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Herbal yeast product, Equi-Strath®, alters the antioxidant status of stallion semen

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ABSTRACT

Effects of a plasmolysed yeast product enriched with herbs, malt, honey and orange syrup on semen characteristics and oxidative status in stallions were evaluated. Twenty stallions (mean age ± standard deviation = 9.5 ± 4.5 years) were randomly divided into a treatment group (n = 10) receiving 0.06 mL/kg bodyweight of plasmolysed herbal yeast, and a control group (n = 10) receiving the same amount of placebo daily in the feed for 10 weeks. Ejaculates were collected weekly from all stallions starting at Week 0. Volume, sperm concentration, motility, and velocity were evaluated immediately, 24 and 48 h after cooled storage at 5 °C. At the two storage time points, membrane lipid peroxidation was determined using the BODIPY-C_{11}. Additionally, blood samples were collected at Weeks 0, 1, 5 and 9, and analysed for antioxidant status, consisting of superoxide dismutase, cholesterol, thiobarbituric acid reactive substances, and non-esterified fatty acids. Due to the nature of the data, the Mann-Whitney U test was applied as preliminary analysis. The BODIPY-C_{11} in the semen was less at 24 h and greater at 48 h after collections in Week 1 to 3 ($P < 0.01$) and Week 1 to 10 ($P < 0.05$) compared with Week 0 in the treatment compared to control group. There were no significant differences between groups for all values for other seminal and blood variables evaluated. In conclusion, feed supplementation with plasmolysed herbal yeast temporarily improved the antioxidant status of stallion semen, which might be of benefit for preservation of cooled semen.

1. Introduction

Oxidative stress because of spontaneous lipid peroxidation is one of the most important factors affecting the longevity of sperm and is known to reduce sperm motility (reviewed by Ball, 2008). Lipid peroxidation of sperm is thought to have effects during the fertilizing process. Mammalian spermatozoa are susceptible to peroxidative damage due to the relatively greater content of polyunsaturated fatty acids in the cell membranes than is present in membranes of most animal cells (Aitken, 1997). Production of reactive oxygen species, in small amounts, is physiological in semen and the reactive oxygen species function in capacitation and the acrosome reaction (de Lamirande and Gagnon, 1993; Aitken et al., 1995, 1998), whereas large amounts of free radicals that exceed

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the sperm antioxidant defence mechanisms, damage the sperm plasma membrane and sperm DNA, potentially initiating apoptotic-like changes (Ball, 2008). Results of some studies in humans indicate there is an association of oxidative damage of sperm with decreased male fertility (Tremellen, 2008).

Results of several studies in mammals (*e.g.*, gilts: Marin-Guzman et al., 1997; boars: Audet et al., 2004; rabbits: Yousef et al., 2003) and humans (*e.g.*, Hunt et al., 1992) indicate the possibility of reduction of oxidative damage with a food supplement containing anti-oxidative substances including polyunsaturated fatty acids (PUFA). In horses, anti-oxidant supplements had an effect on fresh-chilled (Brinsko et al., 2005; Elhordoy et al., 2008; Contri et al., 2011; Schmid-Lausigk and Aurich, 2014) and frozen-thawed semen quality (Elhordoy et al., 2008; Rodrigues et al., 2017), while there was not such an effect in a study of Deichsel et al. (2008) and Grady et al. (2009).

In the present study, a food supplement comprised of plasmolysed herbal yeast, malt, honey and orange syrup was evaluated. Herbal yeast plasmolysate, containing a variety of over 60 important substances, had positive effects on the innate and adaptive immune system in mice (Leslie et al., 1988, 1989) in results of studies focused on the cellular immunological fraction indicating there was an enhanced function of helper/inducer T-lymphocytes, natural killer cells and B-lymphocytes. In addition, daily administration of the product had stress protectant effects in mice and humans (Leslie et al., 1988; Joller, 1988; Joller and Aeppli, 1989; Schwarzenberg et al., 2000). Leslie et al. (1988) postulated that these protectant effects are associated with an unidentified nutritional factor, and based on the report of Joller (1988) hypothesized that this could be regulated by the adaptive immune system.

In horses in a parallel study, however, van Dorland et al. (2018) reported there was no increase of antibody production after vaccination in response to H3N8 influenza vaccination in Equi-Strath®-supplemented animals. Nonetheless, results of recent *in vitro* studies indicated yeast had antioxidative properties (Vargas-Ochoa et al., 2016) when there was assessment of mitochondrial glutathione in thiol-redox regulation (Gostimskaya and Grant, 2016), and measurement of the total antioxidant activity of *Saccharomyces cerevisiae* by analysing 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) during yeast cultivation (Lavová and Urminská, 2013). The results of these studies confirm the suitability of yeast as a nutrition supplement.

In the current study, there was testing of the hypothesis that oral application of Equi-Strath®, a product containing herbal yeast plasmolysate, to healthy Franches-Montagnes stallions would increase the seminal and blood antioxidative status of stallions.

2. Material and methods

2.1. Animals

A total of 20 clinically healthy and sexually experienced Franches-Montagnes stallions (mean age ± 9.5 ± 4.5 years, range 4–21 years) from the Swiss National Stud in Avenches (Switzerland) were used for the experiments. The stallions were housed in individual stalls bedded with straw or wood chips. Feeding consisted of hay or haylage and concentrate (oats, barley, corn and pellets) with feeding occurring three times daily. Water and a mineral salt block were available *ad libitum*. All stallions were regularly exercised and had daily access to a paddock. The experiments were approved by the local animal ethics committee (*Etat de Vaud, Service Vétérinaire*; permit no. 2667.1).

