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AMELIORATING EFFECTS OF GUAVA (*PSIDIUM GUAJAVA*) EXTRACT ON ADRIAMYCIN INDUCED REPRODUCTIVE TOXICITIES

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ABSTRACT

This study was undertaken to investigate the protective effects of guava extract on Adriamycin induced reproductive toxicities. For this purpose, thirty adult male Wistar rats were randomly divided into 6 treatment groups. Group 1, the control, was administered distilled water while group 2 was treated with Adriamycin (ADR) (30 mg.m^{-2}) alone. Groups 3, 4 and 5 were administered combinations of 30 mg.m^{-2} (ADR) and graded doses (125 mg.kg^{-1}; 250 mg.kg^{-1}; 500 mg.kg^{-1}, respectively) of guava extract. Group 6 was treated with the extract (500 mg.kg^{-1}) alone. The treatments were done for seven days with water and feed provided ad libitum. The effects of these treatments on the reproductive characteristics of the male Wistar rats were thereafter investigated. The results showed that the control group (1) had a significantly higher sperm count (96.25 ± 3.84 × 10^6 cells.ml^{-1}) and motility (80.00 ± 4.08 %) compared to the other treatment groups (P < 0.05). Group 3 had a significantly lower sperm count (40.00 ± 0.00 × 10^6 cells.ml^{-1}) compared with the group 4 (67.33 ± 4.81 × 10^6 cells.ml^{-1}) and 6 (62.60 ± 3.09 × 10^6 cells.ml^{-1}). Group 5 had the lowest percentage livability (50 %), which was significant when compared with the control group, but not significant compared with the other treatment groups (P < 0.05). Groups 3, 5 and 6 had over 20 % sperm cell abnormalities. Most prominent of the abnormalities in groups 3 and 5 were curved tails and curved mid pieces in group 6. This work showed that guava extract at 250 mg.kg^{-1} is safe and gave protective cover to ADR induced reproductive toxicities.

Key words: adriamycin; *Psidium guajava*; reproductive; toxicity

INTRODUCTION

Of all the various chemotherapeutic agents, antracyclines, which are tetracyclic chromophore antibiotics, play

a vital role [3]. ADR is one of such antracyclines antibiotics used as an anti-cancer cytotoxic chemotherapeutic drug. ADR has been used effectively in treating; malignant lymphomas, solid tumours, and acute leukemias [15, 34]. However, cancer chemotherapy is often associated with adverse effects [11]. ADR has severe side effects such as; bone marrow suppression, gastrointestinal toxicity, stomatitis, alopecia, cardiomyopathy, and gonadal injury [9, 26]. Gonadal injury by antineoplastic drugs, though commonly observed, has been less investigated than other adverse effects [36]. These side effects have led to research on the development of specific agents to alleviate ADR toxicity. Hence, the quest for safe and effective agents to minimize ADR toxicity is still an active area of research.

Psidium guajava Linn. (Guava), belonging to the family of Myrtaceae, is a native of tropical America and has long been naturalized in Southeast Asia. It is used as foods and also as folk medicine in the subtropical areas of the globe. The positive effects of guava extracts on human ailments have been described [12, 17, 18, 24, 35, 38]. *Psidium guajava* leaf is a phytotherapeutic used in folk medicine to treat gastrointestinal and respiratory conditions and is also used as an anti-inflammatory medicine [2, 13]. The pharmacological and medicinal uses of the aqueous leaf extract include various disturbances such as; diarrhoea, vomiting, gastric pain, and dysentery [17]. Many other effects already reported include; CNS depressor (33), antimutagenic [5, 7], antiproliferative [14] antibiotic [1], anticough [2], immunomodulatory [16], hypotensive [23], and hypoglycemic [19, 20, 23]. Aqueous extracts from *P. guajava* have antioxidant or radical-scavenging activity. Most of the activity is associated with the polyphenols constituents; however, the guava extracts also contain antioxidants, such as ascorbic acid and carotenoids [2, 37]. The main objective of this study was to investigate the hypothesis that guava extract has an ameliorative effect against the development of ADR reproductive injury.

MATERIALS AND METHODS

Animals

Thirty adult male Wistar rats (3—4 months of age, body weight 240—280 g) bred and maintained in a controlled environment at the Experimental Animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan were used in this study. The temperature was kept at 25.0 ± 2.0 °C [25] and experiments were conducted in accordance with the rules and ethics of the Institutional Committee of Animal Care. Water and feed were provided ad libitum. The rats were randomly divided into 6 groups. Group 1 served as a control and given distilled water, while group 2 was administered Adriamycin (ADR) alone at a dosage rate of 30 $mg.m^{-2}$. Treatment groups 3, 4 and 5 were administered a single dose of ADR (30 $mg.m^{-2}$) together with graded dosages of guava extract (12 $mg.kg^{-1}$, 250 $mg.kg^{-1}$ and 500 $mg.kg^{-1}$). The 6th group was given 500 $mg.kg^{-1}$ guava extract alone. The animals were dosed orally once daily for 7 days using a rat cannula.

Preparation of guava extract

The guava extracts were prepared with leaves of *Psidium guajava* L. [8] obtained from the Campus of the University of Ibadan, Ibadan, Nigeria and collected between June and July of 2012 (plants were free of toxic compounds) and authenticated at the Department of Botany at the same institution. Twenty grams of dry leaves were ground in 200 ml of 0.9 % NaCl (100 °C, 5 min). The crude extract was filtered, centrifuged (1500 RPM, 5 min) to obtain the final extract. The supernatant was considered as the concentration at 100 $mg.ml^{-1}$ of guava leaves.

Sample collection

Rats were anaesthetized using diethyl ether and afterward sacrificed by cervical dislocation. Using the open castration method, a midline incision was made and the testicles were milked out of the incision site with gloved hands. Incising the tunica vaginalis exposed the testicles. The spermatic cord was exposed, ligated and incised. Semen samples were thereafter collected from the cauda epididymis. These methods of collection were similar to that described by Oyeyemi and Ubiogoro [27]. The samples were analysed immediately after collection.

Sperm volume, motility and sperm count

The volume was determined by reading out the volume in a calibrated measuring cylinder. The sperm motility was assessed by the method described by Saba et al. [31]. The spermatozoa were counted by a haemocytometer using the improved Neubauer (Deep 1/10 mm, LABART, Germany) chamber as described by Pant and Srivastava [28].

Morphological abnormalities and live/dead ratio

These factors were determined from a total count of 400 spermatozoa in smears obtained with Wells and Awa stains.

The live/dead ratio was determined using 1 % Eosin and 5 % Nigrosin in 3 % sodium citrate dehydrate solution according to the method described by Wells and Awa [31].

Statistical analysis

The data were analysed into descriptive statistics using Graphpad Prism 5. The means were computed together with the Standard Error of the Mean (SEM). Means were compared using the Analysis of Variance. A value of $P < 0.05$ was considered significant.

RESULTS

The secondary metabolites identified in *P. guajava* extracts were; flavonoids, saponins, phenols, terpenes, sesquiterpenes, and tannins, no alkaloid was present (Table1). The control group (1) had a significantly higher sperm count ($96.25 \pm 3.84 \times 10^6$ cells.ml^{-1}) and motility (80.00 ± 4.08 %) compared to the other treatment groups ($P < 0.05$) (Table 2). Group 3 had a significantly lower sperm count (40.00 ± 0.00 cells.ml^{-1}) compared with group 4 (67.33 ± 4.81cells. ml^{-1}) and 6 (62.60 ± 3.09 cells.ml^{-1}) (Table 2). Group 5 had the lowest percentage livability (50 %) that was significant when compared with the control group, but not significant when compared with the other treatment groups ($P < 0.05$). Groups 3, 5 and 6 had over 20 % sperm cell abnormalities (Table 3). Most prominent of the abnormalities in groups 3 and 5 were curved tails and curved mid pieces in group 6 (Table 3). The mean percentage of curved tails and bent mid pieces was higher significantly ($P < 0.05$) in group 2 compared with group 3. The mean percentage of curved tails in group 5, when compared with that of groups 4, 2 and 1, was significantly higher ($P < 0.05$). The mean percentage of curved mid pieces in group 6 was significantly higher when compared with groups 2 and 1 ($P < 0.05$).

DISCUSSION

Table 1. Results of phytochemical screening of *P. guajava* leaf extract

S/No	Constituents	Observation
1	Alkaloids	–ve
2	Flavonoids	++ve
3	Saponins	+ve
4	Phenols	++ve
5	Terpenes	++ve
6	Sesquiterpenes	+ve
7	Tannins	+ve

+ve — present ; ++ve — abundant; –ve — absent

Table 2. Spermatozoa characteristics of the treatment groups

Group	Treatment	Motility [%]	Livability [%]	Volume [cm^3]	Count [10^6.ml^{-1}]
1	CONTROL	80.00 ± 4.08^{abcde}	96.50 ± 0.87^{a}	5.15 ± 0.03	96.25 ± 3.84^{abcde}
2	ADR alone	20.00 ± 8.17^{a}	60.00 ± 7.07	$5.1\ 3 \pm 0.03$	57.00 ± 8.70^{c}
3	ADR + 125 mg.kg^{-1} extract	20.00 ± 0.00^{d}	60.00 ± 0.00	5.10 ± 0.00	40.00 ± 0.00^{a}
4	ADR+ 250 mg.kg^{-1} extract	23.33 ± 14.53^{c}	61.67 ± 13.02	5.10 ± 0.00	67.33 ± 4.81^{ab}
5	ADR + 500 mg.kg^{-1} extract	20.00 ± 11.55^{b}	50.00 ± 25.17^{a}	3.4 ± 1.70	$52.\ 00 \pm 4.36^{d}$
6	Extract alone	34.00 ± 6.00^{e}	77.00 ± 3.00	5.10 ± 0.00	62.60 ± 3.09^{ae}

N = 5; mean values with same superscripts in the same column differ significantly at $P < 0.05$

Table 3. Morphological abnormalities in the treatment groups

Group	Treatment	Tailless head	Headless tail	Rudimentary tail	Bent tail	Curved tail	Bent mid piece	Curved mid piece	Total abnormal	Total normal	Total cell count
1	Control	15 (1.38 %)	16 (1.48 %)	7 (0.64 %)	31 (2.84 %)	29 (2.66 %)d	30 (2.75 %)	28 (2.57 %)^{b}	156 (14.31 %)	934 (85.69 %)	1090 (100 %)
2	ADR alone	17 (1.46 %)	21 (1.80 %)	5 (0.43 %)	33 (2.83 %)	28 (2.40 %)^{ab}	29 (2.49 %)^{a}	30 (2.58 %)^{a}	163 (13.99 %)	1002 (86.01 %)	1165 (100 %)
3	ADR + 125 mg.kg^{-1} extract	5 (2.33 %)	6 (2.79 %)	1 (0.47 %)	7 (3.26 %)	9 (4.19 %)^{b}	9 (4.19 %)^{a}	7 (3.26 %)	44 (20.47 %)	171 (79.53 %)	215 (100 %)
4	ADR+ 250 mg.kg^{-1} extract	14 (1.68 %)	11 (1.32 %)	6 (0.71 %)	25 (2.99 %)	27 (3.23 %)^{c}	24 (2.87 %)	26 (3.11 %)	133 (15.93 %)	702 (84.07 %)	835 (100 %)
5	ADR + 500 mg.kg^{-1} extract	10 (1.97 %)	8 (1.57 %)	5 (0.98 %)	18 (3.54 %)	22 (4.33 %)^{acd}	19 (3.74 %)	21 (4.13 %)	103 (20.28 %)	405 (79.72 %)	508 (100 %)
6	Extract alone	22 (1.94 %)	23 (2.03 %)	6 (0.53 %)	46 (4.06 %)	41 (3.62 %)	46 (4.06 %)	48 (4.24 %)^{ab}	232 (20.48 %)	901 (79.52 %)	1133 (100 %)

N = 5; mean values with same superscript in the same column differ significantly at $P < 0.05$

Antineoplastic drugs, pesticides and heavy metals are known to affect the structure, functions and biochemical composition of reproductive organs [32]. Many chemotherapeutic drugs are therefore limited in their effectiveness due to their toxic side effects [29]. The antioxidant activity of some compounds could be used to prevent various chronic diseases such as; heart disease, diabetes, cancer, arterial thrombosis, cataracts and may provide health-promoting effects [30]. The chemical analysis of guava extracts, revealed the presence of; flavonoids, saponins, phenols, terpenes, sesquiterpenes, and tannins. This corroborates previous reports of Cuellar et al. [10]; Arima and Danno [4] ; Begum et al. [6] that detected the presence of essential oils, tannins, saponins, carotenoids, flavonoids and triterpenes.

