

Effect of supplementation with maca (*Lepidium meyenii*) in libido and semen characteristics in hair sheep rams (*Ovis aries*)

[Efecto de la suplementación con maca (*Lepidium meyenii*) sobre la libido y algunas características del semen en carneros de pelo (*Ovis aries*)]

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Abstract

The aim of the present study was to evaluate the effect of maca (*Lepidium meyenii*) supplementation on some libido and semen characteristics of hair sheep rams (*Ovis aries*). Thirty 15-months old Saint croix males were fed either a control diet (concentrate feed; n=15) for 16 weeks or a maca supplemented diet (233 mg dried hypocotyls of black maca/kg body weight/day added to the concentrate feed) for 8 weeks, followed by 8 weeks without maca supplementation (n=15). Maca supplementation increased (P<0.05) the number of mounts and ejaculations decreasing the time between these events, at the end of week eight. However, no difference was found on reaction time, time until 1st ejaculation, testes circumference, ejaculation volume, sperm concentration and ram efficiency (mounts/ejaculations). These effects persisted 8 weeks later after no supplementation. It was concluded that maca consumption improves some mating behaviors in hair sheep rams, while semen characteristics appeared unaffected.

Keywords: Maca; Breeding rams; Saint Croix; Sexual behavior; Semen; Supplementation

Resumen

El propósito del presente estudio fue el evaluar el efecto de la suplementación con maca (*Lepidium meyenii*) sobre la libido y características del semen de carneros de pelo (*Ovis aries*). Treinta machos Santa cruz con 15 meses de edad se alimentaron con una dieta control (alimento concentrado; n=15) durante 16 semanas o con una dieta suplementada con maca (233 mg de hipocótilos secos de maca negra/kg de peso vivo/día, integrados al alimento concentrado) durante 8 semanas, seguidas de 8 semanas sin suplementación de maca (n=15). La suplementación con maca incrementó (P<0.05) el número de montas y eyaculados disminuyendo el tiempo entre estos eventos al final de la semana ocho. Sin embargo, no se encontraron diferencias en el tiempo de reacción, tiempo al 1er eyaculado, circunferencia escrotal, volumen del eyaculado, concentración espermática ni eficiencia del carnero (montas/eyaculaciones). Efectos que persistieron ocho semanas después, sin suplementación alguna. Se concluye que el consumo de maca incrementa las conductas de cópula en los carneros de pelo, mientras que las características de semen parecen sin cambios.

Palabras Clave: Maca; Carneros reproductores; Santa cruz; Conducta sexual; Semen; Suplementación

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INTRODUCTION

During the last decade, several studies have investigated some alleged fertility-enhancing properties of maca (*Lepidium meyenii*) supplementation in male mammals. Even though, some studies have confirmed positive effects, others have found inconsistent results (Gonzales *et al.*, 2002; Clément *et al.*, 2012). Most of the studies have been conducted in mice and rats, and a few in humans (Gonzales *et al.*, 2003a). To our knowledge, only one study has investigated the effects of maca supplementation in livestock (Clément *et al.*, 2010a). This study established that maca supplementation increased sperm quantity in bulls. However, no further research has been conducted to assess the impact of maca supplementation in bulls or other ruminant's male reproductive performance. Therefore, the aim of the present study was to evaluate the effect of maca supplementation on some libido and semen characteristics in hair sheep rams.

MATERIALS AND METHODS

Subjects

Thirty 15-month old proven Saint croix rams were used in this experiment. The rams had an average weight of 55.44 ± 0.97 kg at the beginning of the experiment.

Throughout the experiment the animals were fed 500 g/ram/day of a commercial concentrate diet formulated for breeding rams, with 16% protein (Nutres®), and free access to water and hay. Food was provided daily at 8:00 h in individual feeding troughs (0.5 m long) to ensure that each ram consumed the amount of food provided. The animals were housed in individual pens (2 x 3 m), 3 months before and during the 4 month experiment.

Maca (*Lepidium meyenii*) supplement

The black maca hypocotyl meal used for the experiment was purchased directly from Macandina® (Peru) who commercializes the product as an organic food supplement for humans. The product is sold as a powder milled from black hypocotyls only, which had been reported to have better effects on sperm production (Gonzales *et al.*, 2001; Gasco *et al.*, 2007) than other ecotypes of maca.