2.2. Experimental design

The stallions were randomly divided into two groups using dicing. The Equi-Strath® was administered to the treatment group (group TG: n = 10; mean age ± standard deviation: 8.6 ± 4.9 years), and an oral placebo was administered to the control group (group CG: n = 10; 10.5 ± 4.1 years). The experiment started in the autumn (October). Before the beginning of the study, extra-gonadal semen reserves were standardized through 7 days of daily semen collection starting before the first experimental collection. After that, ejaculates were collected weekly every Tuesday from all stallions in the study, starting at Week 0. The supplement and placebo were administered daily from Week 1 until 10 (end of the experiment). In addition, blood samples were collected during Weeks 0, 1, 5 and 9, respectively, for analysis of antioxidant status.

2.3. Food supplementation

A plasmolysed herbal yeast food supplement containing 83% plasmolysed herbal yeast (plasmolysate of *Saccharomyces cerevisiae*), 9% malt extract, 3% honey, 5% orange syrup (Equi-Strath® for horses, from Bio-Strath AG, Zürich, Switzerland) was administered. The placebo was specifically produced for the present study with the placebo requirements matching Equi-Strath® as much as could possibly occur in taste, colour and consistency. The placebo consisted of lecithin (0.50%), xanthan gum (0.55%), caramel (0.26%), potassium sorbate (0.12%) and sodium benzoate (0.13%) diluted in water.

Stallions in Group TG were supplemented with 0.06 mL/kg BW of Equi-Strath® daily, between 8:00 and 9:00 o'clock, using an oral syringe. Stallions of Group CG were supplemented daily with the same amount of a placebo using an identical application procedure. The feeding staff were unaware of the group assignments of the stallions.

2.4. Semen collection and evaluation

2.4.1. Semen collection

The semen collection occurred in a building that was separated from the housing stables. A "dummy" mare was used for the stallions to mount to collect the semen in an artificial vagina (type Avenches, National Stud Avenches, Switzerland). The semen collection procedure was always performed in the same way by the same collecting person and stallion handler with the same chronological order of the stallions. Both persons were naïve in respect to the group assignments of the stallions.

2.4.2. Volume, concentration and computer assisted sperm analysis (CASA)

The volume of the ejaculate was measured after removal of the gel fraction. Concentration of the raw semen was then evaluated with the Nucleocounter® SP-100™ system (ChemoMetec A/S, Allerød, Denmark). Total sperm count was calculated as the volume multiplied by the concentration. Semen was then diluted with INRA 96™ (IMV technologies, L'Aîgle, France) to a concentration of 30×10^6 spermatozoa/mL. Diluted semen was placed in a standardized, pre-warmed 20 µm standard count analysis chamber (Standard Count Analysis Chambers SC 20-01-C, Leja, Nieuw-Vennep, The Netherlands) and assessed for total motility and progressive motility of sperm cells in ten fields with a computer assisted sperm analyser (HTM-IVOS, version 12.1, Hamilton Thorne Biosciences, Beverly, MA, USA). In addition, three measures of sperm velocity were determined which were the straight-line velocity (VSL; *i.e.*, the mean distance between the sperm heads' first detected positions to the last), the curvilinear velocity (VCL; *i.e.*, the curvilinear path the sperm heads took), and the average-path velocity (VAP; *i.e.*, the smoothed paths the sperm heads took during an observational period) were determined using procedures described by Burger et al. (2015a, 2015b). Sperm with straightness ≥70% and VAP ≥ 50 µm/s were considered progressively motile. An aliquot of fresh diluted semen was then cooled and stored at 5 °C, using an Equitainer™ (Hamilton Thorne Biosciences, Beverly, MA, USA), and sent to the Clinic for Reproductive Medicine in Zurich, Switzerland, for analysis 24 and 48 h later (after incubation for 15 min at 38 °C each) of total motility, progressive sperm motility, VSL, VCL and VAP.

2.4.3. Flow cytometry analyses

Assessments using flow cytometry 24 and 48 h later were performed using a Call Lab Quanta SC MPL flow cytometer operated using the Cell Lab Quanta SC Software for instrument Control Data Acquisition (Beckman Coulter Inc., Nyon, Switzerland) equipped with a solid state LASER exciting at 488 nm and emission filters detecting green, orange and red fluorescence at 525, 590, and 670 nm, respectively. Applied flow rate was 500 cells/s and for each sample 10,000 events were evaluated after incubation of stained sperm for 15 min at 38 °C. The plasma membrane integrity and acrosomal status of the spermatozoa were determined after double staining with propidium iodide (PI) and peanut agglutinin conjugated with fluorescein isothiocyanate (FITC-PNA) as described in a parallel study by Wach-Gygax et al. (2017); briefly semen was diluted in 238.5 µL Tyrode's solution to a final concentration of 0.6×10^6 spermatozoa/mL; 5 µL of such diluted semen were stained by adding 1.5 µL of 2,99-mM PI (Sigma-Aldrich, Buchs, Switzerland) and 5 µL FITC-PNA (100 µg/mL) (Sigma-Aldrich Buchs, Switzerland) and then assessed using flow cytometry (percentage of membrane and acrosome intact spermatozoa, PMAI%; Nagy et al., 2003). Amount of lipid peroxidation was analysed using a combined staining with the lipophilic fluorescent 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY$^{581/591}$-C11) and PI (described by Gürler et al., 2015); 5 µL of semen were added to 2.5 µL of 1000-µM BODIPY$^{581/591}$-C11 (Thermo Fisher Scientific, Riehen, Switzerland) and 1.5 µL of 2,99-mM PI and then all mixed with 241 µL Tyrode's medium to a final concentration of 0.6×10^6 spermatozoa/mL. For further analysis, mean BODIPY$^{581/591}$-C11 fluorescence intensity of live sperm cells (PI negative) was considered. Plasma membrane intact sperm cells with a large mitochondrial membrane potential (HMMP) were determined using a combined staining with 1.5 µL PI and 2.5 µL JC-1 of 5 µL of sperm previously diluted in 241 µL of Tyrode's solution (Malama et al., 2017). The value of HMMP was defined as the percentage of PI-negative sperm cells with orange fluorescence. All examining persons were naïve in respect to the group assignment of the stallions.