The deleterious effects of the treatment of male Wistar rats with Adriamycin was obvious in this study. Significantly lower motility in all the Adriamycin groups compared to the controls and over 20 % sperm cell abnormalities [21] in groups 3 and 5 indicate a possible testicular degeneration. Noakes et al. [22] had opined that the initial changes in semen quality during testicular degeneration are a decrease in motility and an increase in the percentage of abnormal sperm. Most prominent of the abnormalities in groups 3 and 5 were curved tails and this could be indicative of testicular degeneration [22]. In this work however, it appeared that guava extract did give a semblance of protective covering to Adriamycin-exposed sperm cells at 250 mg.kg^{-1}. At this dosage it had significant percentage livability compared to the ADR + 500 mg.kg^{-1} group, appreciable high sperm counts and acceptable levels of sperm cell abnormalities. The motility was however low, which could be due to the initial reaction to ADR. At this dosage therefore, the effect of ADR against sperm cells seemed to be ameliorated. Below (125 mg.kg^{-1}) and beyond that (500 mg.kg^{-1}) this protective covering seemed to be nonexistent. At 500 mg.kg^{-1} guava extract alone, motility was significantly low ($P < 0.05$) compared with the controls, but the sperm count was appreciable. It therefore appeared that at 500 mg.kg^{-1} guava extract might not be overtly a fertility-inducing agent.

The data presented in this study revealed that guava extract at 250 mg.kg^{-1} is a potent inhibitor of ADR toxicities. Experiments performed to examine the mechanism by which guava extract was exerting its protective effect on ADR toxicities revealed that Guava has an antioxidant property attributed to the polyphenols found in the leaves which

may constitute an important part of its therapeutic effects. This provides evidence that guava extract at 250 mg.kg^{-1} directly protects ADR induced reproductive toxicity.

CONCLUSIONS

In conclusion, the present findings demonstrate that guava extract has multiple therapeutic activities that are beneficial and thus guava extract is a promising agent to ameliorate ADR induced reproductive toxicities. However, additional studies are needed on the chemical characterization of the active principle in the leaf extract of *P. guajava*, that is responsible for the repair of the ADR induced reproductive injury.

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IMPACT OF HUMIC ACIDS ON TRACE ELEMENT CONTENT UNDER DIFFERENT CONDITIONS

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ABSTRACT

The observations in mitochondria share a lot of similarities in trace element distribution with those observed previously in chicken bodies after humic acids supplementation. The redistribution in the levels of some elements, especially the overall increase in iron and decrease in selenium, could have important effects on the use of elements and the overall redox state of the organism. Based on our findings, it is necessary to consider the length of safe use, in addition to the level of dosage. After 42-days of supplementation with humic acid, according to the levels of trace elements found, an improved response of the organism to stress conditions can be expected.

Key words: antioxidant enzymes; humic acids; mitochondria; trace elements; stress

INTRODUCTION

Poultry meat quality has been widely studied, and has become a growing demand in the international market. Parameters that affect meat quality are complex, and occur throughout the production chain. The constant concern with meat quality by the exporting sectors is a response to consumer's demands, and is achieved by increasing efficiency, and investments in personnel training on quality [1]. Poultry meat quality is potentially affected by; management techniques, weather and rearing conditions, genetics, transportation, and the ability of the birds to respond to the environment, and all the variables that may interact, affecting the production cycle [1]. Any of the environmental stress factors can result in changes in the metabolites of muscle. These changes, in turn, are responsible for differences in the ultimate properties of meat. The nature of the

changes depends upon stress and the level of the animal's resistance to stress at the time of death. In poultry, the quality of meat products results from complex interactions between the genotype and the environment, more especially, the stresses undergone before slaughter [4]. Cashman et al. [2] reported that fear levels in birds were mainly determined by transportation and not just by catching and loading. Recently, a study by Cetin et al. [3] provided evidence that humate supplementation to the crowded laying hens diet had a strong anti-stress effect against social stress.

Trace elements are essential for maintenance and production of farm animals, because they are constituents of the hundreds of proteins involved in intermediary metabolism, immune defence systems, and hormone excretion pathways [5]. It has been observed that organic metal complexes, such as humic acid chelates [7], produce higher bioavailability of trace elements in general [6].

So far, a link between the administration of humic acids and the trace element distribution in poultry following the stress conditions from transport to the slaughterhouse has not been described in the currently available literature. A eference from pivotal study [17]

provides information only about the redistribution of elements in common breeding conditions.

The aim of our study was to determine the quantities of some trace elements after a 42-day supplementation with humic acids under common breeding conditions in comparison to stress conditions caused by transportation.

MATERIALS AND METHODS

The experiment was carried out on 36 000 broilers (COBB 500) from a poultry farm in Vinica in the region of Veľký Krtíš (Slovakia). The chicks were divided into 2 groups. The control group (n = 15 700) was fed conventional feed mixtures during 42 days. The experimental group (n = 20 000) was fed conventional feed mixtures enriched by 0.6 % humic acids (Humac® Natur, Humac Ltd, Košice, Slovakia) from the first day of fattening for 42 days. All chicks were subjected to standard management and hygiene practices. Feed and water were provided ad libitum throughout the study period (42 days). Randomly selected 10 chickens from both groups (common breeding conditions and stress conditions) were killed by cervical dislocation (before transport and after transport at the slaughter house), followed by tissue harvesting and the collection of the blood plasma. Selection was performed three times. Liver and kidney mitochondria were isolated by the method described by Fernández-Vizarra et al. [9]. The detection and determination of the total content of iron and zinc (by the flame atomic absorption spectroscopy) and that of copper, manganese and selenium (by the graphite furnace atomic absorption spectrometry (Shimadzu AA7000) were accomplished. The parameters were expressed as the mean ±S.E.M. of three independent measurements. The difference between the two groups was determined using an unpaired Student *t*-test.

RESULTS AND DISCUSSION

The levels of metals detected by atomic absorption spectrometry pointed to significant changes in the amounts of metals (Table 1). We found that the copper content was higher in the liver mitochondria and plasma, but lower in the kidney mitochondria in the group that received the humic acid supplementation, compared to the control group. Copper concentrations are highest in most of farm animals in the liver, and are related to the dietary intake [15]. In the experimental group, the amount of zinc and manganese in the liver and kidneys was lower compared to the control group. An excess intake of zinc, leads to a higher deposition of this metal in the liver, pancreas, kidney, and bone. Generally, livestock do not accumulate extremely high levels of manganese in their tissues, even when excess manganese is fed [14]. The levels of iron were significantly elevated in the mitochondria of the liver, kidney and plasma when compared to the control group. When animals are exposed to excessive amounts of iron, it is preferentially deposited in the liver, spleen, and bone marrow. With very high doses, iron may be deposited in the heart and kidneys [14]. Selenium concentrations were significantly higher in the kidney, but lower in the plasma after 42-days of humic acids supplementation compared to the control group.

The results obtained by Zralý and Písaříková [16] confirmed that feeding sodium humate to animals had no significant adverse effect on the Cu or Zn content in the investigated organs and tissues and cited many other authors with the same findings. On the other hand, elevated concentrations of trace elements in pig tissues were reported by Lopez-Alonso et al. [13].

Table 1. The effect of humic acids administration on trace element (Zn, Cu, Mn, Fe and Se) distribution in the plasma and mitochondria isolated from the liver and kidney before and after transportation to a slaughterhouse (* P < 0.05; *** P < 0.001)

		Common breeding conditions[a]		Stress conditions	
		Control	HA	Control	HA
Zn [μg.g^{-1}] DL = 0.001 mg.l^{-1}	Plasma	59.62 ± 2.97	60.16 ± 4.36	90.03 ± 2.08	9.65 ± 0.48***
	Kidney	49.67 ± 3.10	29.19 ± 2.01***	15.89 ± 0.43	14.12 ± 2.51
	Liver	63.86 ± 4.50	50.35 ± 3.33*	10.28 ± 0.62	45.55 ± 3.64***
Cu [ng.g^{-1}] DL = 0.0004 mg.l^{-1}	Plasma	50.99 ± 0.01	56.27 ± 0.03***	470.00 ± 100.0	392.00 ± 2.00
	Kidney	73.16 ± 0.04	55.21 ± 0.08***	430.00 ± 6.00	508.00 ± 6.00***
	Liver	33.34 ± 0.03	112.58 ± 0.04***	508.00 ± 8.00	626.00 ± 2.00***
Mn [ng.g^{-1}] DL = 0.0002 mg.l^{-1}	Plasma	44.14 ± 0.27	71.03 ± 0.33***	0.24 ± 0.002	0.15 ± 0.006***
	Kidney	127.28 ± 0.65	46.54 ± 0.56***	0.12 ± 0.002	0.33 ± 0.004***
	Liver	124.79 ± 0.11	50.04 ± 0.31***	0.18 ± 0.004	0.36 ± 0.006***
Fe [μg.g^{-1}] DL = 0.004 mg.l^{-1}	Plasma	1.52 ± 0.03	3.84 ± 0.05***	0.148 ± 0.004	0.162 ± 0.007***
	Kidney	0.79 ± 0.01	2.98 ± 0,02***	0.111 ± 0.008	0.171 ± 0.007***
	Liver	2.12 ± 0.08	3.51 ± 0,02***	0.172 ± 0.008	0.171 ± 0.004
Se [ng.g^{-1}] DL = 0.0009 mg.l^{-1}	Plasma	535.13 ± 0.04	199.24 ± 0.03***	under limit	72.65 ± 0.028
	Kidney	107.17 ± 0.05	1163.27 ± 0.04***	under limit	65.75 ± 0.002
	Liver	under limit	51.95 ± 0.01	under limit	23.92 ± 0.014

DL — detection limit; HA — humic acids enriched diet; a — according to Žatko et al. [17]

The highest content of trace elements, except selenium, was detected in the liver which is a depot organ of a higher diagnostic value than the muscular tissue for the assessment of dietary mineral supply to animals. The kidneys, where the highest concentrations of selenium were detected in the present study, are the most important organ involved in selenium disposition [13]. Owing to sodium humate feeding, the levels of Mn and, above all, Se were significantly decreased in the blood serum [16]. Herzig et al. [11] found that humic acid complexes of zinc did not increase the zinc accumulation in muscle, kidney, liver and blood plasma, which means that humic acids do not have a positive effect on the bioavailability of zinc. That negative result, is in contrary to a few previous findings where it was found that humic acids have a high bivalent cation binding capacity in the order of $Cu^{2+} > Fe^{2+} > Mn^{2+} > Zn^{2+}$ and improves their adsorption. However, it also has been found that the interaction of humic acids with metal ions in solution increases, with pH and humic acids concentration [8], whereas these interactions decrease with metal ion concentration.

Stress conditions caused significant changes in the levels of the detected elements (Table 1); see also reference [17]. There was a marked, in the order of a ten-fold decrease, in manganese, selenium and iron, when compared to the unstressed groups. The values of Zn were also decreased. The levels of these elements, however, in the group with humic acids supplementation were significantly higher when compared to the group without the humic acids under the same stress conditions, and Zn, Mn and Fe reached higher values in the mitochondria of the bodies. In the control group, the redistribution of these elements exhibited higher

values in the plasma. Also, of interest are the higher levels of selenium in the humic acids group under stress conditions in the plasma, which was probably the effect of the defence mechanisms against oxidative stress. In the same manner, it may be explained by the increased levels of Cu in the stressed groups. In the study of Hayirli et al. [10], the adverse effects of the increased caging density were ameliorated with humic acids supplementation, suggesting that the benefits of humic acids seemed to be more noteworthy for hens housed in stressed conditions, than for hens housed under standard conditions. Elements such as, iron and zinc are known to be able to participate actively in ligand formation with organic compounds and therefore, the ability of humic acids to act as a ligand creator, may explain their supportive action in transporting ions through biological membranes [12]. It appears, however, that the redistribution of trace elements that supplementation with humic acids manifest with resulting long-term revenues, may not have a beneficial effect on the redox state of the organism.

