables rapid control of anaesthesia depth and is not dependent on hepatic metabolism or renal excretion for drug clearance is considered to be advantageous (Beier et al., 2015; Gimenes et al., 2011; Murrell et al., 2005; Pei et al., 2014). Despite being used extensively in human medicine, there are few studies on its clinical use in dogs (Beier et al., 2015; Lamont and Mathews, 2007; Pei et al., 2014).

Propofol is a short-acting anaesthetic belonging to the alkyl phenol group frequently used in Veterinary Medicine. It provides rapid induction and recovery and repeated administration causes no build-up in the body. However, in the case of high doses or rapid injection, it produces apnoea and significant hypotension (Campbell, 2005; Güzel et al., 2013; Ogurtan et al., 2002).

There is no study on the assessment of the effects of Diazepam/Propofol and Diazepam/Remifentanil combination administered to dogs on clotting time, TT, PT, aPTT and BMBT.

The purpose of the study presented is to investigate the effects of Diazepam/Propofol and Diazepam/Remifentanil combinations on coagulation parameters in dogs aged 5 years and older, requiring surgery for various reasons.

Materials and Methods

The study was conducted in accordance with the ethical principles approved by Istanbul University Animal Experiments Local Ethics Committee (26.04.2018/2018/38).

The study material comprised of 16 dogs aged 5 years and above, presented to the Istanbul University Faculty of Veterinary Medicine Surgery Department and requiring surgery for various reasons. Differences in breed and gender were not taken into account. In terms of anaesthesia risk, cases in the ASA 1 and 2 status were included in the study.

In the pre-operative period, routine physical examination was performed in all cases and haemogram (Erythrocyte-RBC, Haemoglobin-HGB, Hematocrit-HCT, Leucocyte- WBC) and blood biochemical results (Aspartate-aminotransferase-AST, Alanine-aminotransferase-ALT, glucose, urea, creatinine and total protein) were evaluated.

In all cases, food intake was stopped 12 h before and water intake was stopped 1 h before anaesthesia induction. Intravenous injections were administered to the dogs via a 22-gauge cannula placed into the cephalic antebrachial vein. Blood samples for parameter analysis for the study were obtained from the cephalic antebrachial vein in the opposite leg.

Two separate anaesthesia groups were formed, each containing 8 dogs (n=8). Dogs were selected randomly for the groups.

Group I was determined as the Diazepam/Propofol (DP) group (Diazem 10 mg, N05BA01 Deva Holding, Kocaeli/Turkey; Propofol 1%, 10 g, 20 mL, N01AX10, Fresenius Kabi Ltd, Italy). The dogs in this group were administered intravenous (IV) diazepam at a dose of 0.5 mg/kg for premedication. For induction, propofol was given at a dose of 6 mg/kg IV, 5 min after diazepam administration.

Group II was determined as the Diazepam/Remifentanil (DR) group (Diazem 10 mg, N05BA01 Deva Holding, Kocaeli/Turkey; Remifentanil, Ultiva 1 mg, N01AH06, GlaxoSmithKline plc., Italy). Again, diazepam was administered to this group at a dose of 0.5 mg/kg IV. Remifentanil was given 5 min later at a dose of 10 μg/kg via slow IV injection.

For the analysis of the aPTT, PT, and TT parameters, two sodium citrate tubes were each filled with 2 ml of blood collected from all of the dogs (n=16) before anaesthesia. This measurement time was determined as T_0.

In order to establish buccal mucosa bleeding times at the same measurement time (T_0), the right or left buccal mucosae of all dogs were punctured using a penetration device (Contour plus, Bayer, Germany) and measured with the aid of a chronometer and blotting paper. For the purpose of determining simultaneous clotting time, blood was collected into 2 capillary tubes and clotting times were observed starting from 90 sec and checking at 30 sec intervals.

Following collection of blood samples, all cases were intubated using endotracheal intubation tubes of suitable sizes. General anaesthesia was induced with 4% isoflurane and later maintained at 2% concentration.

Fifteen minutes after anaesthesia was identified as measurement time T_1. At this measurement time, all the procedures performed at T_0 were repeated before the surgical incision was made.

Blood samples were taken from the cephalic antebrachial vein of the dogs into the EDTA coated tubes and carried out at the İstanbul University, Faculty of Veterinary Medicine, Department of Physiology Laboratory. The blood samples were analysed using cell counter (Abacus Junior Vet, Austria©).

Statistical analysis

Firstly, the data was examined in terms of normal distribution in order to determine whether or not there was any difference between the effects of the anaesthetics on coagulation parameters. The difference between those demonstrating normal distribution was analysed using the independent T test. The Levene test was used to determine whether or not they exhibited normal distribution. In the comparison of those not displaying normal distribution, the non-parametric Mann Whitney U test was used. The presence of any differences between mean values measured before and after anaesthesia was analysed using the paired samples T test. All statements of significance were based on $p<0.05$ and tendencies were indicated if the P value was between 0.05 and 0.91. Statistical analysis was performed using The Statistical Package for the Social Sciences (SPSS) version 21 for Windows (IBM Corp., Armonk, NY, USA).

Results

The effects of the anaesthetics used in this study on coagulation parameters are shown in Table 1. Prior to anaesthesia (T_0), it was found that there was no difference between the two groups in terms of PT, TT, aPTT and BMBT (p=0.426 p=0,091, p=0.166, p=0.686, p=0.209, respectively).

