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Dietary supplementation with algae and polyphenols in male rabbits: effects on semen quality traits

Introduction

In animal production, poor reproductive performance currently affects livestock productivity. Several efforts toward overcoming this challenge (poor reproductive performance) have culminated in identifying oxidative stress as the main reason because animals' productivity is impaired either directly or indirectly by different unfavourable conditions (Rahal et al., 2014). Oxidative stress has been widely reported as the mechanism behind many pathological development and disease conditions, including reproductive inefficiency (Sikiru et al., 2018). In rabbit production, this aspect is a very important parameter for reproductive performance evaluation, because it determines profitability as well as products yield, and it is one of the major factor determining enterprise productivity and production objectives. In male organisms, pro-oxidant condition affects both seminal qualities and reproductive functions of the spermatozoa. High levels of reactive oxygen substances are also reported to induce oxidative damage of DNA in the sperm plasma membrane, mitochondrion, and nuclear genome. The danger in this damage is not associated with poor reproductive performance alone, but also with cancer development and inherited infertility in the off-springs (Aitken, Krausz, 2001). In recent years, many studies have been focused on natural substances that can affect the health of animals and challenge the new animal welfare prospective.

The present research is based on the claim that exogenous supplementation of antioxidants is a proven tool of reducing oxidative stress associated with reproduc-

tive performance and the need to discover different sources of antioxidants capable of improving reproductive activities. A mix of different extracts was used as a dietary supplement, and it consists exclusively of natural products. Its main components are polyphenols from terrestrial and marine origins and plant polysaccharides. The effect of this supplement on reproduction has not been reviewed in the past, and that is a reason why we decided to test its effect on the reproductive potential of male rabbits.

The aim of the present study is to determine effects of natural mix extracts during a 120-day *in vivo* experiment on selected reproductive traits of male rabbits.

Material and methods

Animals and experimental design

The trial lasted 120 days and was conducted in the Animal Production Research Centre in Nitra, Slovak Republic, on 14 adult New Zealand White rabbit bucks. All experimental procedures and the management of animals were conducted in accordance with European Community guidelines n. 86/609/EEC regarding the protection of animals for experimental purposes. The tested animals (aged 15 ± 3 months) were divided into three homogeneous groups, and the body weights were recorded at the beginning and the end of experiment: the control (CON; n = 5) was fed with commercial feed, the first experimental group (T1; n = 4) received a 0.3% feed additive mix, and the second experimental group (T2; n = 5) received a 0.6% feed additive mix. The mix extract supplement, containing mainly polyphenols from algae and chestnut tannin extracts, was analysed using HPLC-DAD according to Russo et al. (2017) and the following natural compounds were determined as the most prominent: neochlorogenic acid, elaigic acid, syringic acid, cynaroside, and rutin. The natural extract was produced and provided by Lombarda Trading SRL (Casale Belvedere, Cremona, Italy). The ingredients and chemical composition of diets are reported in table 1.

The semen samples were collected on day 0 (basal), and days 30, 60, 90, and 120 of the feeding period with the help of an artificial vagina. The obtained semen samples were diluted with physiological solution in the ratio 1:5. After processing, the samples were incubated at the temperature of 37°C and were analysed immediately in triplicate. Each of prepared samples was evaluated using a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with a microscope (Olympus BX 51, Japan) to assess the spermatozoa motility (Massanyi et al., 2008). Each sample was placed into Makler Counting Chamber (depth 10 μm, Sefi–Medical Instruments, Germany). Using the rabbit specific set up, the following parameters were evaluated: spermatozoa concentration (CONC, 10^6/mL), total motile spermatozoa (%, motility > 5 μm/s), and progressive motile spermatozoa (%, motility > 20 μm/s).