CONCLUSIONS

The observations in mitochondria share a lot of similarities with the trace element distributions with those observed previously in chicken bodies after humic acids supplementation; however, there are also differences which may have a fundamental effect on the overall redox status and the use of the elements. Humic acids supplementation can enhance the ability of chickens to utilize trace minerals, but due to the ability of iron accumulation and a decrease in the levels of selenium, long-term supplementation is not suitable. Due to the emplacement of these elements into biomolecules and their redox properties, the positive effect of humic acids through prolonged supplementation over 42 days should not be expected. After 42-days, according to the levels of trace elements found, still a better response of the organism to stress conditions may be expected.

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EFFECT OF SUPPLEMENTATION OF THE DIET WITH HUMIC ACIDS ON GROWTH PERFORMANCE AND CARCASS YIELD OF BROILERS

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ABSTRACT

The aim of this study was to confirm the effects of the product HUMAC Natur (humic acid source) supplemented to broiler feed during 39 days of fattening on the; final body weight, carcass yield and percentage of the carcasses. Eighty broilers COBB 500 included in the experiment were fed commercial diet. Humic acids were added to the feed of experimental broilers (n = 40) as a 0.6 % concentrate, from the first day of fattening. On day 39 of fattening, the mean body weight of the experimental broilers was lower (2 498.0 ± 244.6 g) in comparison to the control (2 535.0 ± 268.2 g) (P > 0.05). The addition of the humic acid product to feed as a 0.6 % concentrate affected positively the carcass yield (P < 0.05), but had no significant positive effect on the live body weight (P > 0.05). The mean weight of the breast and thigh muscles in the experimental and control groups was comparable.

Key words: broiler; carcass yield; fattening; humic acids

INTRODUCTION

Humic substances are natural organic compounds occurring in water, soil, carbon and other sources. They are heterogeneous high molecular organic substances and their composition differs according the geographic region. According to their solubility in acid or alkaline media, they are divided to humic acids, fulvic acids and humins. Humic acids are considered to be a new suitable alternative of adsorbents, because of various binding sites present in their structure [8], [11]. It has been assumed that humic acids are able to reduce the absorption and systemic availability of bacterial endotoxins, which can be of great importance in the protection of animal and human health [10]. Moreover, many positive effects on the performance and

health of animals have been attributed to humic acids. They inhibit the growth of pathogenic bacteria and moulds and decrease the level of mycotoxins and thus may lead to improved gut health [5], [12]. Humic acids stabilize the intestinal flora, and by this, improve the utilization of nutrients from animal feed, which results in increased weight gains of animals without increasing the amount of feed given to them. Humic acids are said to improve protein digestion, as well as calcium and trace element utilization. They act as dilatators, increasing the cell wall permeability that allows easier transfer of minerals from the blood to bone and cells [5], [9].

Humic acids seem to be a suitable alternative with a favourable impact on production parameters of animals. Replacing antibiotics as growth stimulators by humic acids may affect positively parameters such as weight gain, live performance, carcass weight and feed conversion by better utilization of nutrients from feed [5], [7]. Moreover, humic acids are natural substances and their utilization in animal nutrition excludes the presence of undesirable residues and the development of bacterial resistance similar to antibiotic resistance. As a result of higher food conversion rates and enhanced absorption of nitrogen, the release of odorous compounds are reduced. This is also desirable because the odour presents certain problems.

The aim of this experiment was to investigate the impact of supplementation of a broiler diet with humic acids as a 0.6 % concentrate on the; growth performance, final body weight, carcass, and the breast and thigh muscle yield.

MATERIALS AND METHODS

This experiment was conducted at an approved animal quarters of the Clinic for birds, exotic and wildlife animals, University of Veterinary Medicine and Pharmacy in Kosice. Eighty one-day-old unsexed hybrid broiler chicks COBB 500 were purchased from a commercial hatchery. They were randomly divided into 2 groups (n = 40), 4 replications with 10 birds in each. The chicks were kept in pens with wood shavings in agreement with technological instructions for COBB 500 chickens regarding light regimen, temperature, animal hygiene and feeding. The birds were fed commercial starter and finisher diets (from day 1 to 24 and from 25 to 39, respectively).

The birds were fed as follows:

1. Control group (C) — fed a standard diet without any supplementation.

2. Experimental group (H) — fed a standard diet supplemented with humic acids (HUMAC Natur, purchased from Humac s.r.o., Kosice) as a 0.6 % concentrate, from day 1 of fattening.

Throughout the fattening period, the broilers had free access to water and feed. The temperature was gradually decreased from 33 °C on day 1 to 21 °C on day 21 and kept constant afterword. The lighting regimen provided 24 h of continuous light per day. The relative humidity of the environment was 50—70 %. The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Kosice.

The chicks were weighed at arrival and the mean weight was recorded as the one-day-old weight. The mean live body weight (BW) of the broilers in each group was recorded weekly. The carcass yield (CY) of broilers, and mean weight of the breast and thigh muscles were also recorded. On day 39 of the fattening, prior to stunning, the broiler chicks were slaughtered and processed. To determine the CY and BW of the broilers before slaughtering and carcass weight (CW) without offal was recorded. CY (%) was calculated as a ratio between CW after evisceration and BW of broilers before slaughter. Thigh and breast muscles used for the determination of their proportion to total CW, were deboned and weighted. Proportions of thigh and breast muscles were calculated as a ratio of individual parts and CW after evisceration (%).

The data obtained were analysed statistically using GraphPad Prism 5.0 software. The results are given as means and standard error of the mean (SD). The results obtained for each group were compared by t-test and $P < 0.05$ was considered as the statistically significant difference.

RESULTS AND DISCUSSION

The effects of supplementation of humic acids (as a 0.6 % concentrate) to the diet on mean body weight of broilers is presented in Table 1. During the experiment, no significant difference ($P > 0.05$) was recorded in the BW of the experimental groups in comparison to the control chicks. Our results also showed that humic acids had no influence on body weight and breast and thigh muscle weight

Table 1.The effect of supplementation of humic acids on mean live body weight of broilers during fattening (g)

Group	Day of fattening					
	1	14	21	28	35	39
Control	53.0	493.0	943.8	1611.0	2202.0	2535.0
± SD	± 5.4	± 54.6	± 79.0	± 91.7	± 169.3	± 268.2
Experimental	52.0	480.0	917.7	1537.0	2183.0	2498.0
± SD	± 3.4	± 37.7	± 53.6	± 96.0	± 202.1	± 244.6

Data are presented as means (n = 20) ± standard deviation (SD)

Table 2. The results of carcass, breast and thighs muscles yield

Group	BW	CW	CY	Breast		Thighs	
	[g]	[g]	[%]	[g]	[%]	[g]	[%]
Control	2535.0	1812.0a	71.2 a	486.9	27.1	506.3	28.1
± SD	268.2	130.7	2.1	40.60	1.4	47.84	1.5
Experimental	2498.0	1922.0b	78.1b	516.7	26.5	535.3	27.5
± SD	244.6	145.9	2.2	31.57	1.2	40.00	1.4

Data are presented as means (n = 20) ± standard deviation (SD)
BW — body weight; CW — carcass weight; CY — carcass yield
a, b — means with different superscripts in the same column differed significantly (P < 0.05)

(Table 2). The feed conversion ratio was 1.62 for both groups and the mean feed intake was 4002 g in the experimental group and 4021 g in the control group. Celik et al. [1] reported an increase of the final BW and also higher CY after the addition of humic acids, as a 0.25 % concentrate, to the broiler diet which is inconsistent with our results. Also, other authors [7], [11], [3] reported that the body weight and the feed conversion ratio of the broilers were positively affected by the supplementation of humic acids. On the contrary, Karaoglu et al. [6] reported that humic acid supplementation to diets of broilers (at concentrations of 0.1, 0.2 and 0.3 %) had no effect on the broilers performances.

The results of; carcass, breast and thigh weight and ratio between individual parts and carcass weight are reported in Table 2. There was no influence of humic acids on body weight of the broilers, but the carcass yield was significantly higher in the experimental group compared to the control group ($P < 0.05$). The weights of the breast and thigh muscles were slightly higher in the groups supplemented with humic acids, but the differences were not significant ($P > 0.05$). Kocabagli et al. [7] reported a higher percentage of CY after supplying feed with humic acids (0.25 %) in comparison to the control group ($P < 0.05$), which does not agree with our results.

The supplementation of the broiler diet with humic acids had a positive effect on poultry meat production. Recently, it has been observed that humates included in the feed and water of poultry promoted their growth [2], [4], [5]. Kocabagli et al. [7] investigated the effects of the

supplementation of the diet with humic acids on the live performance, carcass weight and the abdominal fat pad of broilers during different feeding periods. They found that feeding with humic acids had a beneficial effect in terms of growth and feed conversion and that humic acids might constitute a promising group in a search for an agent in terms of immunity enhancement [13].

CONCLUSION

It was reported that the addition of humic acids to diets may positively affect all production parameters in poultry meat production. These compounds are believed to enhance growth, decrease feed consumption and feed conversion ratio. However, in our experiment, the body weight of the chickens fed diets supplemented with humic acids was comparable to control group without humic acids in the diet. The carcass weight and carcass yield was significantly higher in the experimental group. The values of breast and thigh yield were comparable in both groups. The advantage was in the lower feed consumption. There is no doubt that humic acids have many beneficial effects on the production parameters and appear to be a suitable non-nutritive agent in poultry meat production.

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PARASITE DIVERSITY OF COMMON CARP (*CYPRINUS CARPIO* L.) FROM AQUACULTURE SYSTEMS IN EASTERN SLOVAKIA

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ABSTRACT

The common carp (*Cyprinus carpio*) is one of the most commercial freshwater fish species. Carp are the traditional food-fish in Europe (and elsewhere), bred in extensive polyculture pond systems. A total of ninety-four specimens of the common carp, collected from stressful conditions, as import from Hungary or the Czech Republic, or during harvesting or storing from different ponds in Eastern Slovakia, were dissected. We detected six groups of parasite taxa, as: 1. protozoan: *Chilodonella* sp., *Ichthyophthirius multifiliis*, *Trichodina* sp. and *Oodinium pillulare*; 2. helminths: monogeneans *Dactylogyrus* sp., *Gyrodactylus* sp. and *Eudiplozoon nipponicum*; 3. cestodes: from the genus *Caryophylleus* and *Khawia* in the intestine; 4. nematodes: *Philometra* sp.; 5. parasitic crustaceans: *Argulus* sp. and *Lernea cyprinacea*, *Ergasilus* sp.; and 6. acanthocephalans.

Key words: *Eudiplozoon nipponicum*; *Ichthyophthirius multifiliis*; *Lernea cyprinacea*; monogeneosis; ponds

INTRODUCTION

The common carp (*Cyprinus carpio*, Linnaeus 1758) is one of the most commercial freshwater fish species. Carp are the traditional food-fish in Europe (and elsewhere), bred in extensive polyculture pond systems. Carp is also popular for; a large community of sport fishermen, as well as, koi varieties for aquarists. They are naturally distributed in their wild form from the piedmont zone of the Danube River to the Black, Caspian and Aral Sea basins as western dispersants and in central Asia and Siberia as eastern dispersants [1]. However, as the result of translocations and introductions of domesticated and feral forms since Roman times [1], the common carp is now established in ninety-one of one hundred and twenty countries worldwide [4]. This often uncontrolled import leads to the rapid distribution of new parasite species from especially big breeding fishpond systems, from Hungary or Czech Republic, from where the carp is imported to many European countries. These parasites constitute a threat not only for farmed fish, but also for other natural populations [9]. The most complete checklist of carp parasites records a total of three

hundred ten parasite species [2]. The aim of this study is screening of the parasites of the common carp bred in aquaculture systems in the Eastern part of Slovakia and the control of fish introduced to this area from other countries.