2.5. Blood sample collection and determination of antioxidant status

Using jugular venepuncture, blood was collected into EDTA Vacuette® tubes (Greiner Bio-One, Stonehouse, Gloucestershire, UK) 15 min before semen collection. The samples were immediately centrifuged (4000 x *g*, 10 min) at room temperature, and plasma was decanted and frozen (−18 °C) until analysis. Plasma concentrations of total cholesterol, thiobarbituric acid reactive substances (TBARS), non-esterified fatty acids (NEFA), and superoxide dismutase (SOD) activity were analysed enzymatically by using commercial kits on a Cobas Mira analyser (Hoffmann-La Roche, Basel, Switzerland). Kit no. 61,219 (bioMérieux SA, Geneva, Switzerland) was used for total cholesterol analysis, Kit no. FA 115 (Randox Laboratories Ltd., Schwyz, Switzerland) for NEFA determination, and the Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA) was used for measurement of SOD concentration. Furthermore, the TBARS were determined quantitatively through a plate-based colorimetric measurement (530–540 nm) using the TBARS Assay Kit (Cayman Chemical Company).

2.6. Statistical analysis

Data were evaluated for normal distribution, followed by transformation of data where applicable and finally applying a linear mixed-effects models procedure, including treatment (TG or CG), time, and the interaction as fixed effects, and stallion as a repeated subject. Due to there being significant interactions, the overall comparison of the treatment modalities became meaningless, and a different statistical analysis was adopted. The Mann-Whitney U test, a non-parametric test used for comparison of two sample means, was ultimately used and the results considered as part of a preliminary investigation.

To account for pre-existing differences between individual horses, baseline values (Week 0) of each variable were subtracted from values measured during treatment (Weeks 1 to 10), so that data represented changes from baseline. Differences between groups were

Table 1

Data for semen variables (means ± SE) of ejaculates collected from ten stallions supplemented with Equi-Strath® (treatment group TG) and ten stallions supplemented with a placebo (control group CG) comparing the values during the first 3 weeks, and during the entire 10 week study period of supplementation with basal concentrations before supplementation; Differences are indicated with $^{+}$($P <$ 0.10), *(P < 0.05), and **($P <$ 0.01).