Following anaesthesia (T_1), it was found that the buccal mucosal bleeding time in dogs in the D/R group had a tendency to be shorter (p=0.084) than those in the D/P group. Also, PT

Table 1. Differences between Diazepam/Propofol (DP) and Diazepam/Remifentanil (DR) groups regarding coagulation parameters

Before anesthesia	Buccal Mucosa Bleeding Time (second)	Clotting Time (second)	Prothrombin time (second)	APTT (second)	Thrombin time (second)
DP Group	22.0±5.04	258.7±41.25	13.1±0.56	111.1±23.59	15.3±0.80
DR Group	18.0 ±1.75	389.0±55.32	30.8±11.53	126.8±29.1	19.9±3.25
p-value	0.426	0.091	0.166	0.686	0.209
After anesthesia					
DP Group	22.7±4.93	270.0±55.83	12.7±0.49	107.2±24.31	19.9±5.39
DR Group	13.4±2.29	356.0±34.45	14.3±0.42	89.9±3.49	20.1±4.52
p-value	0.084	0.191	0.031	0.467	0.972

APTT: Active partial prothrombin time

Table 2. Changes observed in the coagulation parameters before and after anaesthesia in the group given Diazepam/Propofol combination

	Before anesthesia	After anesthesia	p-value
Buccal Mucosa Bleeding Time (second)	22.0±5.04	22.7±4.93	0.890
Clotting Time (second)	258.7±41.25	270.0±55.83	0.836
Prothrombin Time (second)	13.1±0.56	12.7±0,49	0.563
APTT (second)	111.1±23.69	107.2±24.31	0.341
Thrombin Time (Sn)	15.3±0.80	19.9±5.39	0.437

APTT: Active partial prothrombin time

Table 3. Changes observed in the coagulation parameters before and after anaesthesia in the group given Diazepam/Remifentanil combination

	Before anesthesia	After anesthesia	p-value
Buccal Mucosa Bleeding Time (second)	18.0±1.75	13.4±2.29	0.048
Clotting Time (second)	389.0±55.3	356.0±34.4	0.504
Prothrombin Time (second)	30.8±1.81	14.3±0.42	0.183
APTT (second)	126.8±29.17	89.9±3.49	0.262
Thrombin time (second)	19.9±10.2	20.1±14.3	0.974

APTT: Active partial prothrombin time

in the D/R group was longer (p=0.031) compared to the D/P group. No significant difference was found between the groups with respect to clotting time, TT or aPTT (p=0.191, p=0.467, p=0.972).

Data obtained for pre-anaesthesia (T_0) and post-anaesthesia (T_1) comparison is shown in Table 2 and Table 3. No significant difference was found between the pre-anaesthesia and post-anaesthesia clotting times, PT, TT, aPTT and BMBT in dogs in the D/P group (p=0.890, p=0.836, p=0.563, p=0.341, p=0.437, respectively).

In dogs in the D/R group, however, BMBT was found to be shorter following anaesthesia compared to before anaesthesia (p=0.048). On the other hand, no significant difference was seen between T_0 and T_1 with respect to clotting time, PT, TT or aPTT (p=0.504, p=0.183, p=0.262, p=0.974, respectively).

Discussion

In surgical interventions it is extremely important to control the bleeding at the start of the incision and provide hemostasis (Charlesworth et al., 2012). Anaesthetic drugs may alter the diameters of arterioles and venules and the reaction of these structures to stress. During general anaesthesia, vasodilatation occurs, blood flow decreases and the rate of thrombosis formation increases (Binici et al., 2015).

Diazepam, used extensively in clinical Veterinary Medicine, has no suppressive effect on the cardiovascular system (Kürüm et al., 2013). However, it produces vasodilatation in blood vessels in connection with its muscle relaxant effect (Guzel and McKinstry, 2017; Guzel et al., 2018). Propofol, a general anaesthetic used regularly in Veterinary Medicine leads to a significant degree of hypotension in the event of high doses or rapid intravenous injection (Campbell, 2005; Güzel et al., 2013; Ogurtan et al., 2002). In the first study, group of D/P combination, no suppressive effect was observed to occur on parameters investigated in terms of coagulation. This was because no statistically significant difference was determined between investigations performed before anaesthesia (T_0) and after the anaesthesia protocol administration (T_1).

The endotracheal intubation procedure triggers the cough reflex in patients. This stimulation produces an increase in the sympathetic tone and leads to tachycardia and hypertension (Güzel et al., 2013). In neuroleptanalgesia performed with a tranquilizer and opioid combination, haemodynamic changes caused by endotracheal intubation occur to a lesser degree. In this study, in the anaesthesia administration with D/R, no significant difference was observed between pre-anaesthesia and post-anaesthesia measurement times in terms of clotting time, PT, TT and aPTT. However, in this group BMBT was found to be shorter at T_1 measurement time compared to the D/P group. It has been thought that remifentanil causing a decrease in heart rate and cardiac output as well as its strong hypotensive effects (Beier et al., 2015; Gimenes et al., 2011; Murrell et al., 2005; Pei et al., 2014) may have played a role in this difference emerging. The vasodilatation occurring in the blood vessels is also effective in shortening bleeding time (Binici et al., 2015) by affecting blood viscosity.

A prolonged aPTT in spite of normal prothrombin time indicates the deficiency of factors VIII (Haemophilia A and von Willebrand Disease), IX and XI. Prolongation of both PT and aPTT suggests deficiency of common path coagulation factors (factor X, V and II) or qualitative or quantitative fibrinogen disorder or inhibition (Giurgiu et al., 2009). Lengthening of the PT value alone may be observed in extensive intravascular coagulation, hepatic diseases or patients with Vit K deficiency (Ogurtan et al., 2002). In this study, a statistically significant lengthening was determined in the PT value at measurement time T_1 in the D/R group compared to the D/P group. However, no difference was observed in the aPTT levels in either group. Pre-anaesthesia biochemical analysis was performed in all cases and liver function tests indicated AST and ALT values to be within the normal range. In the authors' opinion, while the PT prolongation emerging only after anaesthesia in the DR group did not develop as a result of liver function disorder, the reason for this lengthening could not be explained, in view of the fact that when evaluated within the group, no statistically significant difference was found between either T_0 or T_1 in terms of PT values.