MATERIALS AND METHODS

A total of ninety-four specimens of the common carp (*Cyprinus carpio*) from Eastern Slovakia, were dissected. We had three sources of samples during the period 2013—2015:

1) Samples collected during harvesting (in winter) and after storing (in spring) from three commercial carp-pond systems Hrhov (A), Perín-Chým (B) and Kusín (C);

2) samples sent by regional Slovak angling organization after import from Hungary and Czech Republic and

3) from small personal pond. The fish were transported alive in aerated cans to the laboratory.

Using complete parasitological dissection we obtained the basic knowledge of the parasite diversity of carp and their epizootological indices according to Bush et al. [3] as the prevalence (P), intensity of parasitic infection (ii) and mean intensity of infection (mi) in relation to season and origin of fish. For protozoan parasites only the prevalence was calculated. The gradual scale for the intensity of infection was according to Kvach et al. [7]:

1) sporadic occurrence, "S" — 1 or few specimens in the examined material;

2) not numerous, "NN" — a few specimens in a few fields of vision;

3) numerous, "N" — up to twenty individuals in the most fields of vision;

4) very numerous, "VN" — more than twenty specimens in most fields of vision. Helminths were isolated, fixed and determined by standard parasitological methods, e.g. Ergens and Lom [5].

RESULTS AND DISCUSSION

We detected six groups of parasite taxa:

1) Ciliates: *Chilodonella* sp., *Ichthyophthirius multifiliis*, *Trichodina* sp. and dinoflagellates *Oodinium pillulare* localized on the gills and skin;

2) helminthes: monogeneans *Dactylogyrus* sp. on the gills, *Gyrodactylus* sp. on the skin and *Eudiplozoon nipponicum* on the base of gill lamellae;

3) Cestodes: from genus *Caryophylleus* and *Khawia* in intestine;

Table 1. Diversity of parasites with parasitological indices of imported carp from Hungary and Czech Republic

Parasite species	Import from Hungary				Import from Czech Republic			
	P	P [%]	ii	mi	P	P [%]	ii	mi
Argulus sp.	–	–	–	–	7/36	19.4	1—3	2
Cestoda	–	–	–	–	6/36	16.6	1—3	1.83
Dactylogyrus sp..	2/7	28.5	NN	NN	22/36	61.1	S	S
Ergasilus sp.	3/7	42.8	8—80	40	–	–	–	–
Eudiplozoon nipponicum	3/7	42.8	1—2	1,3	9/36	25	1–7	4
Gyrodactylus sp.	4/7	57	NN	NN	–	–	–	–
Ichthyophthirius multifiliis	4/7	57	S	S	5/36	13.8	S	S
Trichodina sp..	3/7	42.8	S	–	8/36	22	VN	–

P — prevalence of infection; ii — intensity of parasitic infection; mi — mean intensity of infection
S — sporadic occurrence; NN — not numerous; VN — very numerous

Table 2. Diversity of parasites with parasitological indices of carp after storing

Carp-pond system	A				B				C			
Parasite species	**P**	**[%]**	**ii**	**mi**	**P**	**[%]**	**ii**	**mi**	**P**	**[%]**	**ii**	**mi**
Acanthocephala	–	–	–	–	–	–	–	–	1/2	50	1	1
Cestoda	–	–	–	–	2/6	33.3	1	1	2/2	100	1	1
Dactylogyrus sp.	1/8	12.5	S	S	6/6	100	N	N	2/2	100	S	–
Eudiplozoon nipponicum	1/8	12.5	1	1	–	–	–	–	–	–	–	–
Gyrodactylus sp.	–	–	–	–	1/6	16.6	S	–	2/2	100	NN	–
Ichthyophthirius multifiliis	8/8	100	NN	NN	1/6	16.6	NN	NN	2/2	100	S	-
Lernea cyprinacea	–	–	–	–	4/6	66.6	1—2	1.5	2/2	100	2—3	2.5
Trichodina sp.	–	–	–	–	1/6	16.6	N	–	2/2	100	N	–

P — prevalence of infection; ii— intensity of parasitic infection; mi — mean intensity of infection
S — sporadic occurrence; NN— not numerous; N — numerous

Table 3. Diversity of parasites with parasitological indices of carp after harvesting

Parasite species	A				B			
	P	**[%]**	**ii**	**mi**	**P**	**[%]**	**ii**	**mi**
Argulus sp.	2/23	8.6	3	3	–	–	–	–
Dactylogyrus sp.	8/23	34,7	S	–	8/9	88.8	NN	–
Eudiplozoon nipponicum	3/23	13	1,6	1–2	2/9	22.2	1–3	2
Gyrodactylus sp.	–	–	–	–	2/9	22.2	S	–
Ichthyophthirius multifiliis	–	–	–	–	1/9	11.1	NN	–
Lernea cyprinacea	7/23	30.4	4	1–13	–	–	–	–
Oodinium pillulare	5/23	21.7	N	–	6/9	66.6	NN	NN
Philometra sp.	–	–	–	–	1/9	11.1	1	1
Trichodina sp.	3/23	13	S	S	7/9	77.7	NN	–

Carp-pond system: A — Hrhov, B — Perín-Chým; P — prevalence of infection; ii — intensity of parasitic infection
mi — mean intensity of infection; S — sporadic occurrence; NN — not numerous; N — numerous

Table 4. Diversity of parasites with parasitological indices of carp from the small non-commercial pond

Parasite species	P	P [%]	ii	mi
Eudiplozoon nipponicum	2/3	66.6	5	5
Chilodonella sp.	1/3	33.3	N	N
Ichthyophthirius multifiliis	1/3	33.3	S	S
Trichodina sp.	1/3	33.3	S	S

P — prevalence of infection; ii — intensity of parasitic infection
mi — mean intensity of infection
S — sporadic occurrence; N — numerous

4) Nematodes: *Philometra* sp. in the skin;

5) parasitic crustaceans: *Argulus* sp. and *Lernea cyprinacea* on the skin, *Ergasilus* sp.; and

6) parasites from the phylum Acanthocephala, localised in the intestine in low intensities.

We have not observed markedly pathological changes on the gills or breathing problems in fish with gill parasites. Local haemorrhages at the site of attachment on the skin and fins were caused by anchor worm (*Lernea cyprinacea*) and fish louse (*Argulus* sp.) and in some cases, considerable hyperplasia or fibrosis had developed. Petechial haemorrhages were caused by high intensities of protozoan parasites.

All fish came from stressful conditions, as import, harvested or stored. These conditions, such as: handling high concentration of fish in small volume, sudden changes of temperature and other, can result in decreased resistance by the fish, resulting in the spread of parasite infestation [10]. Single parasitic species where sometimes found in intensities, but may not cause mortalities; however, their multiparasitic combinations as was found in all cases of our study, can lead to high morbidity and mortality within a few days [8]. The neighbouring countries such as Hungary and Czech Republic as the main sources of broodstock, fry or fingerling that are stocked to the free water bodies and or large breeding ponds, are also the sources of introduced pathogens that can spread. Parasites, which are transmitted to our country may be invasive and represent high risk for our farmed and cultured population [6].

CONCLUSIONS

We observed the lowest condition coefficient especially in fish after storing and this fact suggested rapid development of parasites with a direct life cycle.

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THE QUALITY OF DIFFERENT TYPES OF CHICKEN BREAST MEAT (ORGANIC, CONVENTIONAL, TENDERIZED)

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ABSTRACT

The aim of this study was to investigate the qualitative properties of meat from organic and conventional production systems and tenderized breast meat without skin. Fourteen organic, 19 conventional and 30 tenderized chicken breast were obtained from three difference sources and were evaluated for sensorial attributes: (raw, overall impression, colour, odour, consistency and juiciness after a slight compression; baked at 220 °C/25 min: impression, odour, consistency, juiciness, and flavour), colour (lightness, L*; redness, a*; yellowness, b*), texture profile analysis and Warner-Bratzler test in raw and after baking (220 °C/25 min) and chemicals parameters (dry matter, total protein, net protein, net muscle protein, collagen, fat, ash, salt and phosphorus). Tenderized chicken breast was evaluated as the worst in a sensory test. The colour parameters indicated that the classic breast was more yellow and fatter ($P < 0.01$). The organic breast contained more ($P < 0.01$) protein than the tenderized breast, while the ash, salt and phosphorus content on the tenderized breast was higher than the conventional or organic breast. The quality criteria of breast from organic chickens were slightly superior to the quality of the classic and the tenderized breast.

Key words: chicken meat; quality; sensorial and chemical parameters; texture

INTRODUCTION

Chicken meat is very popular among consumers for its versatile culinary uses, taste and dietary character. The economic challenges, involving the consumption of organic food in the Czech Republic, as in other countries is rising [16]. The chicken breast fillet is one of the carcasses portions that have the highest digestibility by consumer [13]. Meat chickens kept in the conventional way in barns is the most common type on the market. Tenderized breast of this meat (semi-product) and chicken meat from organic farms make up only a small part of the market. The consumers believe that organic food is better and healthier than conventional food, due its freedom from harmful ingredients (e.g.

synthetic feed additives, growth promoters or animals offal, pesticides or artificial fertilizers) and the prohibition of GMO (Genetically Modified Organisms) feed components [3, 5]. In order to improve broiler breast tenderness, many technologies have been used, such as, electrical stimulation, wing restraints, marination and combination of these methods [9]. Tenderization is done by injecting or soaking the breast of chickens with sodium chloride or polyphosphates [8]. The objective of marination is to improve the organoleptic properties of meat, mainly the taste, aroma, and texture (tenderness and juiciness) as well as to enhance microbiological safety by reduction of the pH [13]. There are a limited number of studies about the comparison between organic and conventional broilers meat quality [4] and the studies that compare it with tenderized breast of chicken.

The objective of this study was to evaluate the meat quality of chicken breasts without skin from organic and conventional production systems and tenderized breast meat currently provided in markets.

MATERIALS AND METHODS

This experiment was conducted at the Department of Meat Hygiene and Ecology (DMHE), Faculty of Veterinary Hygiene and Ecology University of Veterinary and Pharmaceutical Sciences Brno, CR. A total of 14 organic and 19 conventional fresh chicken breasts, as well as, 30 tenderized breast of chicken (frozen) were obtained from three different sources of chicken meat production in the Czech Republic (organic: Biopark s.r.o., Lipová; conventional: Vodňanská drůbež, a.s., Vodňany; tenderized: Frigoprima s.r.o., Mikulov).

Composition of the tenderized product included: chicken breasts without skin and bones (min. 70 %), neutral marinade, drinking water, edible salt, starch acetate, stabilizers: pyrophosphates, tripolyphosphates, carrageenan, dextrose, potassium chloride, dried glucose syrup, sodium ascorbate, spices, xsanthan, and aroma.

Sensory evaluation was done in accordance with the requirements of the legal standard CSN ISO 8589:2008. A protocol with unstructured 100-mm graphic scales was used during the evaluations. Six panellists (holders of the Sensory Attest Certificate according to Organizational Directive No. 026/2003 of the Czech Agriculture and Food Inspection Authority) were recruited from the DMHE. The evaluations were done by employees in order to determine the attributes of each type of chicken breast meat (without skin) separately following the evaluations. Samples of all three kinds of chicken breast were evaluated in the raw status and after heat treatment (baked in aluminium foil at 220 °C/40 min). Consumer panellists were asked to evaluate the sensorial attributes of the breast meat in the raw status, including their overall impression; colour; odour; consistency and juiciness after a slight compression, while in the case of the baked samples, the following sensorial attributes were evaluated: impression; odour; consistency; juiciness and flavour. The data were statistically analyzed by using Microsoft offices Excel 2003.

Colour parameters. The colour parameters (lightness, L*; redness, a*; yellowness, b*) of the raw surface of breasts were measured by the CIEL*a*b* system by using Minolta CM 2600d (Konica Minolta, Japan). Software (Spectra Magic 3.61) were used for the calculation of the parameters and the mean value ± S. D. of five measurements of each sample are reported.