	Before supplementation		Weeks 1 - 3			Weeks 1 - 10		
Semen variable	TG	CG	TG	CG	*P* value	TG	CG	*P* value
Volume of ejaculate (ml)	25.4 ± 4.2	27.9 ± 4.2	−1.9 ± 1.4	1.5 ± 4.5	0.472	−2.1 ± 1.7	0.3 ± 2.4	0.315
Sperm number per ml (x 10^6)	578.9 ± 66.7	536.8 ± 81.6	−30.4 ± 27.5	−101.8 ± 54.7	0.353	−22.1 ± 24.8	−69.6 ± 38.7	0.353
Total sperm cells (x 10^9)	13.39 ± 1.98	14.11 ± 2.62	−2.19 ± 0.83	−2.38 ± 1.42	0.739	−2.21 ± 0.85	−2.26 ± 1.46	0.971
Total motility (%)	77.0 ± 3.5	81.1 ± 2.0	−2.4 ± 2.2	2.4 ± 0.9	0.063^{+}	−1.3 ± 1.9	3.0 ± 0.7	0.070^{+}
Total motility after 24 h (%)	64.7 ± 4.8	71.7 ± 1.8	2.6 ± 0.6	1.9 ± 1.3	1.000	3.7 ± 1.7	1.8 ± 1.3	0.595
Total motility after 48 h (%)	59.5 ± 5.0	68.6 ± 1.9	−2.4 ± 1.2	2.2 ± 1.4	0.051^{+}	0.6 ± 2.0	0.8 ± 0.5	0.740
Progressive motility (%)	47.8 ± 5.4	54.0 ± 3.4	2.1 ± 1.5	7.2 ± 1.1	0.057^{+}	5.2 ± 1.7	9.2 ± 1.4	0.161
Progressive moti-lity after 24 h (%)	37.9 ± 5.6	50.7 ± 1.6	2.3 ± 2.1	1.3 ± 3.3	0.962	4.5 ± 2.4	0.4 ± 2.2	0.660
Progressive moti-lity after 48 h (%)	37.5 ± 4.9	48.0 ± 2.3	−0.7 ± 1.8	4.3 ± 2.7	0.221	3.4 ± 2.4	1.3 ± 1.9	0.601
VAP (μm/s)	126.9 ± 5.0	122.9 ± 7.6	1.4 ± 1.8	2.9 ± 3.5	1.000	4.2 ± 2.6	3.1 ± 3.7	0.549
VAP after 24 h (μm/s)	85.3 ± 6.1	84.8 ± 5.5	2.1 ± 1.8	−0.8 ± 3.3	0.595	2.4 ± 1.8	0.3 ± 2.6	0.661
VAP after 48 h (μm/s)	90.5 ± 6.1	87.6 ± 6.0	0.4 ± 2.1	2.8 ± 3.0	0.315	−0.04 ± 2.5	1.9 ± 2.6	0.604
VSL (μm/s)	99.9 ± 4.0	96.7 ± 7.2	−0.1 ± 1.6	0.7 ± 3.1	0.905	2.5 ± 2.1	2.7 ± 3.3	0.780
VSL after 24 h (μm/s)	69.5 ± 5.2	68.4 ± 4.7	2.1 ± 1.3	−0.8 ± 2.7	0.315	2.2 ± 1.2	0.8 ± 2.0	0.905
VSL after 48 h (μm/s)	74.4 ± 5.4	70.9 ± 4.9	1.1 ± 1.8	2.7 ± 2.5	0.497	2.7 ± 1.6	2.7 ± 2.0	0.968
VCL (μm/s)	218.4 ± 12.0	211.3 ± 10.5	9.3 ± 5.2	7.8 ± 7.0	1.000	15.7 ± 6.8	7.9 ± 6.6	0.243
VCL after 24 h (μm/s)	167.2 ± 10.1	167.3 ± 9.1	2.3 ± 3.3	−1.2 ± 4.7	0.842	2.5 ± 3.3	−0.6 ± 3.7	0.661
VCL after 48 h (μm/s)	177.3 ± 10.3	171.7 ± 10.6	−1.3 ± 3.3	4.0 ± 4.1	0.315	−11.9 ± 3.6	0.8 ± 4.0	0.095^{+}
PMAI after 24 h (%)	73.8 ± 2.5	80.6 ± 1.6	−1.6 ± 2.2	−0.4 ± 0.8	0.408	−0.6 ± 2.2	−0.8 ± 0.9	1.000
PMAI after 48 h (%)	72.4 ± 2.9	78.7 ± 1.4	−3.4 ± 1.9	0.2 ± 1.3	0.351	−1.1 ± 2.2	−0.8 ± 1.3	0.758
HMMP after 24 h (%)	95.3 ± 0.5	96.3 ± 0.5	−4.3 ± 1.3	−2.0 ± 0.7	0.146	−4.3 ± 0.9	−3.5 ± 0.7	0.633
HMMP after 48 h (%)	94.6 ± 0.6	95.3 ± 0.8	−6.3 ± 1.7	−3.6 ± 1.0	0.203	−6.7 ± 1.3	−4.5 ± 0.5	0.237
BODIPY after 24 h (channels)	117.7 ± 4.7	105.9 ± 1.8	−6.9 ± 4.0	7.4 ± 2.9	0.008**	−10.4 ± 3.7	0.8 ± 2.5	0.022*
BODIPY after 48 h (channels)	152.4 ± 4.7	146.2 ± 9.4	6.7 ± 2.9	−25.5 ± 6.7	0.002**	2.9 ± 3.3	−12.9 ± 5.7	0.023*

Abbreviations: VAP average-path velocity; VSL straight-line velocity; VCL curvilinear velocity; PMAI plasma membrane and acrosomal intact sperma; HMMP sperm with a high mitochondrial membrane potential.

assessed based on the mean change from baseline in Weeks 1 to 3 (short-term, “adaptation”) and 1 to 10 (long-term). Given the exploratory nature of the present study, *P* - values were not adjusted for multiple testing. Differences were considered significant if $P <$ 0.05 and as a trend if $P <$ 0.10.

3. Results

Data are reported in Table 1 for semen values and there is also reporting of corresponding *P* - values obtained from using statistical tests comparing the values at the start of the experiment to those from Weeks 1 to 3 and Weeks 1 to 10 of supplementation, respectively.

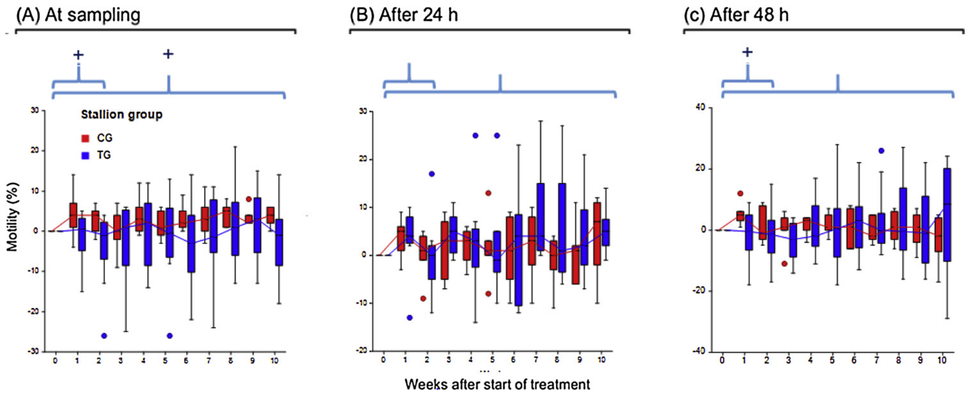

Fig. 1. Changes in semen motility (%) of stallions fed Equi-Strath® (TG; start = Week 0) compared to ten stallions supplemented with a placebo (CG) immediately after sperm collection (A), and after 24 h (B) and 48 h (C) of cooling at 5 °C; Differences between groups TG and CG for the first 3 weeks and during the entire study period of 10 weeks are indicated with $^{+}(P < 0.10)$, $*(P < 0.05)$, and $**(P < 0.01)$.