In a study (Ogurtan et al., 2002) investigating Diazepam/Ketamine combination, it was reported that this combination had

no significant effect on aPTT or BMBT, whereas it caused a significant lengthening in the PT value. In the same study, it was stated that Xylazine/Ketamine combination caused prolongation in aPTT and PT (Ogurtan et al., 2002). In the present study, within-group measurement times in the D/P and D/R groups showed no changes in terms of these parameters. However, when the groups were compared, it was found that only the PT value was longer in the D/R group, compared to the other group. At the same time, in the propofol group, different to the anaesthesia protocols mentioned above, it was evaluated that the reason for PT not lengthening was due to either the antioxidant properties of propofol (Lee, 2012) or the fact that propofol had no adverse effect on thrombocyte function or the coagulation process (Kamal and Kamal, 2008). As a result of this data, while concluding that general anaesthesia is effective on PT, it must be stated that more advanced analysis is required to fully explain the mechanism.

In the D/P group, it was determined that anaesthetic drug administration and the endotracheal intubation procedure did not have a sufficiently significant effect on blood pressure and therefore blood flow rate, to cause coagulation factors to be affected. This finding was evaluated as occurring in relation to the antioxidant properties of propofol (Lee, 2012) or the fact that it has no adverse effect on thrombocyte function or the coagulation process (Kamal and Kamal, 2008).

In dogs, prolonged bleeding time occurs in relation to thrombocytopenia, von Willebrand disease, uremia, aspirin or dextran use and long-term carbenicillin treatment (Forsythe and Willis, 1989; Smith et al., 2005). In this study, no difference was observed either within groups or between groups in terms of bleeding time in either the D/P or D/R group. This data was associated both with the fact that thrombocyte counts were within physiological limits as well as the lack of long-term drug administration in the dogs assessed.

While it is stated that neuroleptanalgesia produces reliable anaesthesia induction in unwell patients, based on the data obtained at the end of the study, it was determined that D/P combination is more reliable in the anaesthesia of patients requiring surgical intervention.

Ethics Committee Approval: The study was conducted in accordance with the ethical principles approved by İstanbul University Animal Experiments Local Ethics Committee (26.04.2018/2018/38).

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Anaesthesia-related Risk Factors in Unwell Patients or Trauma Patients and Anaesthetic Drug Selection: Cats and Dogs

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Abstract

Safe anaesthesia requires careful monitoring and good knowledge of the effects of various anaesthetic drugs on different organ systems. The safest approach in the anaesthesia of critical patients is to select drugs whose effects are easily reversible and to take great care with the dosage. Deaths occurring in the perioperative period are generally caused due to pre-existing diseases, anaesthesic drugs, surgical interventions, or a combination of these factors. Prolonged surgical interventions, hypothermia, fluid loss, and excessive fluid intake are also factors that increase patient deaths. When anaesthetizing an unwell patient, individual requirements are evaluated. Therefore, it is not possible to present a single overall anaesthesia protocol applicable to all patients. There is a series of principles for the administration of anaesthesia to high-risk patients. This review discusses anesthesia administrations required for both diagnostic and surgical interventions in trauma patients and unwell patients. The risk factors and the various complications that may be encountered by clinicians while performing sedation and anaesthesia in such patients and their treatment methods have been explained in detail.

Keywords: Anesthesia, cat, dog, risk factors, trauma

Introduction

General anaesthesia is the state of total unconsciousness produced by a controlled and reversible suppression of the central nervous system using drugs with an anaesthetic effect. Muscle relaxation, loss of reflexes and loss of pain sensation also occurs in general anaesthesia. Clinicians performing anaesthesia are usually anxious about anaesthesia-related complications and, in particular, losing a patient. However, patient loss due to anaesthesia occurs much less than the loss of a patient caused by other reasons.

In this review, anaesthesia administrations required both for diagnostic and surgical interventions in trauma patients and unwell patients have been discussed. Risk factors and various complications that may be encountered by clinicians performing sedation and anaesthesia in such patients and their treatment methods have been explained in detail.

Key points for guidance in anaesthesia administration

1. Safe anaesthesia requires careful monitoring and good knowledge of the effects of various anaesthetic drugs on different organ systems.
2. Anticipating and preparing for probable complications is helpful in achieving successful results.
3. Analgesic treatment in critical patients must be approached on an individual basis, since responses to analgesic drugs may be very different.

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4. The safest approach in the anaesthesia of critical patients is to select drugs whose effects are easily reversible and to take great care with the dosage (Rozanski and Rush, 2007).

Targets for the anaesthesia of unwell and trauma patients

The first goal when performing anaesthesia in unwell and trauma patients must be to optimize tissue perfusion and enable sufficient oxygenation of the vital organs in particular, while achieving analgesia, muscle relaxation and unconsciousness in the patient. The final aim of the treatment, rather than the measured parameters returning to normal, is to provide maximum physiological function (Carroll and Martin, 2007).

Values that are considered normal for healthy patients undergoing anaesthesia produce higher rates of mortality in unwell patients. In humans subjected to trauma, for the chance of post-operative survival, a 110-120% higher blood volume and 50% higher cardiac findings are required. These values are necessary for tissue repair and to meet increased metabolic requirements in trauma and post-surgical patients (Carroll and Martin, 2007).

Determination of the patient's risk status according to the American Society of Anesthesiologists (ASA) classification

The ASA classification is an assessment system useful for identifying the pre-operative risk status of patients and determining a suitable anaesthetic approach and monitoring methods. It is used to classify patients depending on fatal complications arising from general anaesthesia (Redondo et al., 2007; Rozanski and Rush, 2007). According to this method;

Category 1: Normal healthy patients. General anaesthesia poses a minimal risk.

Category 2: Patients with mild systemic disease (localized infection and treated heart disease).