The texture parameters were measured by using an Instron 5544 (Instron Corporation England, United Kingdom). Instron has been used in order to test the breast chicken by texture profile analysis (TPA) and the Warner-Bratzler test. Computer software (Merlin, Series IX) were used to obtained the indicators. For TPA the beaked samples of breast (220 °C/25 min) were prepared in cylindrical shape (1 cm high, 1.25 cm in diameter), then they were compressed with a compression plate of 36 mm in diameter twice to 50 % of their original height at a crosshead speed of 50 mm/min. The hardness and cohesiveness were also evaluated [12]. The Warner-Bratzler test was used to measure the shear force and toughness on the samples of baked and raw samples of breasts (1.0 cm wide, 1.0 cm high and 2.0 cm long), with crosshead speed 80 mm.min^{-1}. The result of each sample was represented as the mean ± SD of five measurements.

Chemical composition. Samples were analysed for chemical composition including dry matter, total protein, net proteins, net muscular protein, collagen, fat, salt (NaCl), ash and phosphorus. The amount of dry matter was determined gravimetrically by drying the samples at +10^3 ± 2 °C (CSN ISO 57 6021). The content of the total protein was determined by using Kjeltec 2300 analyzer (FOSS Analytical AB, Högänas, Sweden) based on the amount of organically bound nitrogen (recalculating coefficient

f1 = 6.25) according to CSN ISO 937:1978, the net protein content was indicated as an amount of nitrogen that was organically bound using a Kjeltec 2300 (FOSS Analytical AB, Sweden) after precipitation with hot tannin solution. The net muscular protein was calculated mathematically by subtracting collagen from net protein, the collagen content was computed from the content of the amino acid hydroxyproline (coefficient factor f = 8). The quantity of hydroxyproline was determined by photometric measurement at 550 nm on a GENESYSTM6 spectrophotometer (Thermo Electron Corporation, USA). The fat content was analyzed by SOXTEC 2055 (Tecator, Högänas, Sweden) with petrol ether as an extraction agent (CSN ISO 1443:1973). The content of salt (NaCl) was determined by titration with silver nitrate (Mohr's method), the ash by burning the sample at 550 °C until black carbon particles disappeared in a muffle oven (Elektro LM 212.11, Germany) according to CSN ISO 936:1978. The amount of phosphorus was determined after conversion to orthophosphate and precipitated as a compound quinoline phosphomolybdenate.

Statistical analysis of data was carried out using Microsoft offices Excel 2003. Tukey-HSD was applied for the determination of the statistically significant differences (*$P < 0.05$, **$P < 0.01$, NS — no significance) among organic, conventional and tenderized breast chickens samples (UNISTAT 6.0 (Unistat® Limited, London, England).

RESULTS AND DISCUSSION

The results of sensorial attributes indicated that no statistical difference ($P > 0.05$) was found between the organic and the conventional chicken breast in the raw and after heat treatment; similar to that found by Husak et al. [6], but a difference was found with tenderized breast of chicken due to the clear changes in the chemical and physical properties that the tenderized chicken breast had been exposed to. The overall impression of the panellists for the breast of organic chicken was the highest ($P < 0.01$) compared to the tenderized breast in the raw and baked status; also the panellists gave the highest score for the flavour properties to the breast of the organic broiler. The tenderized breast of chicken was more ($P < 0.01$) tender than the organic or the classic broiler's breast (Table 1).

The result of this study indicated that the tenderized breast muscles was more dark (L*, $P < 0.01$) than the meat of the organic or the conventional chickens' breasts. The decrease of lightness may be explained by the increase in ionic strength due to adding sodium chloride, which improves the ability of the muscle proteins to bind water, leading to less reflected light due to the presence of less unbind water in the muscle [7]. Also the tenderized meat of breast was more (a*, $P < 0.01$) red than the organic production meat. The conventional meat of chickens' breast was more yellow (b*; $P < 0.01$) than the tenderized or the organic chicken's breast, which could be due to the higher lipid content, resulting from the intramuscular storage of lipophilic pigments [11] (Table 2).

The breast of tenderized chickens (baked) was more ($P < 0.01$) tender, required less shear force (20.01 N) than the breast from the conventional production system (33.66 N), due to the increased water holding capacity by use of salt and polyphosphates as a solution for injection. No statistical difference was observed between the organic and the conventional breast in the raw and baked status, which corresponded with the results found by Wang et al. [14] and Fanatico et al. [4] who found the free-raising system did not influence tenderness. However, Husak et al. [6] and Castellini et al. [2] indicated that the greater motor activity of organic poultry could have had effects on the meat tenderness, increasing the shear value of the meat. The toughness of the conventional baked breast was higher ($P < 0.01$) than tenderized; while no significant difference were observed in hardness and cohesiveness among all types of chicken breast (Table 2). However, there are many factors affecting the meat tenderness including: diet, age, pre-slaughter handling and post-slaughter chilling which makes it difficult to be attributed to the type of production system only [6].

The dry matter content in the tenderized breast was lower ($P < 0.01$) than in the breasts of the organic and conventional chickens (Table 3). The meat of the breast from the organic broilers had the highest total protein content (23.43 %), net protein (21.45 %) and net muscular protein (21.00 %) among all types of chicken's breast with the statistical difference about ($P < 0.01$) higher than the tenderized breast of chicken. The fat content in the breast meat of organic poultry (0.27 %) was lesser ($P < 0.01$) than in the breast meat from conventional chickens (0.71 %). These results support the idea that motor activity prefer myogenesis against lipogenesis [2]. Generally, the ash, NaCl and phosphor content in the tenderized breast was higher than

Table 1. Sensory evaluation of different types of chicken breast meat

Parameters	Chicken breasts in raw status [mean ± SD]			Stat. sign.
	Tenderized [n = 10]	**Organic** [n = 4]	**Conventional** [n = 5]	
Impression	73.93 ± 6.66[a]	86.90 ± 9.50[b]	86.62 ± 12.40[b]	**
Colour	80.26 ± 3.92[a]	88.33 ± 10.50[b]	86.86 ± 11.64[ab]	**
Odour	82.00 ± 5.68[a]	86.88 ± 15.75[ab]	89.34 ± 10.89[b]	*
Consistency	63.73 ± 7.38[a]	69.50 ± 32.60[ab]	78.42 ± 23.63[b]	*
Meat juices	63.22 ± 8.50[a]	89.65 ± 11.24[b]	93.48 ± 5.67[b]	**
	Chicken breasts after heat treatment (baked at 220 °C/40 min)			
Juiciness	76.87 ± 4.14	71.02 ± 34.59	69.70 ± 26.35	NS
Flavour	76.28 ± 6.45[a]	92.23 ± 6.97[b]	88.48 ± 10.39[b]	**
Impression	75.68 ± 6.40[a]	90.65 ± 9.69[b]	84.98 ± 12.68[b]	**
Odour	79.51 ± 2.56[a]	90.15 ± 9.31[b]	88.68 ± 7.15[b]	**
Consistency	68.38 ± 8.33[a]	76.10 ± 22.59[ab]	79.70 ± 18.81[b]	*

Statistical significance * — P < 0.05; ** — P < 0.01
NS — no significance; results with different superscripts in a row differed significantly

Table 2. Colour and texture analysis of different types of chicken breast meat

Parameters	Chicken breasts in raw status [mean ± SD]			Stat. sign.
	Tenderized [n = 10]	**Organic** [n = 10]	**Conventional** [n = 10]	
Shear force	37.34 ± 9.25	27.79 ± 4.97	29.43 ± 5.54	NS
Toughness	417.44 ± 111.51b	190.18 ± 20.41a	330.33 ± 54.37ab	*
L*	52.51 ± 9.69a	57.63 ± 2.36b	58.04 ± 2.57b	**
a*	0.26 ± 1.06b	–0.57 ± 0.65a	-0.30 ± 0.87ab	**
b*	2.50 ± 4.44a	6.68 ± 1.34b	8.15 ± 1.33b	**
	Chicken breasts after heat treatment (baked: 220 °C/25 min)			
Shear force	20.01 ± 3.63a	26.42 ± 3.28ab	33.66 ± 4.84b	**
Toughness	242.05 ± 34.66a	269.68 ± 39.80ab	394.02 ± 39.35b	**
Hardness	29.27 ± 7.96	27.87 ± 6.45	25.41 ± 5.35	NS
Cohesiveness	1.21 ± 0.04	1.22 ± 0.05	1.20 ± 0.04	NS

Statistical significance: * — P < 0.05; ** — P < 0.01
NS — no significance; results with different superscripts in a row differed significantly

Table 3. Chemical analysis of different types of chicken breast meat

Parameters	Breast of chickens in raw status [mean ± SD]			P
	Tenderized [n = 20]	Organic [n = 10]	Conventional [n = 9]	
Dry matter [%]	20.58 ± 1.16a	25.16 ± 0.57b	24.91 ± 0.42b	**
Total protein[%]	16.99 ± 1.56a	23.43 ± 0.45b	22.80 ± 1.08b	**
Net protein [%]	15.35 ± 1.32a	21.45 ± 0.37b	20.44 ± 0.68b	**
Net muscle protein [%]	15.03 ± 1.31a	21.00 ± 0.35b	20.01 ± 0.67b	**
Fat [%]	0.14 ± 0.09a	0.27 ± 0.30a	0.71 ± 0.41b	**
Ash[%]	2.08 ± 0.13b	1.20 ± 0.11a	1.15 ± 0.15a	**
Phosphorus [mg.100 g^{-1})	226.47 ± 10.75b	216.47 ± 5.44a	224.41 ± 10.50ab	*
NaCl [%]	1.07 ± 0.09b	0.20 ± 0.02a	0.20 ± 0.01a	**

Statistical significance: * — P < 0.05; ** — P < 0.01
NS — no significance; results with different superscripts in a row differed significantly

the breast of chicken from the organic or the conventional production systems due to the use of salt and phosphor in the marinate processes during tenderizing the breast of chicken. According to many studies [1, 10, 15], there are many factors which affected the chemical compositions of meat, including: genetics, feed rations, and physical activity. However, in the present research, the samples were obtained as currently provided in the markets for consumers, so that these factors were unknown.

CONCLUSIONS

The results of this study indicated the differences between organic and conventional tenderized breast of chicken in qualitative properties which could be due to many factors, such as: genotypes, diets, ages, production environment, as well as, the technological production methods of the broilers' breast that had been used. The high physical activity of organic poultry had a clear reflection on the high protein content in the breast, while the more yellow colour of the conventional breast was due to the high fat content, these results support the idea that high physical activity prefer myogenesis against lipogenesis. The tenderization process of the breast had a reflection on the results, where the meat of the breast observed: low share force, low dry matter and high content of ash, NaCl and phosphorus. It should be remember that the results of this study is a reflection on the characteristics of these chickens breast as marketed to consumer and do not necessarily reflect only the differences of productions systems on the broilers' properties because the effects of other factors were not controlled in this research.

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IMPACT OF ADDITIVES IN BROILER NUTRITION ON THE QUALITY OF BREAST MUSCLES

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ABSTRACT

The aim of this study was to determine the effects of a modified diet of broiler chickens by the addition of fermented feed, Agrimonia eupatoria extract and humic acids, to quality parameters of breast muscles by using selective instrumental analyses (compression test and Warner-Bratzler shear test — WB). Samples of breast muscles from three experimental groups and a control group were taken, in order to perform the analyses. Based on the mathematical-statistical evaluation of the data obtained, significant differences ($P < 0.05$ or $P < 0.01$) among selected groups of samples in assessing the textural attributes were recorded. From the results of two analyses, aiming to express the tenderness, we can consistently establish the positive effects of changes due to the fermented feed diet of broiler chickens. The feed additives improved the quality of the breast muscles of the broilers. Using the appropriate groups of added ingredients in the broiler nutrition, we can achieve the targeted changes to the indicators of quality meat. In this regard, the application of instrumental texture analysis provides an objective tool for assessing the changes in muscle performance.