3.1. Volume, concentration and CASA semen variables

Total semen motility tended to be less in the treatment group compared to the control group at collection time (Weeks 1–3 compared with Week 0: $P = 0.063$; Weeks 1–10 compared with Week 0: $P = 0.070$) and 48 h (Weeks 1–3 compared with Week 0: $P = 0.051$) after cooled storage. Variations of total semen motility (at sampling and 24 and 48 h later) for the groups TG and CG during the study period are depicted in Fig. 1. Progressive motility tended to be greater in the treatment than control group 24 h after collection time (Weeks 1–3 compared with Week 0: $P = 0.057$). Regarding volume, concentration, total sperm count, VSL, VCL, and VAP there were no tendencies nor significant differences between the groups at all sampling time points.

3.2. Flow cytometry semen variables

Lipid peroxidation (BODIPY-C_{11}) in the TG was less after 24 h (Weeks 1–3 compared with Week 0: $P = 0.008$; Weeks 1–10 compared with Week 0: $P = 0.022$), and increased after 48 compared with the CG (Weeks 1–3 compared with Week 0: $P = 0.002$; Weeks 1–10 compared with Week 0: $P = 0.023$). Variations during the study in lipid peroxidation (BODIPY-C_{11} after 24 and 48 h) of the TG and CG during the study period are depicted in Fig. 2. Regarding plasma membrane and acrosomal intact sperm (Fig. 3) and sperm with a large mitochondrial membrane potential, there were no significant differences between groups.

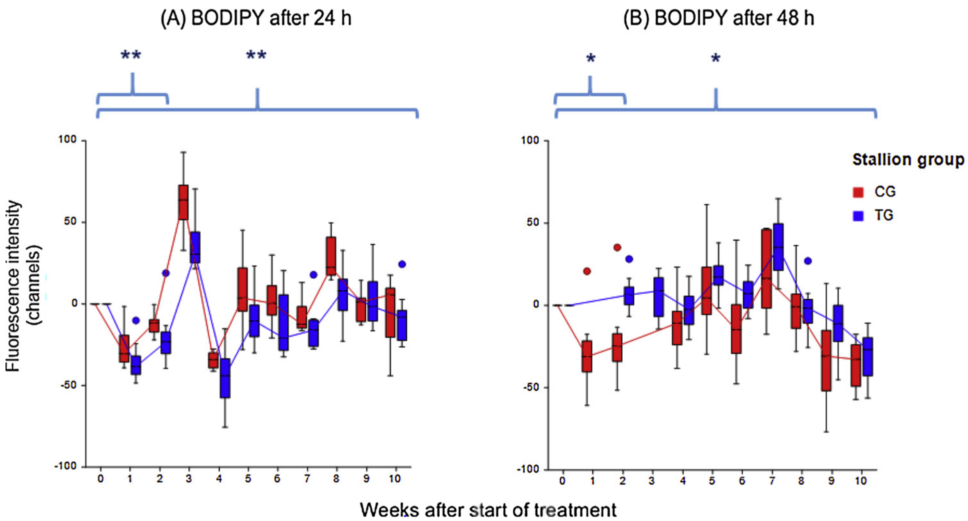

Fig. 2. Changes in lipid peroxidation (channels) of sperm after 24 h (A) and 48 h (B) of cooling at 5 °C in ten stallions fed Equi-Strath® (TG; start = Week 0) compared to ten stallions supplemented with placebo (CG); Differences between the treatment and control group for the first 3 weeks and during the entire study period of 10 weeks are indicated with $^{+}(P < 0.10)$, $*(P < 0.05)$, and $**(P < 0.01)$.

