Effect of phytomelatonin-rich diets on oxidative damage in ram sperm

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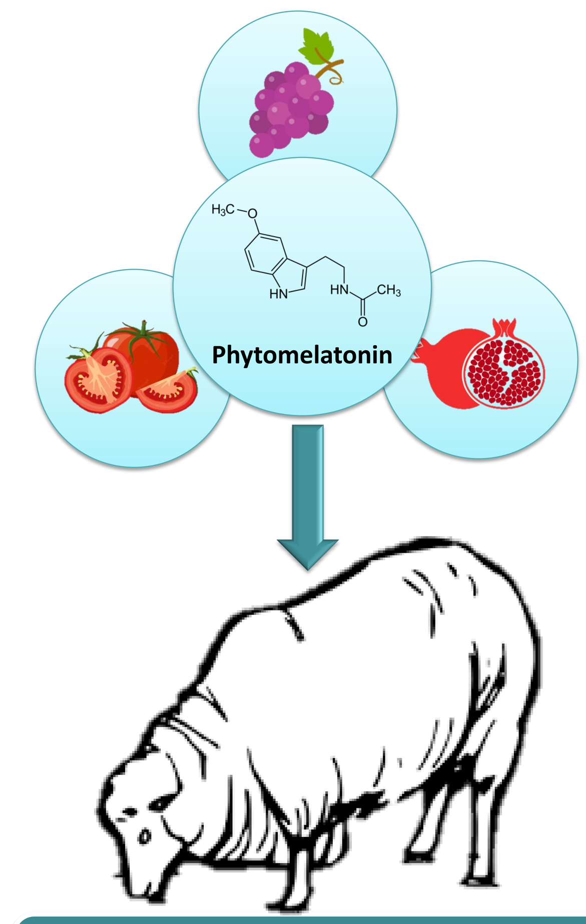
Background:

- **Melatonin** regulates reproductive seasonality in sheep. Sometimes, synthetic melatonin is used in subcutaneous implants to modulate reproductive seasonality in rams
- **Phytomelatonin** is the endogenously-produced melatonin in plants. It could be found in some vegetable by-products from food industries.
- It would be a green **alternative** and within the context of circular economy, to **replace the synthetic melatonin with a natural phytomelatonin**, by **supplementing** the **diet with vegetable by-products** from the food industry.



Aim:

To evaluate the effect of phytomelatonin-rich diets on ram sperm quality and seminal plasma composition.

Methods:

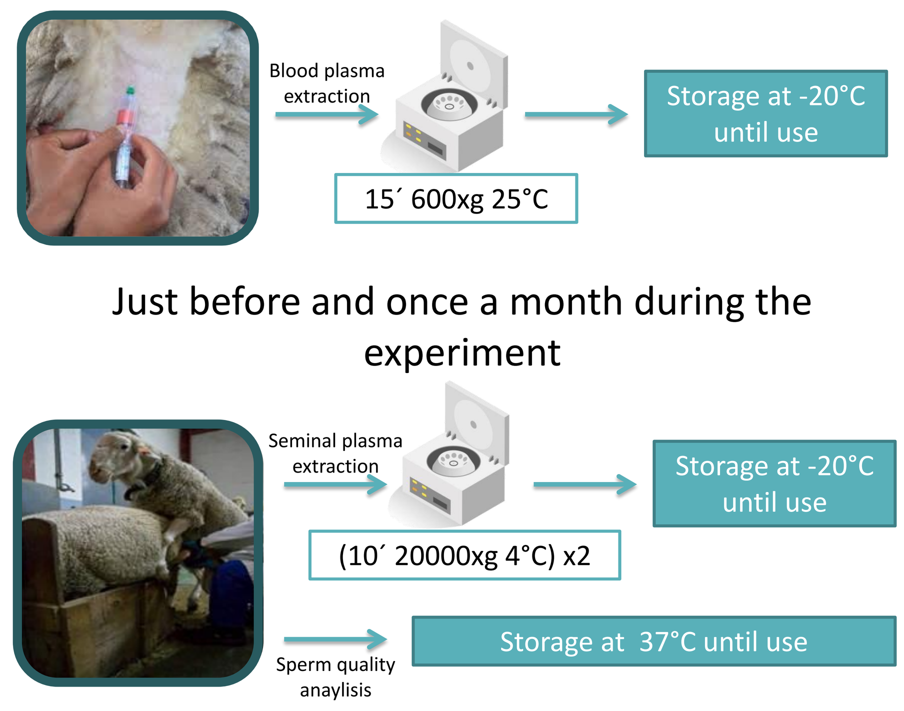
Experimental design

- 9 rams fed with a **commercial feed** (control)
- 9 rams fed with a phytomelatonin-rich diet (**PhyMEL-rich diet**) consisting of:

Commercial feed	80 %
Mixture of vegetable by-products from food industries: • **Pomegranate pulp** • **Tomato pulp** • **Grape pomace**	20 %

5 months (March-July)

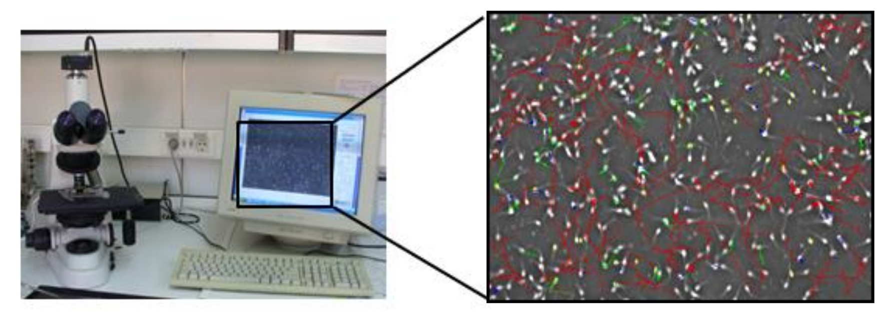
Blood plasma and semen collection



Just before and once a month during the experiment

Just before and twice a month during the experiment

Sperm quality analysis



Sperm motility (ISAS®)

Sperm viability, ROS levels, PS translocation (Flow cytometry)

Enzyme activities and melatonin analysis

Antioxidant enzymes activities (CAT, SOD, GRD, GPx in blood and seminal plasma) assessed with spectrophotometric assays.

Melatonin concentration in seminal plasma measured using a commercial competitive immunoassay (Direct saliva melatonin ELISA kit)

Results:

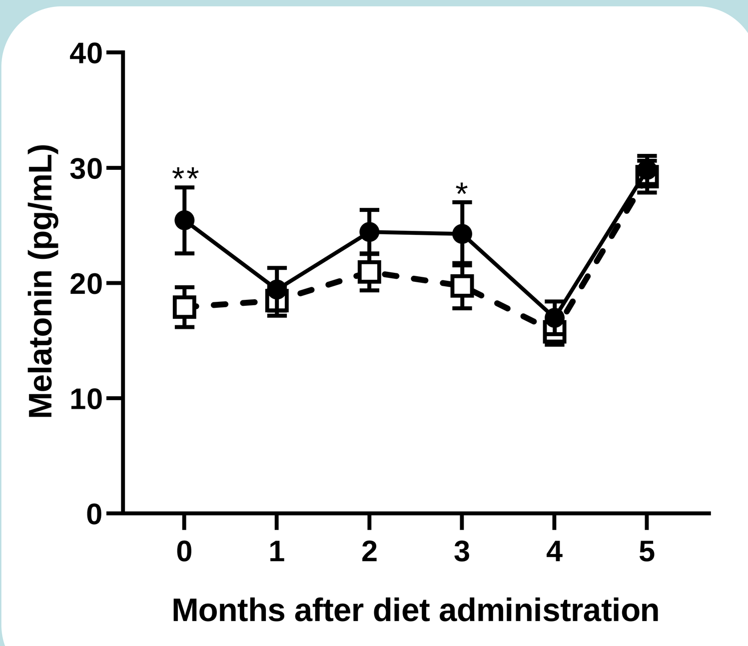


Figure 1: Monthly variations of melatonin levels in seminal plasma of animals fed with the phytomelatonin-rich diet (PhyMEL-rich diet, ●) and animals fed with a commercial diet (control, □) from month 0 (before diet administration) to month 5 (after diet administration). Values are shown as mean ± S.E.M., n=16, and *P<0.05; **P<0.01, indicate significant differences between the two experimental groups.