Category 3: Patients with severe systemic disease (fever, anaemia, mild hypovolemia).

Category 4: Patients with life-threatening severe systemic disease (heart failure, septic shock). Patients not expected to survive without intervention.

Category 5: Patients with life-threatening disease (shock, severe trauma and end stages of fatal infections). Patients not expected to survive for more than 24 h regardless of intervention.

According to ASA criteria, patients in categories 3, 4 and 5 have higher anaesthesia-related death rates (Bille et al., 2012; Brodbelt et al., 2006; Itami et al., 2017). Patient fatalities occur due to problems during anaesthesia in ASA 1 and 2, whereas in ASA 3, 4 and 5, these generate from the poor condition of the patient (Hosgood and Scholl, 2002; Redondo et al., 2007).

Anaesthesia-related mortality risk

The risk of death due to anaesthesia is much lower than the mortality risk arising from a disease or surgical intervention. However, since anaesthesia is achieved via the controlled administration of drugs with toxic properties to the patient, it bears risks such as organ dysfunction, delayed recovery and death (Alef et al., 2008; Evans and Wilson, 2007; Jones, 2001).

Anaesthesia-related mortality rates are higher in Veterinary Faculty hospitals. Playing a role in this are the facts that patients brought to the Veterinary Faculty have multiple problems and diagnostic and surgical interventions are more complicated (Brodbelt, 2009).

Two main factors are influential in patient deaths. Firstly, the physical condition of the patient, and secondly the knowledge, experience and skill of the practitioner. Close monitoring of the patient is important for timely detection of possible risks and necessary intervention (Brodbelt et al., 2008; DeLay, 2016; Evans and Wilson, 2007; Jones, 2001).

The Anaesthesia-related mortality risk is 0.05-0.1% in healthy dogs, 0.24% in healthy cats and 1.33% in ill dogs and cats. The risk of death is higher in cats than in dogs. Also the mortality risk in rabbits and other exotic animal species is higher in comparison to cats and dogs (Bille et al., 2012; Brodbelt, 2009; Brodbelt, 2010; Itami et al., 2017).

The Anaesthesia-related death rate in humans is 0.02-0.05%. The main reason for human mortality rates being lower than animals is the excess of animal species in veterinary anaesthesia. Also, in human anaesthesia, clinical staff receive a high level of training and there is more anaesthesia equipment, monitoring and intensive care facilities (Biboulet et al., 2001; Brodbelt, 2009).

In recent years, factors such as an improvement in veterinary equipment, safer medication and better monitoring in line with technological developments, have significantly reduced anaesthesia-related mortality risk (Brodbelt, 2009; Brodbelt, 2010; DeLay, 2016).

In order to minimize the risk of anaesthesia-related death, prior to anaesthetising the patient it is important to determine a high heart rate, high total leucocyte count, low PCV, low glucose concentrations and levels of inflammatory mediators such as c-reactive protein. Stabilizing the patient before anaesthesia helps to reduce anaesthesia-related death risk (Bille et al., 2012; Brodbelt et al., 2007; Itami et al., 2017; Perkowski, 2000).

Patients at high risk from anesthesia and operative intervention

In small animal practice (particularly in elderly cats with hyperthyroidism) all trauma patients with lung injuries, patients with life-threatening conditions such as haemothorax, pneumothorax and pulmonary haemorrhage, acute head trauma and severe intra-abdominal haemorrhage, carry a high risk with respect to anaesthesia and surgical intervention. Also, newborns, patients with portosystemic shunt occlusion and cardi-

ac, intracranial or intraocular surgery patients are considered to be cases requiring extreme caution (Evans and Wilson, 2007; Evans and Wilson, 2011).

Anaesthesia-related death

Anaesthesia-related death is described as any death occurring in the period starting from anaesthesia induction up to the time when the patient has regained consciousness or returned to its pre-operation status (Brodbelt, 2009).

Peri-operative deaths occurring in the first 48 h including completion of the anaesthetic procedure are deaths related to anaesthesia and sedation. Inoperable surgical patients, patients in the ASA 3, 4, 5 categories and cases with a pre-existing medical condition are not included in anaesthesia deaths (Brodbelt, 2009).

Research has revealed that among anaesthesia-related deaths, 0.1% occur at premedication, 6-8% during induction, 30-46% during maintenance and 47-60% in the post-operative period and the first 48 h. The first 3 post-operative h are important (Brodbelt et al., 2006; Brodbelt et al., 2008; Itami et al., 2017).

Reasons for death

Deaths occurring in the peri-operative period are usually caused by pre-existing diseases, anaesthetic drugs, surgical interventions or a combination of these factors. Deaths due to physiological causes may be multifactorial. Multiple organ or system failure leads to the loss of the patient (Brodbelt, 2009; Brodbelt, 2010).

The majority of patient losses observed in the post-operative period occur as a result of cardiovascular and respiratory system complications. Gastrointestinal, neurological, liver and kidney problems are also among the reasons for death (Brodbelt, 2010; Hosgood and Scholl, 2002).

The main cardiovascular reasons include, a weakened pumping ability of the heart and vascular collapse leading to insufficient blood supply to vital organs. Cardiac arrest occurs as a result of cardiac arrhythmias due to increased catecholamines in the circulation, myocardial hypoxia, specific anaesthetic drugs, pathological conditions present in the patient, interventional procedures such as vagal traction or eye enucleation and myocardial depression due to high-dose anaesthetic drug administration (Brodbelt, 2009; Jones, 2001).

Hypovolaemia and circulation failure are also among the main reasons for cardiovascular collapse (Brodbelt, 2009; Muir et al., 2007).