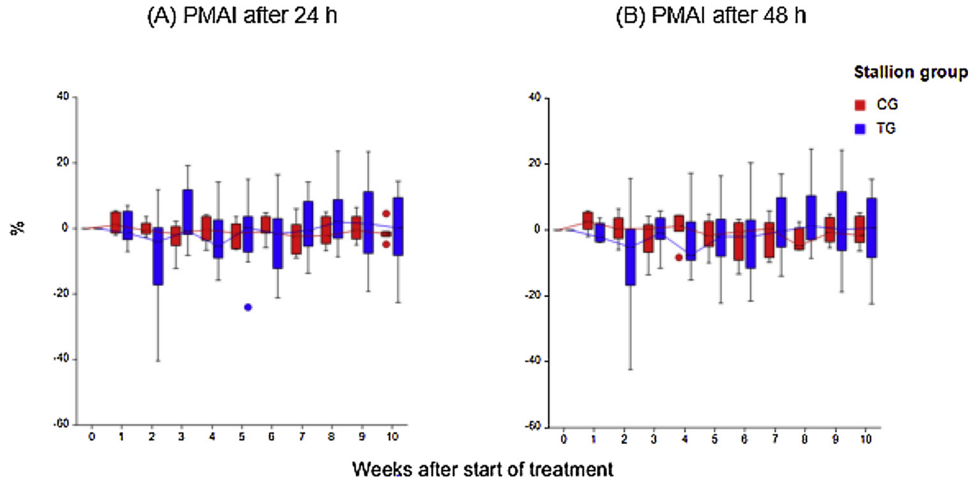

Fig. 3. Changes in plasma membrane and acrosomal intact sperma (PMAI; %) of sperm after 24 h (A) and 48 h (B) of cooling at 5 °C in ten stallions fed Equi-Strath® (TG; start = week 0) compared to ten stallions supplemented with placebo (CG); There are no significant differences between the treatment and control group.

3.3. Antioxidant status variables in the blood

There were no significant differences in the mean plasma concentrations of TBARS, SOD, total cholesterol and NEFA between the TG and CG.

4. Discussion

There were large inter- and intra-individual variabilities of antioxidant status in stallion semen with significantly lesser BODIPY-C_{11} values 24 h after collection during Equi-Strath® supplementation and greater BODIPY-C_{11} values 48 h after collection. There were no other significant differences in the semen and blood results between the TG and CG.

The analysis of BODIPY-C_{11} is an important marker for oxidative stress expressed as amount of lipid peroxidation (Aitken et al., 2014). Results of the present study are consistent with those of Gürler et al. (2015) in which there was significant inter-individual variation in values of bull ejaculate variables, but there was not intra-individual variation in these variables during the 10 weeks of the present study. In addition, Ortega Ferrusola et al. (2009) reported that in frozen-thawed stallion semen there was an increased lipid peroxidation as assessed using BODIPY-C_{11} as the marker compared to these variations in fresh semen; while there was no significant inter-individual variation of lipid peroxidation in fresh semen with variations after freezing and thawing being largely stallion-dependent. The extent of lipid peroxidation in fresh compared with frozen-thawed semen was not correlated (Ortega Ferrusola et al., 2009). Due to infrastructural reasons, there was no BODIPY-C_{11} analysis in the present study directly after semen collection.

To the best of our knowledge, however, repeated semen BODIPY-C_{11} evaluations of cooled stored semen after 24 and 48 h, as occurred in the present study, have not yet been published. It can be hypothesied that the feed supplementation with plasmolysed herbal yeast results in a decrease of lipid peroxidation after 24 h; due to increased amount of metabolically active sperm cells which was associated with a greater progressive motility of the sperm after 24 h in the treatment group, however more oxidative stress may have led to an increase of lipid peroxidation 48 h after semen collection. Indeed, it has also been speculated, that oxidative stress is more pronounced in ejaculates with greater sperm concentration due to metabolically active sperm producing more reactive oxygen species, thus, the relative amounts of antioxidants compared to the sperm number are generally less in such ejaculates (Gürler et al., 2015). The relationship of oxidative stress of spermatozoa and semen variables is not sufficiently understood. Oxidative stress is associated with a loss of sperm motility and an increase of sperm membrane and DNA damage in various species (Tremellen, 2008; Ferramosca et al., 2013; Aitken et al., 2014). In frozen-thawed semen of stallions, oxidative stress was associated with decreased intact membranes and lesser mitochondrial membrane potential (Ortega Ferrusola et al., 2009). There were no similar effects in the present study regarding sperm motility, sperm membrane integrity nor relatively greater mitochondrial membrane potential. Inconsistent with previous findings, Gibb et al. (2014) reported there was a positive association between lipid peroxidation and fertility, sperm motility and velocity, suggesting that subclinical oxidative stress might be a positive indicator of sperm viability (*e.g.*, for mitochondrial metabolism). Jeannerat et al. (2018) also reported there were positive correlations between lipid peroxidation, sperm motility and viability when bringing stallions a short time before semen collection into contact with genetically compatible mares. Whether the increasing lipid peroxidation (BODIPY-C_{11}) in the semen at 48 h after sampling of the treatment stallions in the present study represents a favourable process that should be further assessed by conducting studies involving fertility experiments.

Indeed, there are some consistent observations in the present study that support the hypothesis that there is an increased seminal antioxidant capacity in the group of supplemented stallions: while total and progressive motility of sperm of the treated stallions tended to be less after semen collection, but not after 48 h (progressive motility), and with some values for variables being inconsistent with value for fresh semen (*e.g.*, total motility). Interestingly, there was no measurable effect of the feed supplementation with plasmolysed herbal yeast on the values for the variables that were assessed in this study regarding antioxidant status in the blood. It would be worthwhile to evaluate the amounts of antioxidants directly in the seminal plasma originating from the accessory glands, similar to what has occurred in bulls in a previous study by Gürler et al. (2015) where there were significant variations between the ejaculates within the males.

