TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2022, vol. 7, No 2(13): 104–109

INFLUENCE OF ELECTROACTEVATED REDUCED WATER ON THE BLOOD BIOCHEMICAL PROFILES OF WHITE PEKIN DUCK

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ABSTRACT

The aim of the current study was to determine the influence of electroactivated reduced water (catholyte) on the blood biochemical parameters of domestic duck (*Anas platyrhynchos domesticus*). Blood samples from 20 ducks divided in two groups (experimental, n=10 and control group, n=10) were taken twice to estimate the dynamics of the potential alterations. The activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), creatinine (CREAT), urea, calcium (Ca), phosphorus (P) and potassium (K) in serum were measured. Results showed serum biochemistry values within the physiological range. Differences between groups were insignificant. It can be therefore concluded that treatment with electroactivated reduced water had neutral effect on blood biochemical profiles of the tested animals.

Key words: electroactivated reduced water, catholyte, biochemical parameters, *White Pekin duck*.

Introduction

Water subjected to unipolar electric influence possesses some unique biological, microbiological, biochemical, biophysical characteristics. Electro- and electrochemically activated substances are formed that can be useful in the prophylaxis and treatment of disease (Atanasov et al., 2014, Karadzhov et al., 2014, Ignatov et al., 2015, Petrova & Popova, 2018). There are two types of solutions that can be obtained during the activation process. The anolyte is a bright, clear liquid with chlorine odor with antiseptic, anti-allergic, anti-inflammatory, antiedematous and anti-itching properties. It can destroy bacteria as well as many viruses and fungi. Local effects are explained by direct influence on bacteria and the inflammatory process. Unlike the other product – the catholyte, the anolyte retains its properties for longer and can be stored in closed glassware for months (Aschbach, 2008; Popova et al., 2016). The catholyte or "living water" presents a solution of alkaline nature that is similar to normal water by its taste, smell and color. However, it is characterized by specific properties such as oxidal-redox potential, alkality, the presence of active micro- and macro- elements. Depending on activation pH varies between 7 to 12 which is suitable for maintaining the balance between acid-forming and alkali-forming products (Aschbach, 2008; Gluhchev et al., 2015; Ignatov et al., 2015). Antioxidant, immune stimulating, regenerative properties have also been described. The aim of this study was to access the presence or absence of relevant effects on biochemistry parameters in experimental domestic ducks (*Anas platyrhynchos domesticus* – White Pekin breed).

Materials and methods

***Animals and study design*.** Twenty domestic ducks, White Pekin breed at the age of 8-weeks, female, with mean body weight of 1,663 kg were included. All were clinically healthy and dewormed in the beginning of the study period. They were fed with combined fodder suitable for the above-mentioned age (combined feed for ducklings was used – Grower (8 – 9 weeks of age; *Viand AD – Clone, Id. № αBG2230075)*. The population was divided into two groups – Group I (n=10) and Group II (n=10). The experimental group was treated with catholyte as drinking water available *ad libitum* for one month (the experiment was an investigation of a growth effect of catholyte).

Catholyte. The experimental group of animals was watered with catholyte (electrolyzed reduced water, produced by drinking tap water without added salt), obtained by activation of the 3rd degree in the apparatus (water tap related model) Aschbach – ionisiertes Wasser, with average parameters pH 9.29 (± 0,03), ORP -139 (± 0) mV (Manual multi-parameter analyser Consort C1010 (Consort bvba, Belgium) for pH, mV and temperature measurement). The catholyte was given *ad libitum* for 1 month. The control group of animals under the same conditions (*ad libitum*) received tap drinking water (pH 7).

***Sample collection and handling*.** Blood samples were obtained aseptically by venepuncture of *v. saphena medialis* with 20G needles. Blood was left to clot at room temperature. The serum was separated using centrifugation at 3000 rpm for 20 minutes and then transferred in Eppendorf tubes. Biochemical analysis was performed the same day. Blood serum was used for the evaluation of selected parameters of liver activity (ALAT, ASAT, ALP), renal function (CREAT, UREA), blood protein state (TP, ALB) and mineral and electrolyte profile (Ca, P and K). Automatic biochemistry analyzer BA-2800 Vet (Mindray, China) was used.

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Statistical analysis was performed using *Microsoft®Office Professional Plus Excel 2013 (15.0.4569.15060).* The results were processed mathematically and were calculated mean values (AV) and standard deviation (SD). To test statistical reliability of the results, Student's t-test analysis for independent samples was applied. The level of statistical significance (P) was assigned to be 0.05.