Anaesthesia deaths originating in the respiratory system usually arise from unsuitable endotracheal intubation, upper respiratory tract trauma and insufficient ventilation. The principle cause of respiratory complications, seen in brachycephalic patients in particular, is respiratory obstruction. As well as the small size of the feline respiratory tract, cats are also more vulnerable to trauma, spasm and oedema formation compared to dogs. In this species, the death rate due to endotracheal intubation is as high as the death rates of a respiratory and cardiovascular origin. Therefore, extreme caution must be observed during endotracheal intubation in cats (Brodbelt, 2010; Jones, 2001).

Other causes of patient loss in the peri-operative period include; post-operative renal insufficiency, iliac thrombosis, regurgitation, aspiration of gastric content, anaphylactic reactions, failure to regain consciousness and other unknown reasons (Brodbelt, 2009; Muir et al., 2007).

In cats, the most important risk factors for anaesthesia-related deaths are; general poor health, advanced age, endotracheal intubation and insufficient monitorisation (Brodbelt, 2010; Jones, 2001).

Determining the risk factors leading to anaesthesia-related deaths will reduce the rate of death. In pediatric, elderly, unwell and trauma patients, sterilization procedures, Terriers, Spaniels and brachycephalic breeds, the death rate is usually high (Brodbelt, 2009; Brodbelt, 2010; Muir et al., 2007).

Prolonged surgical interventions, hypothermia, fluid loss or excessive fluid intake are also factors increasing patient deaths (Devey, 2013; Hosgood and Scholl, 2002; Muir et al., 2007).

Possible complications in trauma patients and unwell patients

During the first few days, tissue breakdown without direct relation to trauma, suppression of the immune system and complications due to metabolic disorders may be observed in trauma patients and unwell patients. Complications may be either septic or aseptic, or originating from both (Carroll and Martin, 2007; Rozanski and Rush, 2007).

Cardiopulmonary collapse and arrest may develop in trauma patients and unwell patients. Acute circulatory failure is caused by severe myocardial ischaemia, arrhythmias with poor prognosis, hypoxaemia due to pulmonary or respiratory tract injury, haemorrhagic shock and acid-base and electrolyte disorders. In such patients, cardiopulmonary resuscitation is commenced immediately and the sympathetic system is stimulated. Insufficient resuscitation, anaesthesia and major trauma such as surgery usually result in death (Campbell, 2005; Carroll and Martin, 2007; Jones, 2001).

Planning for possible complications

Firstly, possible complications are identified and treatment is planned. Necessary devices for monitoring the patient, drugs and other equipment are prepared in an easily accessible position (Dyson, 2008; Rozanski and Rush, 2007).

When the patient is presented to the emergency department; the airway, respiration, circulation and neurological condition is assessed initially. Parameters including respiratory rate and motion, heart rate and rhythm, blood pressure and pulse quality, capillary refill time, central nervous system functions and pain are regularly checked to monitor patient condition and prognosis (Carroll and Martin, 2007; Devey, 2013; Pachtinger, 2013).

In anaesthetized patients, access to respiratory and intravenous routes is obligatory. In the presence of cardiovascular depression, oxygen support is provided to maintain tissue oxygenisation. In the event of the patient requiring assisted ventilation, endotracheal intubation is performed. Intravenous access is essential to administer emergency medication and fluid therapy. Furthermore, it is beneficial to place multiple intravenous catheters in most of the patients at risk. This procedure will make it easier to administer serum, blood, injectable analgesic and anaesthetic drugs to the patient (Quandt, 2013; Rozanski and Rush, 2007).

In order to reduce pain and stress in trauma patients, in the first instance endogenous encephalins, endorphins and other amino peptides are released producing a moderate level of sedation and analgesia. Therefore, anaesthetic drugs are used at lower doses in these patients (Carroll and Martin, 2007).

Approach to general anaesthesia in critical patients

When anaesthetizing critical patients, individual requirements are assessed. Therefore, it is not possible to present a single overall anaesthesia protocol applicable to all patients. There is a series of principles for anaesthesia administration in high-risk patients (Rozanski and Rush, 2007). These are;

Stabilization of the patient

Prior to anaesthesia, the patient is stabilized as much as possible. Most anaesthetic drugs suppress the cardiovascular and respiratory systems. This, in turn, reduces perfusion and oxygenisation of organs such as the liver, kidneys, heart and brain. It leads to the development of many side effects arising from anaesthesia (Bednarski et al., 2011; Rozanski and Rush, 2007).

In healthy patients with no heart or respiratory problem, the cardiovascular depression caused by anaesthesia does not produce significant complications. However, prior to anaesthesia if oxygen transport is decreased (heart disease, anaemia, pulmonary disease or electrolyte disorders) or if there are circulation disturbances due to organ failure (kidney failure or traumatic brain damage) the depressant effects of anaesthetic drugs will cause a drop in tissue oxygenisation (Rozanski and Rush, 2007).

In order to stabilize the patient before anaesthesia, intravenous fluid therapy, blood infusion, establishment of electrolyte balance and correction of lung functions by treating pleural effusion and pneumothorax must be achieved. Also, reducing intracranial pressure, warming hypothermic patients, treating hypoglycaemia and fluid therapy to lower azotaemia in patients with kidney failure are among the necessary steps (Quandt, 2013; Rozanski and Rush, 2007).

Drug selection for heart and respiratory functions

Drugs with a minimal effect on heart and respiratory functions must be selected. In order to develop pre-anaesthesia tissue perfusion, anaesthetic drugs that cause myocardial, vascular and respiratory depression should be avoided. The drugs of choice should be those best maintaining cardiovascular and respiratory functions such as opioids (morphine, fentanyl), benzodiazepines (midazolam, diazepam), ketamine and etomidate (Demirkan et al., 2002; Mathews and Dyson, 2005; Rozanski and Rush, 2007).

Propofol, thiopental and inhalation anaesthetics such as isoflurane and sevoflurane decrease cardiac contractility and cause vasodilatation and hypoventilation (Mathews and Dyson, 2005; Rozanski and Rush, 2007).