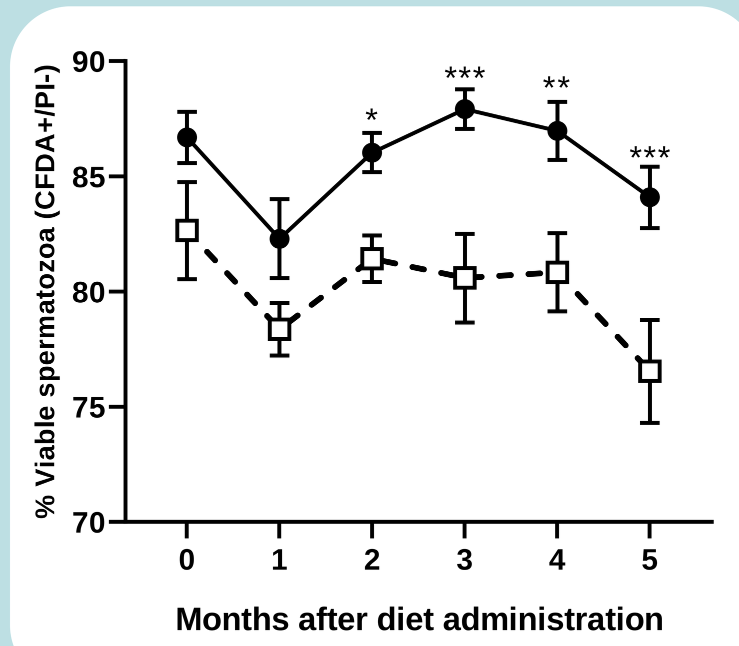


Figure 2: Percentage of viable sperm cells in samples from animals fed with the phytomelatonin-rich diet (PhyMEL-rich diet, ●) and animals fed with a commercial diet (control, □) from month 0 (before diet administration) to month 5 (after diet administration). Values are shown as mean ± S.E.M., n=16. *P<0.05; **P<0.01; ***P<0.001, indicate significant differences between the two experimental groups.