Semen quality and especially motility variables do not exclusively predict a fertility prognosis, but remain important variables of semen quality assessment in commercial programs involving the use of cooled and shipped semen (Aurich, 2008). Findings in the present study, therefore, could be of interest to the equine industry. These findings also support results from other studies on dietary antioxidant supplementation with similar observations regarding sperm motility (Brinsko et al., 2005; Contri et al., 2011; Schmid-Lausigk and Aurich, 2014), but with other feeding supplements used as compared to yeast and with other semen variables other than BODIPY-C_{11} being analysed. Whether yeast products also affect freezing capacity and thawing results of stallion semen, as reported by Rodrigues et al. (2017) with dietary polyunsaturated fatty acid supplementation, remains to be further elucidated. Results of the present study are consistent with those from *in vitro* studies where yeast was reported to have antioxidative properties (Gostimskaya and Grant, 2016; Lavová and Urminská, 2013) in the human food industry.

A limitation of the present study was the use of a relatively small number of stallions. Substantial efforts, however, were made to standardize study conditions by testing healthy fertile males all of the same breed and with very similar housing and feeding conditions at one location. Also the feeding of hay or haylage to the stallions was balanced between the two groups. This represents a quite unique condition in research with horses and feeding supplementation experiments. In addition, substantial efforts were made to utilize the most stringent statistical methods in the present study. In this study, Equi-Strath® did not have a definitive overall effect on the variables that were assessed using a linear mixed-effects models procedure, and there was not a definitive accumulated effect of Equi-Strath® during the study period. Application of a more simple statistical method allowed for identification of variables that should be starting points for evaluation in subsequent studies. A repeated study design using each stallion in both groups was unfortunately not possible due to the long-term availability of animals required for this purpose. Another limitation was the lack of capacity in the present study to investigate more detailed potential underlying mechanisms that contribute to the findings (*e.g.*, specific antioxidant variables in the seminal plasma). In this respect, results of the present study are preliminary with regard to the topic investigated.

5. Conclusion

In conclusion, results from this preliminary investigation indicate a plasmolysed herbal yeast product treatment as an oral antioxidant feed supplementation temporarily affects values for semen variables, in particular BODIPY-C11. Further studies including more animals, as well as studies involving fertility data analysis (*e.g.*, benefits for the preservation of cooled stored sperm) when feeding plasmolysed-herbal-yeast supplemented in stallion diets would be of interest.

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References

Aitken, R.J., Paterson, M., Fisher, H., Buckingham, D.W., van Duin, M., 1995. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. J. Cell. Sci. 108, 2017–2025.
Aitken, R.J., 1997. Molecular mechanisms regulating human sperm function. Mol. Hum. Reprod. 3, 169–173.
Aitken, R.J., Harkiss, D., Knox, W., Paterson, M., Irvine, D.S., 1998. A novel signal transduction cascade in capacitating human spermatozoa characterised by a redox-regulated, cAMP-mediated induction of tyrosine phosphorylation. J. Cell. Sci. 111, 645–656.
Aitken, R.J., Smith, T.B., Jobling, M.S., Baker, M.A., De Iuliis, G.N., 2014. Oxidative stress and male reproductive health. Asian J. Androl. 16, 31–38.
Audet, I., Laforest, J.P., Martineau, G.P., Matte, J.J., 2004. Effect of vitamin supplements on some aspects of performance, vitamin status, and semen quality in boars. J. Anim. Sci. 82, 626–633.
Aurich, C., 2008. Recent advances in cooled-semen technology. Anim. Reprod. Sci. 107, 268–275.
Ball, B.A., 2008. Oxidative stress, osmotic stress and apoptosis: impacts on sperm function and preservation in the horse. Anim. Reprod. Sci. 107, 257–267.
Brinsko, S.P., Varner, D.D., Love, C.C., Blanchard, T.L., Mark, B.C., Wilson, E., 2005. Effect of feeding a DHA-enriched nutraceutical on the quality of fresh, cooled and frozen stallion semen. Theriogenology 63, 1519–1527.
Burger, D., Dolivo, G., Marti, E., Sieme, H., Wedekind, C., 2015a. Female major histocompatibility complex type affects male testosterone levels and sperm number in the horse (Equus caballus). Proc. R. Soc. Lond. Biol. Sci. 282, 20150407.
Burger, D., Dolivo, G., Wedekind, C., 2015b. Ejaculate characteristics depend on social environment in the horse (Equus caballus). PLoS One 10, e0143185.
Contri, A., De Amicis, I., Molinari, A., Faustini, M., Gramenzi, A., Robbe, D., Carluccio, A., 2011. Effect of dietary antioxidant supplementation on fresh semen quality in stallion. Theriogenology 75, 1319–1326.
Deichsel, K., Palm, F., Koblischke, P., Budik, S., Aurich, C., 2008. Effect of a dietary antioxidant supplementation on semen quality in pony stallions. Theriogenology 69 940-940.
De Lamirande, E., Gagnon, C., 1993. Human sperm hyperactivation and capacitation as parts of an oxidative process. Free Radic. Biol. Med. 14, 157–166.
Elhordoy, D.M., Cazales, N., Costa, G., Estévez, J., 2008. Effect of dietary supplementation with DHA on the quality of fresh, cooled and frozen stallion semen. Anim. Reprod. Sci. 107, 319–327.