Reversal of drug effects

While opioids, benzodiazepines and ketamine have minimal side effects, in cases with severe cardiovascular, respiratory or neurologic depression, these drugs may cause patient instability or prolonged sedation (Armitege-Cahn et al., 2007; Rozanski and Rush, 2007).

The effects of opioids may be reversed using an antagonist such as naloxone. The effects of benzodiazepines are antagonized using flumazenil. Since the plasma half-life of naloxone and flumazenil is short, it can be repeated as required. These drugs must always be administered slowly. If naloxone is administered rapidly at a higher dose than necessary, it causes cardiovascular side effects. Diluting antagonist drugs in saline at a rate of 1:10 enables easy slow intravenous administration of a small dose of the drug (Quandt, 2013; Rozanski and Rush, 2007).

Drug doses and route of administration

In most patients in a critical condition, sensitivity to anaesthetic drugs increases. In these patients, a larger amount of the blood is directed towards the brain in order to keep the patient alive. Therefore, a much greater amount of medication reaches the brain. Especially in patients where the blood-brain barrier is disrupted, anaesthetic drugs reach the central neurons more rapidly and cause depression in the central nervous system (Rozanski and Rush, 2007).

In patients with hypoproteinaemia, binding of the anaesthetic drug to proteins decreases and the free active part of the drug increases. The required drug dose is also reduced in these patients. However, it is difficult to pre-estimate the lower dose amount. Therefore, "effective" drug administration is advised (Rozanski and Rush, 2007).

In effective drug administration, the drugs are given in a controlled and slow manner via the intravenous route, continuing the injection until the desired sedation or level of anaesthesia has been achieved. In subcutaneous or intramuscular anaesthetic drug administration however, the full calculated dose is given to the patient. This may lead to an unintentional high dose administration of the drug to the patient (Bednarski et al., 2011; Rozanski and Rush, 2007).

Monitoring the cardiovascular and respiratory systems

Heart rate, respiratory rate and blood pressure must be monitored in anaesthetized and unwell patients. These parameters give information about the patient's cardiac output, plasma volume and respiratory functions. In addition, a pulse oximeter shows the decreases in arterial oxygenisation, while capnometry displays the changes in arterial CO_2 pressure. Measuring body temperature is important for the timely detection of the adverse effects of hypothermia on the cardiorespiratory system (Pachtinger, 2013; Rozanski and Rush, 2007).

While ECG monitorisation detects arrhythmias and electrolyte balance disorders; pulse quality, mucosa colour and blood pressure provide important data for the assessment of cardiac output. Urinary catheterization is required to evaluate kidney function, while central venous catheterization is essential for central venous pressure, blood gases and electrolyte analysis (Haskins, 2007; Pachtinger, 2013; Perkowski, 2000; Rozanski and Rush, 2007).

In ASA 3, 4, 5 category patients, heart rate and cardiac arrest incidence is extremely high. In these patients, fever, pain, hypoxia, hypercapnia and heart failure increase the heart rate. Hypovolaemia, peripheral vasodilatation or a decrease in myocardial contractility, on the other hand, produces hypotension and bradycardia. This increases the risk of anaesthesia. In such patients, fluid input and positive inotropic support must be provided to continue sufficient tissue perfusion (Jones, 2001; Redondo et al., 2007).

General anaesthesia increases the formation of atelectasis in peripheral lung areas, thus decreasing ventilation and causing hypoxaemia. Hypoxaemia, in turn, leads to various arrhythmias and disruption of the body's acid-base balance. Giving a high input of oxygen to patients with normal lung structure prevents the development of hypoxaemia (Guzel et al., 2013a; Itami et al., 2017).

Since patients in the high-risk category are generally inclined towards hypoventilation, mechanical ventilation is advised for these patients. However, mechanical ventilation increases intrathoracic pressure and obstructs venous return (Redondo et al., 2007). In their study carried out in patients in the ASA 1 and 2 categories, Guzel et al. (2013a) reported that there was no statistical difference between heart rate and blood gas parameters in dogs with spontaneous respiration and those given mechanical ventilation.

The oxygen saturation of haemoglobin (SpO_2) values in high-risk group patients are significantly less than patients in the ASA1 category. The main reason for hypercapnia developing during anaesthesia is the suppression of the respiratory centre due to anaesthetic drugs. In the case of end tidal CO_2 ($EtCO_2$) values being higher than 60 mmHg, the patient must be given either manual or mechanical respiratory support (Pachtinger, 2013; Redondo et al., 2007).

In order to minimize anaesthesia related deaths, during the anaesthesia period it is extremely important to closely monitor the patient, observe oxygen saturation using a pulse oximeter and determine CO_2 levels with the use of capnography (Brodbelt, 2009; Brodbelt, 2010; Quandt, 2013).

Anaesthesia in trauma patients and unwell patients

In unwell or trauma patients it is important to provide sedation, anaesthesia and pain control for diagnostic or therapeutic surgical interventions. All anaesthetic drugs have potential cardiopulmonary suppressing properties. Therefore, in emergency patients, a balanced anaesthesia technique is used to perform safe general anaesthesia. With this method, the amount of anaesthetic drug required is reduced (Bednarski, 2011; Campbell, 2005).

An experienced anesthetist is able to determine 140 different anaesthesia protocols suitable for the condition of the patient. For this purpose, the anaesthetist prepares and administers appropriate combinations of drugs including atropine, acepromazine, medetomidine, xylazine, ketamine, propofol, thiopenthal, halothane, isoflurane, sevoflurane, opioids and non-steroidal anti-inflammatory drugs (NSAIDs) (Redondo et al., 2007).