Key words: additive; meat; nutrition; poultry; texture

INTRODUCTION

The quality of breast muscles of broiler chickens is usually assessed in terms of colour, pH, retention capacity, softness and overall sensory acceptability. These named descriptors are crucial in terms of culinary quality and technological properties of chicken [8, 13, 20]. In view of the consumer preferences, it can be considered ideal when the

meat is juicy, soft and not too pale [6, 11, 15, 18]. Nutrition in a given direction, acts directly on the quality of the muscle, affecting the amino acid composition and the fatty acids in the muscle of broilers. The fatty acids contained in animal feed are stored in the muscles of chicken [21]. Thus, the fatty acid composition of the feed influences the content of fatty acids in the chicken muscles, which greatly affects the oxidation potential of the meat and its shelf life [1, 2, 17]. The ability of the rapid growth of broilers, with short generation intervals and with the use of feed antibiotics in animal feed have resulted in the improvement of the product. While this method of nutrition on the one hand has a positive impact on the quality of production, it also may prove to have risks of adverse health effects on the consumers [4]. In order to improve the general health of chickens and to fulfil consumer demands with regard to product quality, poultry producers increasingly use natural feed additives, such as herbs [5]. A major problem encountered by the processing industry in poultry production is pale, soft and exudative meat (PSE). PSE meat is caused by a rapid decrease in the pH immediately after slaughter, when the temperature of the carcass is still quite high [7]. The opposite, but equally undesirable effect, is the appearance of firm meat. The reason for this qualitative change is the growing demand from consumers for the availability of processed products that leads poultry producers to seek opportunities for expansion of their production, but often at the expense of quality. To keep pace with market requirements, processors need to develop more methods to speed up the production of deboned meat without affecting the quality requirements of the consumer. The development of methods for increasing the efficiency of processing operations include: reducing energy bills and job growth; and an increase of the production volume and total gain [9]. To achieve these aims, it is the important application of new methods with the aim to improving the quality of the final product. Currently, a number of factors affect the overall softness of fillets made from chicken breast muscle. The classical scheme of processing involves the chilling of the carcasses by the use of double-phase cooling. This method increases the efficiency of cooling depth of cuts in approximately 1—1.5 hours. The carcasses are aged subsequently on ice for 4—6 hours for achieving maximum softness. Stewart et al. [16] pointed out the need to comply with that period of maturation, due to biochemical processes associated with the completion of post-mortem development.

The aim of this study was to determine changes in selected quality indicators of breast muscles of broiler chickens by the use of instrumental analysis, after the experimental administration of selected components in the diet of broilers.

MATERIALS AND METHODS

Implementation of nutrition in broiler chickens

The scheme of nutrition in chickens from the experimental and the control group was carried out by the commercial feed (KKZ) HYD 01, HYD 02, HYD 03 and HYD 04 during the 38 days of fattening. The amount of added components sequentially reduced the daily dose of KKZ in selected experimental groups. The experimental group GLA: from the 17th day of fattening chickens KKZ was enriched by the addition of fermented feed with a higher proportion of gamma-linolenic acid (produced by fermentation of Cuninghanela on wheat bran) in the amount of 10 %. The experimental group GLA+R: Starting from the first day of fattening the chickens, the source of drinking water in the morning and evening was enriched by Agrimonia eupatoria extract added in a 0.2 % concentration. From the 17th day of fattening except KKZ was also fed fermented feed with a higher proportion gamma-linolenic acid (produced by fermentation of Cuninghanela on wheat bran) in the amount of 10 %. The experimental group HC: Started on the first day of fattening the chickens, they were fed with a combination of KKZ and humic acids with a 0.6 % concentration.

Preparation of samples for instrumental analysis

Samples were stored frozen for 1 month (–18 °C), and then defrosted prior to the analysis. Part of the breast muscles without bone (2 cm thickness × 4 cm width x 4 cm length) was analysed using a compression test by the use of a spherical probe (SMS P/1S), immediately after thawing, and warming the sample to room temperature. The remaining part of the breast muscle, after thawing, was heat treated as follows: the samples were individually wrapped in aluminium foil and placed in an electric oven until the temperature reached 76 °C in the core of muscles (checked by a needle thermometer). After heat treatment and the

unpacking of the samples for the purpose of cooling, fillets were prepared by the use of scalpel to dimensions: 1 cm height × 1 cm width × 4 cm length. Thus, prepared samples were then individually placed in the fridge for overnight storage at 4 °C. The next day, the prepared fillets of chicken breast muscle, after warming to the room temperature, were analysed for softness using the Warner-Bratzler (WB) shear test. Energy and force required to cut and compress the muscle samples were continuously recorded and then analysed using the software Exponent ver. 5.0.9.0.

Settings of the instruments

Hardware equipment: Textural analyser: TA XT2 Plus (Stable Microsystems, UK); Probe: Spherical probe SMS P/1S and WB knife (Stable Microsystems, UK); Platform HDP/90 (Stable Microsystems, UK).

Compression test: Pre-Test Speed: 1.0 mm.s^{-1}; Test Speed: 1.1 mm.s^{-1}; Post-Test Speed: 10.0 mm.s^{-1}; Distance: 20 mm; Load cell: 5 kg.

WB shear test: Pre-Test Speed: 2.0 mm.s^{-1}; Test Speed: 2.0 mm.s^{-1}; Post-Test Speed: 10.0 mm.s^{-1}; Distance: 1.5 mm; Load cell: 5 kg.

Under the compression test, 15 repetitions were carried out within one sample. The Warner-Bratzler test was carried out using ten repetitions per sample.

Analysis of the data

For processing data from the compression and WB test,

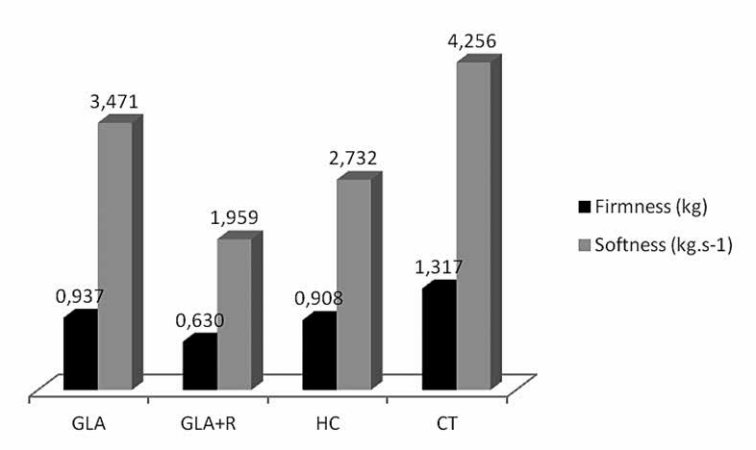

Fig. 1. Compression test — mean values of parameters determining firmness and overall softness of muscle

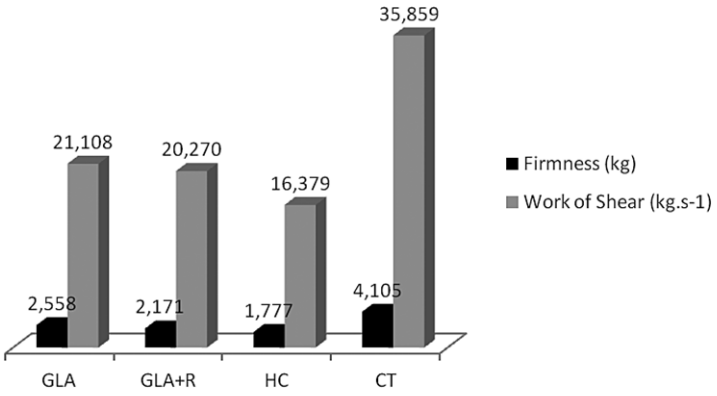

Fig. 2. WB test — mean values in the parameters of hardness and overall work for cutting of muscle

Exponent software ver. 5.0.9.0 and Tanagra software — Wilcoxon Signed Ranks non-parametric test was used to identify any significant differences in the measured parameters of the tested samples.

RESULTS AND DISCUSSION

The softness of the meat is a major determinant of quality, representing the most significant indicator of the sensory quality of the meat [3]. Therefore, the softness of the meat is the endpoint for a number of experimental studies. Currently, there are several methods in order to evaluate the softness of the breast muscles of broiler chickens, such as: instrumental analyses, descriptive sensory analysis, or a combination of both. In the context of instrumental analyses, Allo-Kramer shear-compression system (multiple blade), Warner-Bratzler shear knife and texture profile analysis represents the ordinary methods used in the poultry industry in order to determine the softness of the breast muscle of broilers [14].

The descriptive sensory analysis is a method utilized in the experimental area for setting the attributes that determine the softness of poultry meat. This method of quality evaluation of the meat is one reliable method, the results of which are correlated with outputs of instrumental analysis, although the sensory evaluation is one of the time consuming methods [9, 10, 12, 19]. The methods for comparing the measured outputs of the instrumental analysis of samples through statistical program (Tanagra) identified significant differences in values: GLA *vs.* GLA + R ($P < 0.05$), and CT (Control) *vs.* GLA + R ($P < 0.01$) in the parameter of the total firmness of the sample after thawing, through the compression test for fresh uncooked chicken breast muscle. An accompanying output of compression tests was the analysis of the total work involved in compressing the sample to 50 % of its thickness, in order to assess the overall softness. The results of this part of the compression analysis correlated with the results of the analysis of the total hardness: GLA *vs.* GLA + R ($P < 0.01$), and CT *vs.* GLA + R ($P < 0.01$). For samples subjected to heat treatment (simulating standard conditions of culinary preparation of this type of muscle) by analysis under the method of the Warner-Bratzler shear test in the form of fillets of defined dimensions; they have identified significant differences in the values for the parameter of total hardness: GLA *vs.* HC ($P < 0.01$); GLA *vs.* CT ($P < 0.01$); GLA + R *vs.* HC ($P < 0.05$); GLA + R *vs.* CT ($P < 0.01$); HC *vs.* CT ($P < 0.01$). The identified differences correlated with the additional output of WB test for the value of the total work involved to cutting of the sample: GLA *vs.* HC ($P < 0.01$); GLA *vs.* CT ($P < 0.01$); GLA + R *vs.* HC ($P < 0.01$); GLA + R *vs.* CT ($P < 0.01$); HC *vs.* CT ($P < 0.01$). On the basis of the average values (Exponent software), the parameters determining the firmness and overall softness of the sample, obtained by the compression test (Fig. 1) can be used to sorting the samples according to the average softness/firmness from the softest to toughest in the following order: GLA + R, HC, GLA and CT.

In accordance with the average values of the parameters determining hardness and overall work required to cut samples obtained via WB shear (Fig. 2), samples can be sorted by average energy (from lowest to highest) needed to cut samples of defined dimensions as follows: HC, GLA + R, GLA and CT.

CONCLUSIONS

In both analyses, focusing to the expression of the overall softness of muscle, as one of the most important parameters evaluated in this type of samples can be described the effect of changes in the diet of broiler chickens by the feed additives on the resulting values, determining the quality of breast muscles of broilers. The use of additives in nutrition may lead to changes in the quality parameter in the desired direction.

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VARIATIONS IN THE ARTERIAL BLOOD SUPPLY TO THE THORACOLUMBAR SPINAL CORD IN THE LABORATORY MOUSE

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ABSTRACT

The aim of this study was to describe the blood supply to the thoracolumbar spinal cord in the laboratory mouse using the dissection technique. The study was carried out on 10 adult mice (strain ICR). The arterial system of the thoracolumbar spinal cord was injected by using Batson's corrosion casting kit No. 17. The branches entering the ventral spinal artery along the entire thoracolumbar spinal cord, were observed as left-sided in 64.2 % of the cases and as right-sided in 35.8 % of the cases. In 60 % of the cases, the artery of Adamkiewicz branched from the left-sided fourth lumbar artery and in 30 % of the cases from the right-sided fourth lumbar artery. In 10 % of the cases, the artery of Adamkiewicz originated from the right-sided, as well as from the left-sided fifth lumbar artery. On the dorsal surface of the spinal cord, two irregular longitudinal dorsal spinal arteries were present in 70 % of the cases, in 30 % of the cases they were absent, and in 10 % there were three arteries. The dorsal branches along the entire thoracolumbar spinal cord, were observed as left-sided in 60.8 % of the cases and in 39.2 % of the cases as right-sided. The dorsal and ventral branches of the lumbar arteries were present irregularly, more frequently absent than in the thoracic part. This finding allowed us to assume a higher risk of irreparable ischemic injury to the lumbar part of the spinal cord.