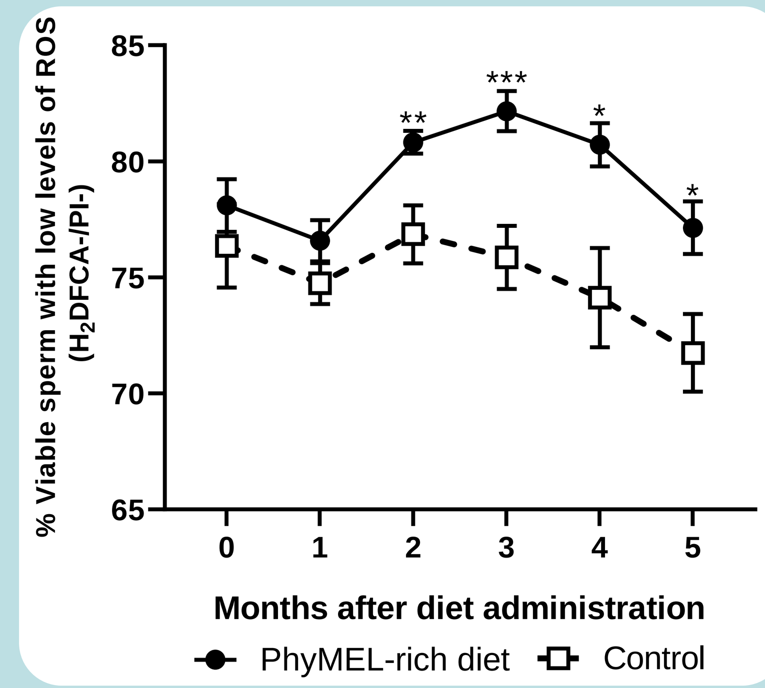


Figure 3: Percentage of viable with low levels of ROS sperm cells in samples from animals fed with the phytomelatonin-rich diet (PhyMEL-rich diet, ●) and animals fed with a commercial diet (control, □) from month 0 (before diet administration) to month 5 (after diet administration). Values are shown as mean ± S.E.M., n=16. *P<0.05; **P<0.01; ***P<0.001, indicate significant differences between the two experimental groups.

Blood plasma					
Month		CAT (nmol/min·mL)	GPx (nmol/min·mL)	GRD (nmol/min·mL)	SOD (nmol/min·mL)
0	Diet	17.859 ± 5.074	0.010 ± 0.002	0.018 ± 0.002	4.938 ± 0.673
	Control	24.168 ± 4.998	0.017 ± 0.002	0.011 ± 0.001	4.745 ± 0.709
1	Diet	23.740 ± 4.859	0.012 ± 0.001	0.020 ± 0.003	5.761 ± 1.396
	Control	16.892 ± 2.350	0.014 ± 0.000	0.029 ± 0.002	6.687 ± 0.507
2	Diet	27.429 ± 4.493	0.013 ± 0.001	0.031 ± 0.005	6.481 ± 0756
	Control	16.700 ± 3.468	0.010 ± 0.001	0.044 ± 0.006	6.687 ± 1.021
3	Diet	22.614 ± 3.335	0.012 ± 0.002	0.063 ± 0.009	6.713 ± 0.518
	Control	35.729 ± 6.972	0.022 ± 0.006	0.056 ± 0.003	7.755 ± 1.332
4	Diet	32.928 ± 8.257	0.033 ± 0.004	0.023 ± 0.004	6.250 ± 0.672
	Control	28.935 ± 4.824	0.026 ± 0.001	0.019 ± 0.001	4.762 ± 0.67
5	Diet	16.903 ± 1.654	0.022 ± 0.004	0.017 ± 0.003	5.556 ± 0.495
	Control	16.789 ± 3.396	0.018 ± 0.002	0.020 ± 0.005	5.671 ± 0.688

Seminal plasma				
Month		CAT (nmol/min·mL)	GPx (nmol/min·mL)	GRD (nmol/min·mL)
0	Diet	11.752 ± 1.994	0.016 ± 0.002	0.022 ± 0.002
	Control	15.306 ± 2.572	0.017 ± 0.003	0.028 ± 0.002
1	Diet	12.528 ± 1.931	0.014 ± 0.002	0.025 ± 0.002
	Control	20.603 ± 3.455	0.015 ± 0.002	0.021 ± 0.002
2	Diet	15.197 ± 3.753	0.012 ± 0.002	0.024 ± 0.004
	Control	8.547 ± 1.497	0.013 ± 0.002	0.021 ± 0.002
3	Diet	12.747 ± 1.764	0.022 ± 0.001	0.025 ± 0.002
	Control	12.652 ± 2.581	0.022 ± 0.002	0.029 ± 0.002
4	Diet	25.418 ± 3.218	0.024 ± 0.002	0.028 ± 0.003
	Control	24.190 ± 3.854	0.019 ± 0.003	0.033 ± 0.002
5	Diet	23.250 ± 3.768	0.018 ± 0.001	0.020 ± 0.003
	Control	19.939 ± 2.847	0.017 ± 0.002	0.025 ± 0.002

Table 1: Monthly variations of antioxidant enzyme activities in blood plasma and seminal plasma of animals fed with a phytomelatonin-rich diet (Diet) and animals fed with a commercial diet (Control) from month 0 (before diet administration) to month 5 (after diet administration). Values are shown as mean ± S.E.M., n=16.

Conclusions:

Phytomelatonin-rich diet increases ram sperm viability and decreases intracellular ROS levels.

It would exert this effect directly, not through the modulation of antioxidant enzyme activity.

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