Drugs used for premedication

This is the administration of a single or multiple drugs (anticholinergic, tranquilizer, sedative) via different routes (subcutaneous, intramuscular, intravenous) in order to prepare the patient's metabolism prior to general anaesthesia (Bednarski, 2011; Koc and Sarıtas, 2004).

Anticholinergic drugs are used to control possible excessive secretions in the patient and the cardiopulmonary effects of the vagal tone. However, since anticholinergics such as atropine or glycopyrrolate increase heart rate and myocardial oxygen consumption and lead to arrythmias, these drugs are not routinely used in unwell patients (Bednarski, 2011; Carroll and Martin, 2007; Guzel and Perk, 2002).

Tranquilisation is described as the behavioural changes in the relaxed patient where the patient is aware of the surrounding events. Sedation is a state of drowsiness with cloudy consciousness as a result of central nervous system suppression. During tranquilisation and sedation, the patient is indifferent to events around it, however, it responds to painful stimuli. Both groups of drugs are used to alleviate the patient's sense of fear, make pre-oxygenisation easier and minimize sympathoadrenal stimuli (Guzel, 2003; Thurmon and Short, 2007).

There is no perfect way to sedate a patient. The safest sedative drugs for emergency patients are those that can be titrated, have reversible effects and no suppressive effect on the cardiopulmonary system (Campbell, 2005).

Phenothiazines and α_2 agonists (xylazine, medetomidine, dexmedetomidine) produce hypotension by significantly suppressing the cardiovascular system. Therefore, these drugs are unsuitable for the sedation of patients that are either unstable or hypotensive (Armitege-Cahn et al., 2007; Campbell, 2005; Demirkan et al., 2002). In unwell patients (ASA 3, 4 and 5), medetomidine increases the anaesthesia-related mortality risk (Brodbelt et al., 2006; Brodbelt et al., 2007).

Due to its lower cardiopulmonary depressing effect compared to other drugs, acepromazine reduces anaesthesia-related mortality risk. At the same time, it exhibits a protective effect against rhythm disorder by increasing the arrhythmia threshold produced by catecholamines (Brodbelt et al., 2006; Brodbelt, 2010). Despite this, since acepromazine can cause thrombocyte dysfunction and sequestration of erythrocytes in the spleen, it is not used in patients with anaemia or bleeding (Campbell, 2005).

Neuroleptanalgesia achieved with the combination of benzodiazepine (diazepam or midazolam) and opioid (morphine or fentanyl) is one of the most reliable sedative combinations for the anaesthesia of emergency patients (Guzel, 2003; Guzel et al., 2013b; Liao et al., 2017). Both drug groups are reversible. The clinical doses in use have scarcely any depressive effect on cardiovascular and respiratory functions. In patients with cardiovascular or central nervous system problems, or for interventions such as radiography or thoracocentesis, satisfactory sedation can be achieved with this combination (Guzel, 2003; Liao et al., 2017; Mathews and Dyson, 2005).

Neuroleptanalgesia produces excellent premedication prior to anaesthesia. As well as being beneficial in decreasing the dose of the main anaesthetic drug required for anaesthesia, it also produces pre-emptive analgesia for surgery or other painful interventions (Guzel, 2003; Liao et al., 2017). This combination has several contraindications. In patients with central nervous system depression, opioids may cause hypoventilation. This leads to an increase in intracranial pressure. Therefore, such patients must be closely monitored regarding respiratory functions (Rozanski and Rush, 2007).

Intravenous morphine and meperidine administration in dogs causes dose-related histamine secretion and causes severe hypotension. Slow intravenous injection of these drugs is acceptable on condition that blood pressure is monitored (Carroll and Martin, 2007; Mathews and Dyson, 2005). Morphine causes mydriasis, excitation and aggressive behaviour in cats. Oxymorphone, hydromorphone or fentanyl does not lead to histamine secretion. Also, its use in critical patients has been proven to be safe (Campbell, 2005; Mathews and Dyson, 2005).

Drugs used for anaesthesia induction

Patients exposed to trauma are usually in a state of acidaemia and hypoproteinemia. Therefore, the need for the induction drug is greatly reduced (Carroll and Martin, 2007). Propofol, thiopenthal, etomidate and ketamine/benzodiazepine combination is used for anaesthesia induction. In unwell patients, the dosage to be used is significantly reduced and the drug is administered via the intravenous route. This way, overdosing is avoided (Campbell, 2005; Liao et al., 2017).

In traumatised or unwell patients, intravenous induction anaesthetics are preferred for the immediate control of airways. Mask induction is not recommended with volatile anaesthetics. In mask procedures, it is difficult to follow the depth of anaesthesia and the amount of anaesthetic drug needed is higher. This leads to cardiovascular depression (Bednarski et al., 2011; Carroll and Martin, 2007).

Propofol provides rapid induction and recovery. There is no accumulation in the body as a result of repeated usage. However, in the event of high dosage or fast injection, it produces apnoea and a significant degree of hypotension (Campbell, 2005; Guzel et al., 2006). In the anaesthesia induction of patients aged 10 years and older, diazepam/alfentanyl combination and propofol anaesthesia was compared and it was reported that propofol presented more stable results regarding heart and respiratory system functions and intraocular pressure (Guzel et al, 2013b). In the anaesthesia of patients in categories 4 and 5 according to ASA criteria, a propofol injection followed by isoflurane administration reduced anaesthesia-related mortality rate (Bille et al., 2014).

In central nervous system disorders, due to their properties of reducing brain activity and intracranial pressure, barbiturates such as thiopenthal may be used for the purpose of increasing oxygen transport to the brain, especially in patients with cerebral ischaemia. Therefore, seizure activity will be decreased and neurons protected (Armitege-Cahn et al., 2007).