Results

The results of tested blood biochemical parameters of the investigated ducks are shown on table 1**.**

Biochemical analysis of the groups at D12 was characterized by insignificant differences. This tendency was stable during the experimental period. It was noticed that urea, creatinine, albumin, total protein levels were increased at D30 in both groups but remained within the reference range. Variations in enzyme activity between groups and the day of sampling were also visible with increase in alanine aminotransferase and decrease in alkaline phosphatase at D30 compared to D12. There was also a significant increase in phosphorus concentration. The experimental group showed a bit higher level APH at D12 and D30 while the level of ALT and AST were similar. Review of available literature found no data to explain observed fluctuations within or between groups. There are no differences in the blood biochemical parameters, and they are within the reference range for both groups that shows indirectly no toxic effect of the catholyte, taken *per os* and catholyte does not change the normal biochemical blood parameters of the studied animals. Only in the phosphorus

values there are slight increases in the experimental group of animals, and they are only with a statistical reliability, which is most likely related to the growth phase (more intensive growth process in experimental ducks, which corelates with the higher levels of live mass and slightly higher average growth values in the length of the tarso-metatarsal bone of the ducks from the experimental group, measured on the 30th day).

Table 1: Serum biochemistry values of experimental and control group at 12th day (D12) and at 30th day (D30) of the experimental period.

Parameter	Unit	Catholyte D12 (n=10)		Water D12 (n=10)		P <0.05	Catholyte D30 (n=8)		Water D30 (n=8)		P <0.05
		Mean (X)	Standard deviation (SD)	Mean (X)	Standard deviation (SD)		Mean (X)	Standard deviation (SD)	Mean (X)	Standard deviation (SD)	
UREA	mmol/l	1.35	±0.44	1.3	±0.57	**0.830**	2.83	±0.52	2.55	±0.77	**0.399**
CREAT	µg/l	18.32	±8.69	15.64	±8.88	**0.373**	23.3	±10.11	24.78	±5.94	**0.726**
ASAT	U/l	10.63	±7.87	11.87	±6.74	**0.692**	13.77	8.27	16.21	±3.92	**0.468**
ALAT	U/l	31.03	±19.49	27.90	±19.86	**0.726**	29.06	±15.42	31.42	±8.3	**0.710**
ALP	U/l	401.51	±178.85	355.11	±234.77	**0.625**	176.33	±97.31	154.92	±48.04	**0.589**
ALB	g/l	13.00	±6.61	12.61	±7.43	**0.902**	15.71	±1.02	14.96	±1.16	**0.192**
TP	g/l	24.87	±13.14	28.99	±15.50	**0.634**	29.01	±11.38	31.38	±1.78	**0.577**
Ca	mmol/l	2.39	±1.17	2.32	±1.22	**0.960**	2.46	±0.75	2.72	±0.1	**0.354**
P	mmol/l	1.84	±0.96	1.57	±0.89	**0.523**	2.34	±0.44	1.93	±0.32	**0.048**
K	mmol/l	5.40	±2.81	5.72	±2.95	**0.806**	4.25	±2.98	3.97	±0.48	**0.804**

Legend: *CREAT – Creatinin, ASAT- Aspartate aminotransferase, ALAT – Alanine aminotransferase, ALP – Alkaline phosphatase, ALB – Albumin, TP – Total protein, Ca – Calcium, P – Phosphorus, K – Potassium*

Discussion

Electroactivated water – catholyte shows a stimulating effect on the metabolism of the experimental ducks, which is proven by the investigated indices productivity / feed consumed (the birds of the experimental group showed a higher average growth for the period at lower values of feed consumption, energy expenditure and crude protein consumption per kilogram of growth than those of control birds). The blood biochemical parameters are within the reference range for both groups (Okeudo et al. 2003; Café et al. 2012; El-katcha et al. 2017). Possibly because of the short period, pathogenic changes did not appear in control group and the biochemical blood parameters stayed in referent degree (till enough compensatory effect of the systems in the body). The active ingredient in the catholyte is dissolved dihydrogen, which eliminates the free radical $HO^{\bullet}$ *in vivo*. H_2 selectively eliminates hydroxyl $HO^{\bullet}$ radicals, against which the body is usually without enzymatic protection. The nanoparticles present in catholyte have a similar effect to superoxide dismutase and catalase,