Ferramosca, A., Provenzano, S.P., Montagna, D.D., Coppola, L., Zara, V., 2013. Oxidative stress negatively affects human sperm mitochondrial respiration. Urology 82, 78–83.
Gibb, Z., Lambourne, S.R., Aitken, R.J., 2014. The paradoxical relationship between stallion fertility and oxidative stress. Biol. Reprod. 91, 1–10.
Gostimskaya, I., Grant, C.M., 2016. Yeast mitochondrial glutathione is an essential antioxidant with mitochondrial thioredoxin providing a back-up system. Free Radic. Biol. Med. 94, 55–65.
Grady, S.T., Scott, B.D., Brinsko, D.W., Forrest, D.W., Sawyer, J.E., Cavinder, C.A., 2009. Dietary supplementation of 2 sources of omega-3 fatty acids and subsequent effects on fresh, cooled, and frozen seminal characteristics of stallions. J. Equine Vet. Sci. 29, 333–343.
Gürler, H., Calisici, O., Bollwein, H., 2015. Inter- and intra-individual variability of total antioxidant capacity of bovine seminal plasma and relationships with sperm quality before and after cryopreservation. Anim. Reprod. Sci. 155, 99–105.
Hunt, C.D., Johnson, P.E., Herbel, J., Mullen, L.K., 1992. Effects of dietary zinc depletion on semen volume and zinc loss, serum testosterone concentrations, and sperm morphology in young men. Am. J. Clin. Nutr. 56, 148–157.
Jeannerat, E., Marti, E., Berney, C., Janett, F., Bollwein, H., Sieme, H., Burger, D., Wedekind, C., 2018. Stallion semen quality depends on major histocompatibility complex matching to teaser mare. Mol. Ecol. 27 (4), 1025–1035.
Joller, P., 1988. Reinforcing immune defence against bacterial infections using a yeast preparation. Swiss Med 10 (6), 30–35.
Joller, P., Aeppli, R.E., 1989. Influence of prophylactic administration of a yeast preparation (Bio-Strath®) on the defence response. Swiss Med 11 (7), 66–71.
Lavová, B., Urminská, D., 2013. Total antioxidant activity of yeast saccharomyces cerevisiae. J. Microbiol. Biotechnol. Food Sci. 2, 1927–1933.
Leslie, G.B., Frasdillla, J.C., Salmon, G.K., Sturman, G., 1988. The effect of lifetime treatment with a herbal yeast food supplement (Bio-Strath®) on survival and infection resistance in mice. Swiss Med 10 (6), 36–38.
Leslie, G.B., Canham, D., Frasdilla, J.C., 1989. The effect of 30 days treatment with a herbal yeast food supplement (Bio-Strath®) on infection resistance in old mice. Swiss Med 11 (8), 30–31.
Malama, E., Zeron, Y., Janett, F., Siuda, M., Roth, Z., Bollwein, H., 2017. Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality. Theriogenology 87, 79–90.
Marin-Guzman, J., Mahan, D.C., Chung, Y.K., Pate, J.L., Pope, W.F., 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality, and subsequent fertilization rates in mature gilt. J. Anim. Sci. 75, 2994–3003.
Nagy, S., Jansen, J., Topper, E.K., Gadella, B.M., 2003. A triple-stain flow cytometric method to assess plasma- and acrosome-membrane integrity of cryopreserved bovine sperm immediately after thawing in presence of egg-yolk particles. Biol. Reprod. 68, 1828–1835.
Ortega Ferrusola, C., González Fernández, L., Morrell, J.M., Salazar Sandoval, C., Macías García, B., Rodríguez-Martinez, H., Tapia, J.A., Peña, F.J., 2009. Lipid peroxidation, assessed with BODIPY-C11, increases after cryopreservation of stallion spermatozoa, is stallion-dependent and is related to apoptotic-like changes. Reproduction 138, 55–63.
Rodrigues, P.G., de Moura, R.S., Rocha, L.G.P., Bottino, M.P., Nichi, M., Maculan, R., Bertechini, A.G., Souza, J.C., 2017. Dietary polyunsaturated fatty acid supplementation improves the quality of stallion cryopreserved semen. J. Equine Vet. Sci. 54, 18–23.
Schmid-Lausigk, Y., Aurich, C., 2014. Influences of a diet supplemented with oil and antioxidants on quality of equine semen after cooling and cryopreservation during winter. Theriogenology 81, 966–973.
Schwarzenberg, M., Joller, P., Cogoli, A., 2000. Stress-compensation by a food supplement based on yeast plasmolysate in mitogen activated T-lymphocytes under simulated low gravity. Biol. Sci. Space 14 (1), 3–8.
Tremellen, K., 2008. Oxidative stress and male infertility—a clinical perspective. Hum. Reprod. Update 14, 243–258.
Yousef, M.I., Abdallah, G.A., Kamel, K.I., 2003. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci. 76, 99–111.
Van Dorland, H.A., Zanoni, R., Gerber, V., Jeannerat, E., Wiederkehr, D., Burger, D., 2018. Antibody response to Influenza vaccination in Franches-Montagnes stallions supplemented with Equi-Strath®. Vet. Med. Sci. 4 (2), 133–139.
Vargas-Ochoa, B., Mejía-Barajas, J., Clemente-Guerrero, M., Manzo-Avalos, S., Salgado-Garciglia, R., Saavedra-Molina, A., 2016. Evaluation of antioxidant activity from different yeast extracts. Faseb J. 30 (1) Supplement 1174.22.
Wach-Gygax, L., Burger, D., Malama, E., Bollwein, H., Fleisch, A., Jeannerat, E., Thomas, S., Schuler, G., Janett, F., 2017. Seasonal changes of DNA fragmentation and quality of raw and cold-stored stallion spermatozoa. Theriogenology 99, 98–104.