

Reproductive health effects in the male dog following oral *lepidium meyenii* supplementation

Author

Viola Zappone – Department of Veterinary Sciences, University of Messina, Messina, Italy

Debora Teresa Gattuso – MGVet CSB (Canine Semen Bank), Reggio Calabria, Italy

Cristina Tomasella – BIOGENE, Veterinary Diagnostic Center, Catania, Italy

Carmelo Cavallo – Department of Veterinary Sciences, University of Messina, Messina, Italy

Santo Cristarella – Department of Veterinary Sciences, University of Messina, Messina, Italy

Sara Pastore – Department of Veterinary Medicine, University of Perugia, Perugia, Italy

Alessandro Troisi – School of Biosciences and Veterinary Medicine, University of Camerino, Macerata, Italy

Marco Quartuccio – Department of Veterinary Sciences, University of Messina, Messina, Italy

Citation

Zappone, V., Gattuso, D.T., Tomasella, C., Cavallo, C., Cristarella, S., Pastore, S., Troisi, A., Quartuccio, M. Reproductive health effects in the male dog following oral *lepidium meyenii* supplementation.

Male infertility can have serious financial implications for dog breeders. A major threat to semen quality, both in vivo and in vitro, is the reactive oxygen species (ROS) accumulated by spermatozoa during spermatogenesis and steroidogenesis. Sperm function is not impaired when ROS and antioxidant levels are in balance, as this ensures that no significant damage occurs [1]. However, metabolic oxidative stress caused by excessive ROS production

or low antioxidant status, or both, can lead to impaired sperm function. Therefore, ensuring low levels of ROS is crucial for proper fertilisation, particularly for capacitation, hyperactivation and the acrosomal reaction [1]. A promising approach to improve semen quality and male reproductive health is the use of antioxidants that regulate ROS levels. *Lepidium meyenii* (Maca) is a plant that has become popular due to its antimicrobial, antioxidant and anti-inflammatory activities [2]. Maca is rich in valuable nutrients and secondary metabolites such as macamides, alkaloids and glucosinolates. Macamides and glucosinolates reduce free radicals and protect cells from oxidative stress [3]. The aim of this study was to assess the effects of oral supplementation of *Lepidium meyenii* on the quality of canine semen and its storage under refrigerated conditions at 5°C, and on serum levels of testosterone and canine prostate-specific esterase (CPSE). Forty male dogs were enrolled in the study. Subjects with semen parameters incompatible with adequate reproductive capacity and who had experienced at least one reproductive failure in the 6 months prior to the study, either by natural mating or artificial insemination, were classified as subfertile (n=20). Subjects with semen parameters consistent with adequate fertility and a normal reproductive history in the 6 months prior to the study, with a numerically representative litter from both natural mating and artificial insemination, were classified as normo-fertile (n=20). The subjects in the subfertile (n=20) and normofertile (n=20) groups were further divided into a control group (SC group, n=10; NC group, n=10) and a treatment group (ST group, n=10; NT group, n=10). Dogs in the treatment groups received Maca in their diet in a capsule formulation (75 mg/kg), while the control groups received placebo (starch) capsules. Three semen samples were collected from each subject at three points in the semen cycle: immediately before the start of oral supplementation (T_0), after 31 days (T_{31}), and after 62 days (T_{62}). Blood samples were collected at the time of sperm collection and used to assess testosterone and CPSE concentrations. Sperm collection was performed after removal of the extragonadal reserve to minimise defects in sperm stored in the epididymis. The ejaculate was fractionated by discarding the third fraction and immediately assaying the first two fractions. Semen was evaluated for volume, concentration, motility, morphology and membrane integrity. An aliquot of the fresh semen analysed above was centrifuged

and the supernatant seminal plasma removed to optimise storage at 5°C. The remaining pellet was diluted appropriately with CaniPRO™Chill10 extender. Samples were immediately refrigerated and stored at 5°C. Total motility, progressive motility and membrane integrity were assessed at 3 (T_3), 24 (T_{24}), 48 (T_{48}) and 72 hours (T_{72}). The results of the study indicate that oral supplementation of 75 mg/kg Maca extract in dogs results in an overall improvement in male reproductive health, with beneficial effects on both seminal parameters and serum testosterone and CPSE levels. In fact, sperm parameters such as ejaculate volume, total sperm count, motility, morphology, and membrane integrity improved significantly. Furthermore, semen parameters evaluated after storage at 5°C showed less decline over time in sperm samples from subjects who had received the oral Maca supplementation compared to those from subjects in the control groups. Finally, an increase in serum testosterone levels and a decrease in CPSE levels were also observed. In conclusion, the results of the present study indicate that oral supplementation of Maca as part of the diet of male dogs resulted in significant improvements in their reproductive health. The observed positive effect on male canine fertility suggests that the use of Maca as a dietary supplement could be a promising strategy to improve semen quality and support reproductive health in the canine population.

Keywords: *Lepidium meyenii*, Maca, Dietary supplementation, Canine semen, Cooled semen, Testosterone levels, CPSE levels.

References

1. Vieira NMG, Losano JDA, Angrimani DSR, Kawai GKV, Bicudo LC, Rui BR, da Silva BDCS, Assumpção MEOD, Nichi M. Induced sperm oxidative stress in dogs: Susceptibility against different reactive oxygen species and protective role of seminal plasma. *Theriogenology*. (2018) 108:39–45. doi: 10.1016/j.theriogenology.2017.11.020
2. Póttorak A, Marcinkowska-Lesiak M, Lendzion K, Moczkowska M, Onopiuk A, Wojtasik-Kalinowska I, et al. Evaluation of the antioxidant, anti-inflammatory and antimicrobial effects of catuaba, galangal, roseroot, maca root, guarana and polyfloral honey in sausages during storage. *LWT*. (2018) 96:364–70. doi: 10.1016/j.lwt.2018.05.035
3. Tafuri S, Cocchia N, Carotenuto D, Vassetti A, Staropoli A, Mastellone V, et al. Chemical analysis of *Lepidium meyenii* (Maca) and its effects on redox status and on reproductive biology in stallions. *Molecules*. (2019) 24:1–12. doi: 10.3390/molecules24101981