Tab. 1. Ingredients and chemical composition of the diets [g/kg]

Ingredients	Experimental diet		
	CON	T1	T2
Maize	282	281	280
Alfalfa hay	305	305	305
Sunflower meal	135	135	135
Palm seed oil	8	8	8
Soybean oil	7	7	7
Wheat	80	80	80
Cane molasses	20	20	20
Carob bean meal	90	90	90
Oat	53	53	53
Calcium carbonate	7	7	7
Sodium Chloride	3	3	3
Dicalcium phosphate	2	2	2
Methionine (99%)	2.5	2.5	2.5
Lysine (78.5%)	1.6	1.6	1.6
Choline (75%)	1.4	1.4	1.4
Vitamin and mineral premix*	2.5	2.5	2.5
Experimental supplement**	0.00	3	6
Chemical composition***			
Crude protein	184.0	183.6	183.5
Ether extract	35.7	35.5	35.5
Crude fibre	187.0	186.8	187.0
Ash	86.0	85.7	85.8
Nitrogen free extract	507.0	507.1	506.9
NDF	302.1	301.5	301.7
ADF	195.8	195.4	195.3
ADL	39.9	39.5	39.5

Notes: *Supplied per kg diet: 13.500 I.U. vitamin A (trans-retinyl acetate); 800 I.U. vitamin D3 (cholecalciferol); 35 mg vitamin E (α-tocopherol min 91%), 35 mg copper (cupric sulphate pentahydrate), 150 mg aminoside sulphate; ** quantities of plant extract, T1 – experimental group fed 0.3% of natural mix supplement and T2 – experimental group fed 0.6% of natural mix supplement; *** analyses determined in triplicate

The superoxide dismutase levels (SOD), which catalyses the dismutation of superoxide radical reaction in hydrogen peroxide and molecular oxygen, together with glutathione peroxidase (GPx) determination, were determined using a commercial colorimetric kit-assay provided by Randox (Randox Laboratories Ltd., United Kingdom). SOD activity was expressed in units per milligram of protein [U/mg], and GPx activity was expressed in units per gram of protein [U/g]. The ferric reducing antioxidant power (FRAP) test, developed by Benzie and Strain (1996), measures the antioxidant capacity of plasma and is used to assess the ability to reduce the ferric iron complex in an acidic environment. One unit FRAP is expressed in mmol/ml and

indicates the number of moles of ferric ion (Fe^{3+}) reduced to ferrous ion (Fe^{2+}) from one mol of tested antioxidants.

Statistical analysis

Obtained data was statistically analysed with the help of the PC program Excel and a commercially available statistics package SAS 8.0 (SAS Institute Inc., USA) using Student's t-test and Scheffe's test. Statistical significance was indicated by p values of less than 0.05, 0.01, and 0.001.

Tab. 2. Semen characteristics (x ± SD) of control (CON, n = 5) and experimental (T1, n = 4; T2, n = 5) buck rabbits

Items	Dietary treatment[1]			
	CON	T1	T2	P-value[2]
	Concentration [$10^6 \times ml^{-1}$]			
Basal	0.656±0.270	0.695±0.428	0.728±0.532	n.s.
30d	0.638±0.280	0.635±0.602	0.607±0.490	n.s.
60d	0.647±0.1754	0.699±0.345	0.871±0.387	n.s.
90d	0.640±0.245	0.538±0.307	0.586±0.461	n.s.
120d	0.663±0.251	0.685±0.123	0.609±0.213	n.s.
	Motility [%]			
Basal	79.640±2.240	81.630±2.760	82.959±4.040	n.s.
30d	84.760±2.694	85.840±9.272	77.700±12.740	n.s.
60d	85.090±7.526	87.210±8.245	89.380±8.023	n.s.
90d	82.840±4.511	87.630±4.567	77.330±12.710	n.s.
120d	92.100±4.209	88.670±5.257	85.680±7.959	n.s.
	Progressive motility [%]			
Basal	66.440±3.130	66.530±13.690	62.490±12.940	n.s.
30d	74.510±4.948	69.940±18.980	62.420±14.260	n.s.
60d	74.280±12.600	79.070±13.890	81.280±11.370	n.s.
90d	70.890±4.401	77.160±4.913	64.390±18.440	n.s.
120d	84.610±6.610	82.040±6.592	76.020±14.470	n.s.