enhance the antioxidant action of dissolved H_2 and are responsible for the elimination of ROS (Marc & Chambron 2013). Reduced water can reduce the number of free radicals in biological systems and has been reported to exhibit an antioxidant effect on highly unsaturated fats and oils. (Miyasbita et al. 1999). The acid-alkaline balance of the blood during acidification of the body is achieved at the cost of changing the indicators of other systems of the body. Catholyte is an affordable and simple method of maintaining the balance between acid-forming and alkaline-forming products, since catholyte has a pH of 7 to 12, depending on the activation. The reduced water produced by electrolysis enhances the antioxidant effects of proton donors. Reduced water produced by electrolysis of tap water has a higher pH (9.0–10.0), lower oxidation reduction potential, lower dissolved oxyge and higher dissolved hydrogen from the non-electrolyzed water. Therefore, the results suggest that catholyte may prevent oxidative DNA damage, possibly through enhanced antioxidant effects against superoxide anion radicals, as originally shown by Shirahata et al. (1997). Thus, it appears that consumption of electrolyzed reduced drinking water may potentially serve to prevent DNA damage induced by mitochondrially produced oxygen free radicals due to an increase in oxidative stress (Hanaoka et al., 2004).

The organism is characterized by a redox potential. Every liquid that the body takes has and is characterized by its own redox potential, which means that together with food and water, the body receives not only a set of vitamins, minerals, trace elements, but also oxidizers and restorers, protons and electrons. Prilutsky & Bahir (1995) provide measurement and calculation data for redox potential of blood and internal tissues and calculate for arterial blood (pH = 7.4) redox potential -57 mV, and for venous blood redox – potential – -7 mV. Acidosis is one of the forms of disturbed acid-alkaline balance of the body, characterized by an absolute or relative excess of acids, i.e., substances donating hydrogen ions (i.e. protons). In compensated acidosis, the pH of the blood moves to the lower limit of the physiological norm (7.35). As a result of metabolism (substance exchange), many acids are formed in the body in two forms: volatile (carbon) and non-volatile (fixed). Volatile carbonic acids formed during cell metabolism, they are formed and are released from the cells in the form of H^+ ions, which hemoglobin binds and carries to the lungs. In it, hemoglobin releases H^+ ions, they combine with bicarbonates to form carbon dioxide, which is released during breathing. As a result of the metabolism of proteins and other acid-forming products, non-volatile acids such as sulfuric and phosphoric acids are formed and if they are not constantly neutralized and released, the pH value of the blood would drop to pH 2.7 – the high concentration of these decay products causes poisoning of the central nervous system, cirrhosis of the liver, decompensation of heart activity, insufficient supply of oxygen during breathing, and others. (Aschbach 2014). A lack of oxygen also leads to acidosis, because in this case the oxidation is incomplete, the body cannot remove the under-oxidized products from the reactions. Acidosis, as a disease state of the body, changes the biochemical properties of the blood, leads to a change in the blood flow rate and causes the aggregation of the erythrocytes. The increased load on the hemoglobin buffer of the blood causes a change in the properties and characteristics of the blood, a slowing of the blood flow, an increase in the aggregation of the erythrocytes and a decrease in the supply of tissues with oxygen. It leads to the fact that the body does not receive enough nutrients, vitamins, oxygen, slags are not removed from the cells. In Germany, studies by Dr. Irlacher show the influence of catholyte on the properties of the blood and the treatment with reduced water showed that the use of the catholyte prevents the blood from thickening. (www.dr-ada-fischer.de/dunkel.html; Aschbach, 2014).

The theory of the oxidation (acidification) of the organism explains many of the causes of and complications of many diseases (especially their chronicity). The danger of acidic soils (result of

acid rain, because of intensive industrial and economic activity globally) is that the main elements of plant nutrition (nitrogen, phosphorus, potassium) become unavailable to them. At the expense of this, the ions of manganese, iron, aluminum, heavy metals, radionuclides consumed by plants in large quantities are available, for this reason it becomes impossible for plants to provide the necessary minerals, nutrients, flavonoids, vitamins. What plants do not receive, animals also do not receive, and twice as much – man.

Conclusion

Results can neither confirm nor deny possible wellness effects of catholyte. The values of ASAT, ALAT, AP in the experimental group were in referent values like these of the ducks in the control group, so it can be concluded that catholyte have no toxic effect for the liver and kidneys. The results of the biochemical parameters that catholyte had no negative effects on the organism of ducks and it can be safely used under experimental as well as productive conditions.

Acknowledgments

The present study was funded by the University of Forestry because of the implementation of Contract № NIC-B-1004/ 26.03.2019 for a scientific project "Treatment effect of electrochemically activated water solutions on selection parameters and health status in farmed birds and rabbits".

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