Key words: artery of Adamkiewicz; dorsal spinal artery; laboratory mouse; ventral spinal artery

INTRODUCTION

The laboratory mouse is one of the more frequently chosen species of laboratory animals used in experimental studies dealing with spinal cord injuries [1, 6, 13]. The blood supply to the spinal cord has been the object of several studies in different species of laboratory animals and in man [2, 11, 15, 17, 18, 19]. However, only a few of these studies have described the arterial blood supply to the spinal cord in the mouse [8, 14].

The aim of this study was to describe the anatomical variations of arteries participating in the blood supply of the

thoracolumbar spinal cord in the laboratory mouse using the dissection technique. This part of the spinal cord is most often affected by the risk of serious neurological damage.

MATERIALS AND METHODS

This study was carried out on 10 adult mice (age 80—100 days). We used mice (strain ICR) of both sexes (female n = 5; male n = 5) weighing 30—40 g in an accredited experimental laboratory of the University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C, relative humidity 45 %, 12-hour light period), and fed with a granular feed mixture (FANTASIA PREMIUM). Drinking water was available to all animals ad libitum. The animals were sacrificed by the intracardiac injection of embutramide (T-61, 0.3 ml.kg^{-1}).

Immediately after euthanasia, the vascular network was perfused with a saline solution. During the manual injection through the ascending aorta, the right atrium of the heart was opened in order to lower the pressure in the vessels to ensure an optimal injection distribution. Three ml of Batson's corrosion casting kit No.17 (Dione, České Budějovice, Czech Republic) was used as the casting medium. After the polymerization of the medium (1 h), the mice were placed in 10% formaldehyde to fix the spinal cord. After 1-week fixation, the vertebral canal was opened by removing the vertebral arches in the thoracic, lumbar and sacral spinal regions. The prepared spinal cords were fixed in 10% formaldehyde. The study was carried out under the authority of decision No. 2647/07-221/5.

RESULTS

The spinal branches arising from the dorsal intercostal and lumbar arteries entered the vertebral canal through the intervertebral foramina. Their passage through the foramen was associated with the respective spinal nerve roots. The spinal branches divided inside the vertebral canal into the dorsal and ventral branches. The ventral branches joined the ventral spinal artery which was located in the ventral median fissure of the spinal cord. The occurrence of ventral branches at each spinal cord segment is shown in Table 1. The left-sided branches entering the ventral spinal artery in the thoracic part of spinal cord were present in 64.8% of the cases and the right-sided in 35.2% of the cases. The left-sided branches entering the ventral spinal artery in the lumbar part of the spinal cord were present in 63.5% of the cases and the right-sided in 36.5% of the cases. Generally, in the entire thoracolumbar spinal cord, the left-sided branches were present in 64.2% of the cases and right-sided in 35.8% of the cases, which is most likely related to the left-sided localization of the aorta. The artery of Adamkiewicz was present as a bigger feeding artery arising from the spinal branch. In 60% of the cases, it branched off of the left-sided fourth lumbar artery (Figure 1) and in 30% of the cases off of the right-sided fourth lumbar artery (Figure 2). In 10% of the cases, it was observed as a doubled artery of Adamkiewicz, originating from the ramus spinalis of the right- and left-sided fifth lumbar artery (Figure 3). In

Table 1. Frequency of occurrence of ventral branches of arterial spinal branches in the thoracolumbar region of spinal cord

Occurrence of arterial spinal branches [%]		
Level	**Right**	**Left**
Th1	40	60
Th 2	0	30
Th 3	0	0
Th 4	30	70
Th 5	0	50
Th 6	30	60
Th 7	0	50
Th 8	20	80
Th 9	0	30
Th 10	50	50
Th 11	30	0
Th 12	60	60
Th 13	50	100
L 1	50	60
L 2	40	80
L 3	50	90
L 4	40	100
L 5	50	70

L — lumbar segment of the spinal cord
Th — thoracic segment of the spinal cord

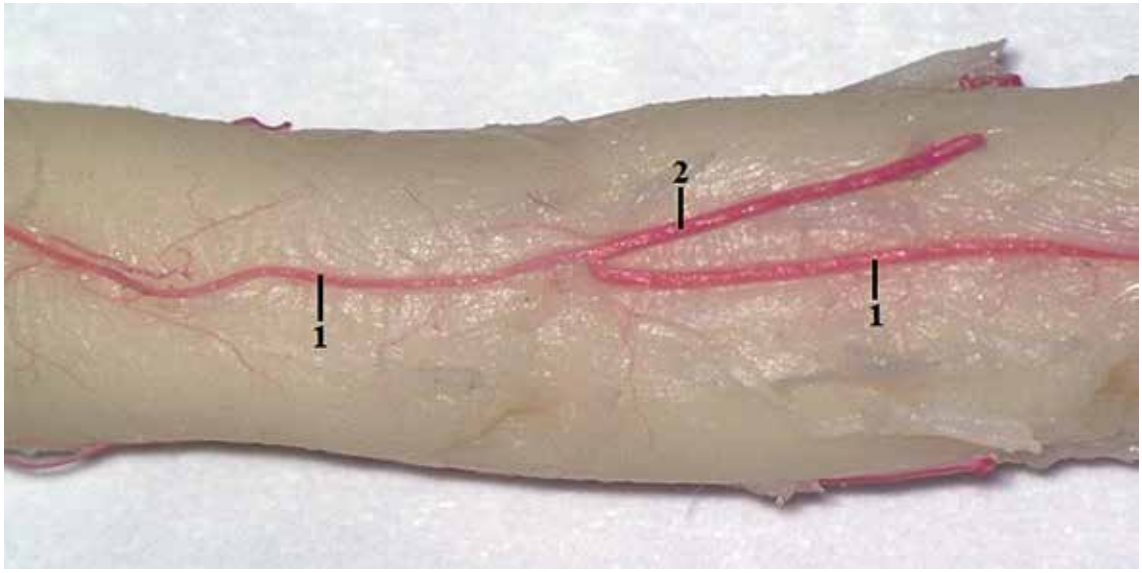

Fig. 1. Left-sided localization of the artery of Adamkiewicz
(1) arteria spinalis ventralis; (2) artery of Adamkiewicz
Ventral view. Magn×12.5

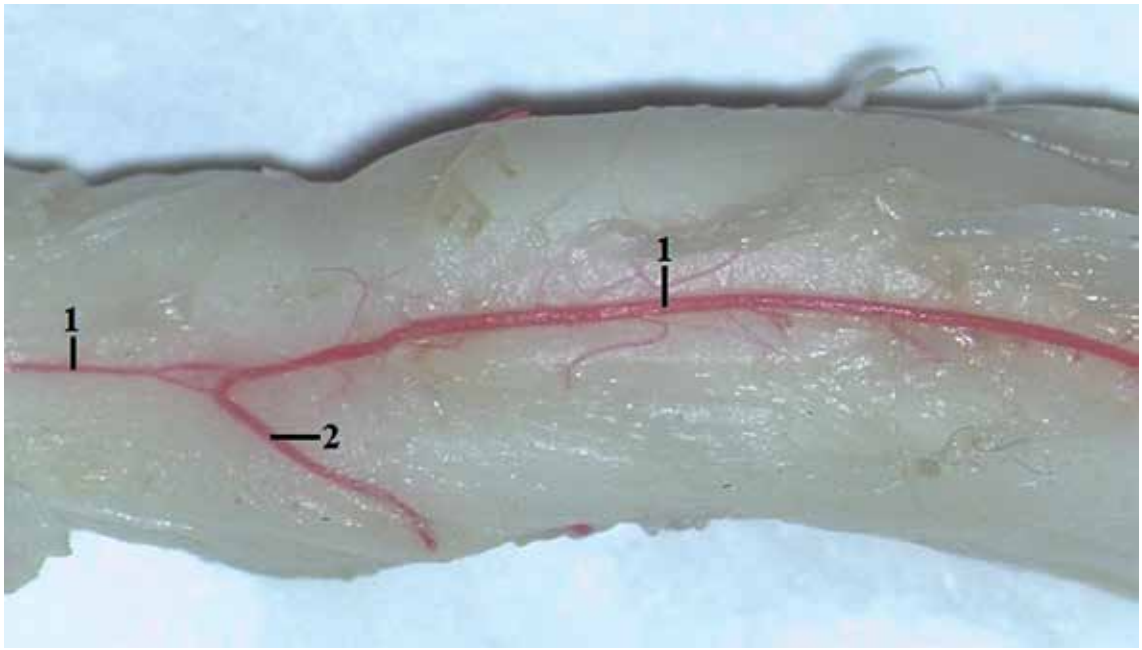

Fig. 2. Right-sided localization of the artery of Adamkiewicz
(1) arteria spinalis ventralis; (2) artery of Adamkiewicz.
Ventral view. Magn ×12.5.

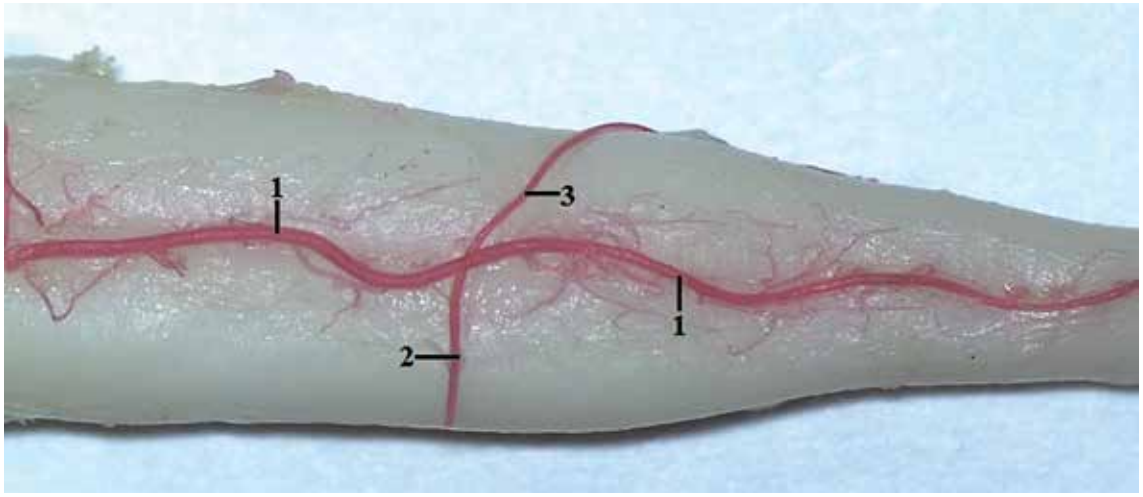

Fig. 3. Doubled artery of Adamkiewicz
(1) arteria spinalis ventralis; (2) right-sided artery of Adamkiewicz; (3) left-sided artery of Adamkiewicz
Ventral view. Magn ×12.5

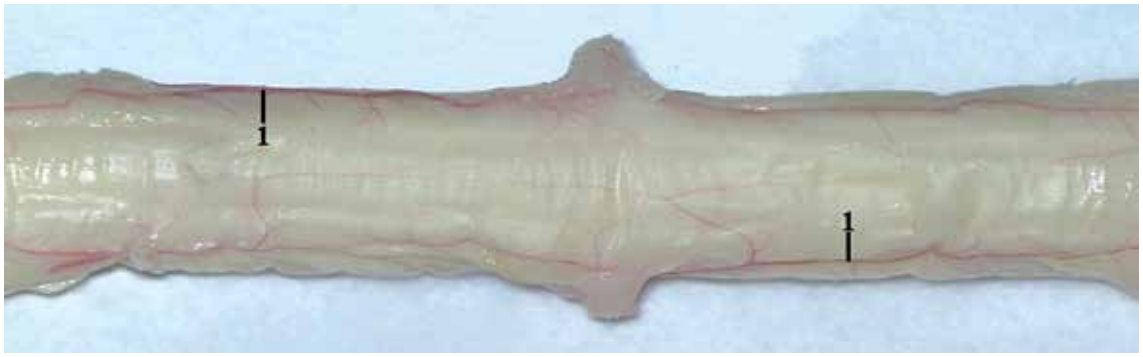

Fig. 4. Presence of two longitudinal dorsal spinal arteries
(1) dorsal spinal artery. Dorsal view. Magn ×12.5