Note: [1](CON) – Control group fed with commercial feed; T1 – experimental group fed 0.3% of natural mix supplement; T2 – experimental group fed 0.6% of natural mix supplement; [2]p – value: n.s. = not significant

Results and discussion

The dietary supplementation with the natural extracts mix did not cause any changes in the animal body weights, and it did not induce any evident clinical signs in rabbits over the 120-days of the experimental period. The concentration of spermatozoa was not significantly different between experimental groups and the control group after 4 months of dietary treatment (Tab. 1). Mourvaki et al. (2010) also found no effect with use of flaxseed dietary supplementation on the volume and spermatozoa concen-

tration in rabbit. However, Okab et al. (2013), feeding rabbit bucks with dried seaweed (2%), showed a significant decrease in spermatozoa concentration, the percentage of live spermatozoa, and ejaculate volume. The spermatozoa motility parameters (motility and progressive motility; Tab. 1) were not significantly different between the control group and experimental group with natural extracts mix supplementation. Controversially, Yousef et al. (2003) observed an improvement of spermatozoa motility parameters after the dietary supplementation of ascorbic acid and vitamin E, alone and in combination, in male rabbits. Since a lack of effects has been observed in our experimental study, further research is needed in order to test different doses of natural extracts mix supplements.

Tab. 3. Antioxidant seminal plasma markers (x ± SD) of control (CON, n = 5) and experimental (T1, n = 4; T2, n = 5) buck rabbits

Items	Dietary treatment[1]			
	CON	T1	T2	p–value[2]
	SOD [U × mg^{-1} TP]			
Basal	0.408±0.085	0.502±0.117	0.477±0.091	n.s.
30d	0.422±0.097	0.664±0.575	0.353±0.059	n.s.
60d	0.296±0.194	0.443±0.268	0.346±0.150	n.s.
90d	0.381±0.123	0.296±0.102	0.421±0.088	n.s.
120d	0.264±0.114	0.429±0.240	0.311±0.094	n.s.
	GPx [U × g^{-1} TP]			
Basal	42.517±10.225	40.120±0.311	39.370±2.221	n.s.
30d	44.630±15.370	36.330±0.476	30.370±9.601	n.s.
60d	23.280±12.330	41.760±28.010	35.790±16.640	n.s.
90d	32.960±12.300	32.750±14.000	44.420±15.620	n.s.
120d	26.600±11.250[1]	65.580±19.310[2]	30.530±4.525[1]	**
	FRAP [µmol Fe2+ × g^{-1} TP]			
Basal	85.125±29.119	93.225±17.455	89.541±7.853	n.s.
30d	97.420±48.960	112.800±18.400	60.600±12.100	n.s.
60d	75.590±54.340	106.300±64.980	69.750±26.350	n.s.
90d	59.240±15.330	73.330±15.540	68.020±28.520	n.s.
120d	69.440±18.510[1]	103.000±36.100[2]	67.110±7.527[1]	*

Note: [1](CON) – Control group fed with commercial feed; T1 – experimental group fed 0.3% of natural mix supplement; T2 – experimental group fed 0.6% of natural mix supplement; [2] p–value: n.s. = not significant; * ($p < 0.05$); ** ($p < 0.01$)

Tables 2–3 contain data regarding the tested oxidative markers in seminal plasma. At the end of the dietary treatment (after 120 days), all three parameters (SOD, GPx and FRAP) were positively altered, although the statistical significance was reached only for GPx ($p < 0.01$) and FRAP ($p < 0.05$) values. In fact, group T1 data showed the highest content of the two parameters when compared with group CON. In literature, it has also been reported that SOD activity survey in seminal plasma could be a useful tool

for determining sperm fertilization potential and could improve the diagnosis of male infertility (Shiva et al., 2011). In general, antioxidant dietary supplementation leads to an improvement of the antioxidant markers profile in the seminal plasma, in particular, when algae-based feed additive is supplemented in the diet (Murphy et al., 2017).