Ketamine is an anaesthetic drug with indirect cardiovascular stimulating properties. In healthy patients, it increases blood pressure, heart rate and cardiac output. It is a good choice for the anaesthesia induction of patients with weak myocardial contractility, cardiogenic shock and myocardial insufficiency. In unwell or trauma patients, ketamine and diazepam may be rapidly given consecutively at low intravenous doses (Guzel and Perk, 2002; Guzel, 2003). Ketamine is not used in cats with hypertrophic cardiomyopathy or patients with head trauma or eye damage (Carroll and Martin, 2007; Rozanski and Rush, 2007).

Etomidate has no depressive effect on either cardiovascular or respiratory functions. It enables the continuity of cerebral and heamodynamic homeostasis. Therefore it is a suitable anaesthetic drug for use in unwell patients. It is combined with benzodiazepine or an opioid in order to minimize side effects such as myoclonus (muscle spasm) and vomiting. Repeated use of

etomidate in cats causes haemolysis due to propylene glycol (Armitege-Cahn et al., 2007; Guzel and Perk, 2002; Guzel, 2003; Guzel et al., 2006).

Drugs used for the maintenance of general anaesthesia

Maintenance of general anaesthesia is achieved using volatile anaesthetics such as halothane, isoflurane and sevoflurane. Isoflurane and sevoflurane are equally hypotensive. However, they do not make the myocardium susceptible to the effects of catecholamines. Halothane produces a significant level of myocardial depression and vasodilatation. These undesired effects may be reduced by maintaining low concentrations (Armitege-Cahn et al., 2007; Carroll and Martin, 2007; Thurmon and Short, 2007).

Propofol induction may be used as an alternative to volatile anaesthetics. In the case of total intravenous anaesthesia being preferred, it is beneficial to give the patients oxygen or assisted ventilation. Therefore, endotracheal intubation must be performed (Bednarski, 2011; Rozanski and Rush, 2007).

Analgesia in unwell or trauma patients

In general, opioid agonists are used as an analgesic in these patients. The analgesic effects of these drugs are strong and reversible and the incidence of side effects is low. However, use of opioid agonists in extremely unwell patients may produce an unexpected depth of sedation or cardiorespiratory depression. Therefore, each patient must be assessed individually and drug selection and dosage must be decided accordingly. Epidural administration of opioid analgesics is also useful. In this route of administration, side effects arising in systemic usage are not observed (Alef et al., 2008; Armitege-Cahn et al., 2007; Mathews and Dyson, 2005; Pekcan, 2016).

In patients with sufficient tissue perfusion, non-steroidal anti-inflammatory drugs (NSIADs) may also be used as analgesics. If NSAIDs are used together with opioids, the opioid dose must be reduced (Alef et al., 2008; Mathews and Dyson, 2005; Pekcan, 2016).

Recovery from anaesthesia and the recuperation process of patients

During and after anaesthesia, intravenous fluid support must be given to patients. In the post-operative period, intestinal and urinary bladder functions, hypothermia, lung functions and skin integrity must be checked regularly. Eye ointment is used to protect the eyes from drying and against external effects. The environment must be quiet. Suitable social communication regarding their individual needs must be made with hospitalised patients (Armitege-Cahn et al., 2007; Brodeur et al., 2017; Quandt, 2013).

Conclusion

In unwell and trauma patients, from the moment the patient arrives at the emergency department, it is important to compose a detailed situation plan and effectively use a well-prepared protocol and time resource. At the same time, the team performing the procedure should delegate roles, assess the patient as quickly as possible and commence treatment. The process will therefore begin without delay and the chance of patient survival will increase.

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44th Volume Index

REWIEWER LIST

January 2018-December 2018

Abdullah KAYAR
Ahmet DOĞAN
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Akın YAKAN
Alev Gürol BAYRAKTAROĞLU
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Ali BUMİN
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Arif KURTDEDE
Artun YIBAR
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Banur BOYNUKARA
Behlül BEHLÜLİ
Bilal AKYÜZ
Burhan ÇETİNKAYA
Cem VURUŞANER
Derya YEŞİLBAĞ
Duygu KAYA
Elmutaz Atta AWAD
Emrullah EKEN
Fulya ÜSTÜN ALKAN
Georgios AMOIRIDIS
Gürsel SÖNMEZ
Gökşen ÇEÇEN
Hakan BİRİCİK
Hamza AVCIOĞLU
Handan AYDIN VURAL
Hüseyin YILMAZ
İbrahim Mert POLAT
İlknur PİRYAĞCI
İllias GIANNENAS
İsa ÖZAYDIN
İsmail ABAŞ
Ioannis GRIVAS
Kamil ÜNEY
Kazakos GEROGIOS
Kamil ÜNEY
Kemal METİNER
Kerem ÖTER
Kürşad TURGUT
Levent ALTINTAŞ
Levent AYDIN
Mehmet Rıfat VURAL
Mustafa ARICAN
Mustafa Doğa TEMİZSOYLU
Murat YILDIRIM
Mustafa OĞAN
Müjdat ALP
Nazan GEZER İNCE
Necmettin ÜNAL
Neşe KOCABAĞLI
Nihat DENEK
Nihat TOPLU
Nihal Yaşar GÜL SATAR
Nürettin GÜLŞEN
Oğuz KUL
Oktay DÜZGÜN
Oya BULUT
Özlem GÜZEL
Özge Özgen ARUN
Pınar Alkım ULUTAŞ
Pınar SAÇAKLI
R. Gözde ÖZALP
Rizah AVDIC
Serap SAVAŞAN
Serkan ERDOĞAN
Seyfullah HALİLOĞLU
Soner ALTUN
Şerife Şule CENGİZ
Şükrü KIRKAN
Tuba Çiğdem OĞUZOĞLU
Tülay AKAYLI
Tülay BAKIREL
Uçkun Sait UÇAN
Ulaş ACARÖZ
Vedat ONAR
Yaşar AKAR
Zafer OKUMUŞ
Zehra SELÇUK
Zeynep PEKCAN