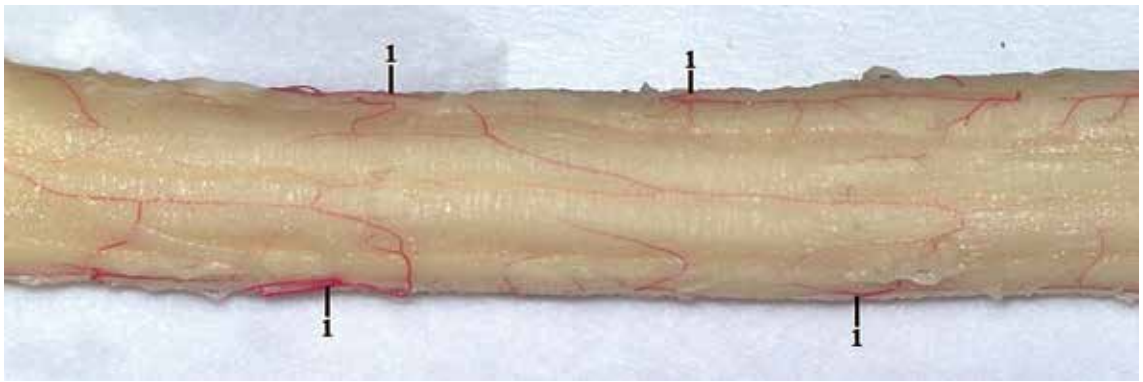

Fig. 5. Absence of dorsal spinal arteries
(1) dorsal branch of spinal artery. Dorsal view. Magn ×12.5

Table 2. Frequency of occurrence of dorsal branches of arterial spinal branches in the thoracolumbar region of spinal cord

Occurrence of arterial spinal branches [%]		
Level	**Right**	**Left**
Th1	50	50
Th 2	30	40
Th 3	40	60
Th 4	40	0
Th 5	50	50
Th 6	20	60
Th 7	30	60
Th 8	0	20
Th 9	10	50
Th 10	20	40
Th 11	20	60
Th 12	30	70
Th 13	60	80
L 1	50	70
L 2	40	60
L 3	30	60
L 4	60	80
L 5	40	50

L — lumbar segment of the spinal cord
Th — thoracic segment of the spinal cord

all of the cases, the artery of Adamkiewicz was found entering the arteria spinalis ventralis.

Two irregular longitudinal dorsal spinal arteries were lying in the sulcus lateralis dorsalis bilaterally in 70% of the cases (Figure 4). The dorsal branches of the spinal branches entered the dorsal spinal arteries and in 30% of the cases, the dorsal spinal arteries were absent (Figure 5). In 10% of the cases, three irregular longitudinal dorsal spinal arteries were present. The third dorsal spinal artery was located in the sulcus dorsalis. The occurrence of the dorsal branches at each spinal cord segment is shown in Table 2. In some of the animals with two irregular dorsal spinal arteries, these arteries were formed only by fusion of the small cranial and caudal branches arising from the dorsal branches. The left-sided dorsal branches in the thoracic part of the spinal cord were present in 61.5% of the cases and the right-sided in 38.5%. The left-sided dorsal branches in the lumbar part of the spinal cord were present in 59.3% of the cases and the right-sided in 40.7%. Generally, in the entire thoracolumbar spinal cord, the left-sided dorsal branches were present in 60.8% of the cases and right-sided in 39.2% of the cases. According to our results, it can be concluded that the blood supply of the thoracolumbar spinal cord in the mouse shows a rather high variability.

DISCUSSION

Pigs, dogs, guinea pigs and rats have been used as experimental models in the study of the spinal cord damage. Possible variations in the density of arteries forming the spinal arterial ring and the frequency of occurrence of spinal branches originating from the radicular arteries have also been described in dogs [12]. The most frequently used animal which serves as an experimental model in the study of spinal cord injury is the rat. Its blood supply to the spinal cord is probably the most documented, but the results of many studies differ [4, 15, 16, 20]. Two dorsal spinal arteries [9] or their occurrence as a less constant vessel was found in albino Wistar rats [19]. The anatomical variations and the existence of extrasegmental arteries of the spinal cord have been described in the pig [17]. In our group of mice, we found two, three or no irregular longitudinal dorsal spinal arteries (in human the posterior spinal arteries). The posterior spinal arteries in humans were present as cranially to caudally continuing trunks [5]. The dorsal spinal arteries in dogs were found as pairs of lateral dorsal spinal arteries with larger diameter and a pair of thinner medial dorsal spinal arteries [12]. In rats, Woollam and Millen observed the dorsal spinal arteries as a less constant vessels, with the formation of irregular connections between each other [20].

Works dealing with the study of the spinal cord blood supply in mice have been published sporadically. Less than ten ventral branches entering the ventral spinal artery have been described [14]. The dorsal spinal arteries differ in number from one study to another. They were described as two uninterrupted trunks [8, 14] or only one dorsal spinal artery was present [3]. In our study, two, three or no dorsal spinal artery was present.

The artery of Adamkiewicz was found as a doubled vessel originating from the spinal branch of the third or fourth lumbar artery [14]. In our study, the artery of Adamkiewicz

was present as a single or doubled artery with a different level of origin. In dogs, the artery of Adamkiewicz was found in 50% of all the specimens [12]. In rats, the artery of Adamkiewicz was described in all specimens [4, 7, 16, 20], but many authors doubt its presence at all [15, 19]. The artery of Adamkiewicz was present in all studied human spinal cords [10] and absent in all spinal cords of pigs [17].

The occurrence of the dorsal and ventral branches of the spinal arteries bringing blood to the spinal cord is very variable. The frequency of dorsal and ventral branches was higher on the left than on the right side. The lumbar segmental arteries were present irregularly with a higher frequency in their absence compared with the thoracic part. This finding allowed us to assume a higher risk of irreparable ischemic injury to the lumbar part of the spinal cord in the mouse, if the aortic occlusion is located cranially to the origin of the artery of Adamkiewicz.

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SEGMENTAL BRANCHES OF THE DESCENDING AORTA IN THE LABORATORY MOUSE

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ABSTRACT

The aim of this study was to contribute to the anatomical description of the segmental branches of the descending aorta which supply the thoracic and lumbar part of spinal cord in the laboratory mouse. The study was carried out on 20 adult mice (strain ICR). We prepared corrosion casts of the arteries of the thoracolumbar spinal cord by using Spofacryl (polymethylmethacrylate). We found 13 pairs of the arteriae intercostales dorsales. Seven pairs of these arteries, as paired branches, arose from the dorsal surface of the thoracic aorta and were present in 80 % of the cases; 8 pairs in 15 % of the cases; and 9 pairs in 5 % of the cases. The remaining arteriae intercostales dorsales originated from the arteria intercostalis suprema. Five pairs of the arteriae lumbales arose from the dorsal surface of the abdominal aorta and were present in all of the cases. The arteriae lumbales originated as a common trunk with division in the right-left direction and were present in 70 % of the cases. The independent origin of the right- and left-sided arteries at the same level were observed in 30 % of the cases. The nearly regular segmental blood supply of the thoracic and lumbar parts of the spinal cord in all of the studied mice, reveals why the laboratory mouse serves as a simple model for investigations involving ischemic damage to the thoracolumbar part of spinal cord.

Key words: corrosion cast; dorsal spinal artery; laboratory mouse; lumbar artery

INTRODUCTION

Spinal cord injuries (SCI) affect more than 2.5 million people worldwide and 130,000 new cases are reported each year [12]. Although advances have been made through research, currently there are not available fully restorative therapies for spinal cord injuries, and therefore, safety measures especially in the sports industry have been put in place to reduce the risk of developing any new cases [8]. Spinal cord injury can lead to devastating long term adverse effects.

There is a vast amount of research that is available on different models that are used to present experimental SCI

Fig. 1. Origin of the arteriae intercostales dorsales
(1) aorta thoracica; (2) independent origin of arteriae intercostales dorsales; (3) origin of arteriae intercostales dorsales by means of a common trunk with division in right-left direction. Dorsal view. Magn. ×5

Fig. 2. Origin of arteriae lumbales
(1) aorta abdominalis; (2) independent origin of arteriae lumbales; (3) origin of arteriae lumbales by means of a common trunk with division into right-left direction. Dorsal view. Magn. ×5

that are also reproducible, reliable and in some ways resemble the injury seen in humans [7]. Laboratory mice are frequently used in studies of the spinal cord injury [1, 3, 9].

The aim of this study was to point to some anatomical variations in the segmental arterial blood supply of the spinal cord in the thoracic and lumbar regions in the laboratory mouse.

MATERIALS AND METHODS

The study was carried out on 20 adult mice (age 80—100 days). We used mice (strain ICR) of both sexes (female n = 5; male n = 5) with an average weight of 35 g in an accredited experimental laboratory of the University of Veterinary Medicine and Pharmacy in Kosice. The animals were kept in cages under standard conditions (temperature 15—20 °C, relative humidity 45 %, 12-hour light period), and provided with a granular feed mixture (FANTASIA PREMIUM). Drinking water was available to all animals ad libitum. The animals were sacrificed by the intracardiac injection of embutramide (T-61, 0.3 ml.kg^{-1}).

Immediately after euthanasia, the vascular network was perfused with a saline solution. During the manual injection through the cannula inserted into the ascending aorta via the left ventricle, the right atrium of the heart was opened in order to lower the pressure in the vessels to ensure the best possible perfusion. Spofacryl (polymethylmethacrylate, Spofa Dental, Czech Republic) in a volume of 3 ml was used as the casting medium. The maceration was carried out in a 2—4 % KOH solution for a period of 2 days at 60—70 °C. The study was carried out under the authority of decision No. 2647/07-221/5.

RESULTS

The oxygenated blood is delivered to the thoracic spinal cord by means of spinal branches arising from arteriae intercostales dorsales (Figure 1). In all experimental mice, 13 pairs of the arteriae intercostales dorsales were present. They originated from the thoracic aorta in 7 pairs in 80 % of the animals; in 8 pairs in 15 % of the animals; and in 9 pairs in 5 % of the animals. The remaining arteriae intercostales

dorsales were present as branches of the arteria intercostalis suprema. The origin of the arteriae intercostales dorsales at the same level as a common trunk with division into right-left direction was present in 40 % of the cases (Figure 1). The independent origin of arteries at the same level from the thoracic aorta was found in 60 % of the cases (Figure 1).

The spinal branches originating from the paired arteriae lumbales supplied the lumbar spinal cord. In all animals, there were 5 pairs of the arteriae lumbales which arose from the dorsal surface of the abdominal aorta. The first 4 pairs of them arose from the abdominal aorta and the last pair was present as a branch of the arteria sacralis mediana. The arteriae lumbales originating as a common trunk with their division into the right-left direction, were present in 70 % of the cases (Figure 2). The independent origin of the right- and left-sided arteries at the same level was present in 30 % of the cases (Figure 2).

DISCUSSION

During a surgical intervention on thoracoabdominal aneurysms, the anatomical arrangement of the segmental dorsal intercostal and lumbar arteries are very important [2]. The correctly performed re-implantation of segmental arteries decreases the risk of spinal cord ischemia which can lead to the paraparesis or paraplegia [6]. Thirteen pairs of the arteriae intercostales dorsales had independent origins. They were described as branches of the thoracic aorta. The first three pairs originated from arteria intercostalis suprema [10]. Five pairs of lumbar arteries, with independent origin from the abdominal aorta, have been described by Popesko et al. [10].

Experimental animals, especially rodent models, help to forecast functional outcomes of neurological failures and damages. Many of the behavioral results have comparable clinical symptoms described in human patients to a remarkable degree. Understanding the strengths and limitations of animal models will allow more relevant analysis of the injury, behavioral sequela, and therapeutic approaches. Each part of the experiment should be planned before a study is begun [5]. Detailed knowledge of the spinal cord blood supply plays a significant role in the prevention of spinal cord ischemia or infarction during several surgical procedures to the spine [4, 11].

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