Conclusion

Since oxidative stress is considered a biochemical process negatively affecting reproduction, the reduction of its effects is highly important for the promotion of animal welfare. The use of polyphenols and tannins in rabbit diets is a source of natural antioxidants, and we can conclude that supplementation of 0.3% of natural mix did not significantly negatively affect any of the studied reproductive parameters of male rabbits, but we have found some improvement in several antioxidant parameters.

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Abstract

In recent years, many studies have been focused on natural substances that can affect the health of animals. A mix of different extracts was used as a dietary supplement, and it consisted exclusively of natural products. Its main components were polyphenols from terrestrial and marine origins and plant polysaccharides. The effect of this supplement on reproduction has not been reviewed in the past, which is why its effect on the reproduction potential of male rabbits was tested. The aim of the present study is to determine the effects of the natural mix during a 120-day *in vivo* experiment on selected reproductive traits of male rabbits. Natural mix was supplemented in two different concentrations (T1 – 0.3% and T2 – 0.6%) with the basal ingredients of the conventional rabbit feed in pellet form. In our experiments, emphasis was placed on both the spermatozoa concentration and its motility parameters as well as on the properties of seminal plasma and antioxidant activity. The dietary supplementation with the natural extracts mix positively altered the quality traits of rabbit spermatozoa, but these changes were statistically not significant. In experimental group T1, a significant increase of GPx and FRAP content, both regarding the antioxidant markers profile in seminal plasma, was recorded. We can conclude that the supplementation of 0.3% of natural mix did not significantly negatively affect any of the studied reproductive parameters of male rabbits, but some improvement in several antioxidant parameters was found.

Key words: extract, rabbit, spermatozoa, mobility, seminal plasma, antioxidants

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Suplementacja diety algami i polifenolami u samca królika: wpływ na cechy jakościowe nasienia

Streszczenie

W ostatnich latach wiele badań dotyczy substancji naturalnych, które mogą wpływać na zdrowie zwierząt. Jako suplement diety wykorzystano mieszankę różnych ekstraktów, składającą się wyłącznie z naturalnych produktów. Jej głównymi składnikami były polifenole pochodzenia lądowego i morskiego oraz polisacharydy roślinne. Oddziaływanie tych suplementów na reprodukcję nie zostało w przeszłości poddane analizie, co było powodem dla którego zbadano ich wpływ na potencjał reprodukcyjny samców królików. Celem niniejszego badania było określenie wpływu naturalnej mieszanki podawanej podczas 120-dniowego eksperymentu *in vivo* na wybrane cechy reprodukcyjne samców królika. Naturalną mieszankę w dwóch różnych stężeniach (T1 – 0,3% i T2 – 0,6%) uzupełniono podstawowymi składnikami tradycyjnego pokarmu dla królików w postaci śrutu. W doświadczeniach nacisk położono, zarówno na koncentrację plemników, jak i na ich parametry ruchowe, a także na właściwości plazmy nasiennej oraz aktywność przeciwutleniającą. Suplementacja diety mieszaniną naturalnych ekstraktów pozytywnie zmieniła cechy jakościowe plemników królika, ale zmiany te nie były statystycznie istotne. W grupie doświadczalnej T1 odnotowano istotny wzrost zawartości, zarówno GPx, jak i FRAP, pod względem profilu markerów antyoksydacyjnych w plazmie nasienia. Można zatem stwierdzić, że suplementacja 0,3% naturalnej mieszanki nie wpłynęła znacząco negatywnie na żaden z badanych parametrów reprodukcyjnych samców królików, a nawet odnotowano tu pewną poprawę kilku parametrów antyoksydacyjnych.

Słowa kluczowe: ekstrakt, królik, plemniki, ruchliwość, plazma nasienna, przeciwutleniacze

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