Experimental design

At the beginning of the experiment, animals were randomly allocated to either a control diet (standard

concentrate feed) for 16 weeks or a maca supplemented diet (233 mg dried maca-hypocotyls/kg body weight/day mixed into the standard concentrate diet) for 8 weeks, followed by 8 weeks of a control diet (residual effect). The level of maca supplementation used in the present study is similar to that reported in bulls (Clément *et al.*, 2010a) and rats (Gasco *et al.*, 2004) where maca supplementation resulted in increased sperm quality and quantity.

The 16-week period included two complete spermatogenic cycles (48 days cycle in rams) (Zeng *et al.*, 2006).

Evaluations

Semen collection and sexual performance tests were conducted at the end of each cycle on Monday, Wednesday and Friday in weeks 8 and 16. A vaginal collection vial (Wulster-Radcliffe *et al.*, 2001) was used to collect the semen from the first ejaculation of each ram and sexual performance was assessed individually for 20 min.

The rams were individually exposed to a single restrained estrous-induced female in a service crate. Each male was visually isolated from other members of the flock and the order of testing was at random, alternating treated and non treated rams. The following characteristics were measured during the sexual performance tests:

The reaction time and time until 1st ejaculation, defined as the period between the introduction of the ram to the test arena and the first mount and first serve, respectively. Time between ejaculations, was the period between the first and the second ejaculation, regardless of the number of ejaculations obtained. The number of mounts and ejaculations were the total events that occurred during the 20 min sexual performance test. Ram efficiency was a relative measure obtained from the quotient of the number of mounts and ejaculations of each ram.

Scrotal circumference was measured 1 h before the sexual performance tests and was done by pushing the testicles to the bottom of the scrotum, and then measuring the greatest circumference with a flexible plastic tape.

Semen volume and concentration were evaluated within 15 min of ejaculation. The vaginal collection vial was removed immediately after first ejaculation, during the male sexual refractory period. No semen collection device was used for the rest of the test.

Semen volume was measured in a conical glass tube graduated with 0.1 mL optically visible intervals, and sperm concentration was determined at a dilution of 1:400 in 0.2% glutaraldehyde in saline using a calibrated spectrophotometer at 540 nm (Hafez and Hafez, 2000).

Statistical analysis

The Friedman two-way analysis of variance (Siegel and Castellan, 1988) was used to analyze ram efficiency, number of mounts and ejaculations data. To compare semen volume, sperm concentration, scrotal circumference, reaction time, time until 1st ejaculation and between ejaculations among treatments, a two-way analysis of variance was used (SAS, 1985). In the analysis, Factor A was considered: week of testing (8 and 16), while factor B: were the three sexual performance tests carried out each of the

two weeks. Interaction within both factors was also evaluated.

RESULTS AND DISCUSSION

Maca supplementation increased ($P < 0.05$) the number of mounts and ejaculations, decreasing the time between ejaculations, at the end of week 8. However, no difference was found in reaction time, time until 1st ejaculation, testes circumference, ejaculation volume, sperm concentration and ram efficiency (mounts/ejaculations). These effects persisted 8 weeks later (residual effect) after no supplementation in the treated animals (Table N° 1). In addition, no difference was found in any variable in the control group when data within and between weeks 8 and 16 were compared. Thus, average results from the six sexual performance tests are presented in Table 1 for this group.

Table N° 1
Effect of Maca supplementation at the end of two 8 week periods on some libido and semen characteristics in hair sheep rams.

	Control (N° supplementation)	Treated (8 weeks of supplementation)	Residual (treated animals after 8 weeks of no supplementation)
Reaction time (s)	0.27 ± 0.05	0.58 ± 0.16	0.25 ± 0.05
Time until 1st ejaculation (s)	2.10 ± 0.40	2.57 ± 0.91	1.34 ± 0.32
Time between ejaculations (s)	7.10 ± 1.06 b	3.36 ± 0.39 a	5.27 ± 1.64 b
Number of mounts	6.70 ± 0.63 a	12.30 ± 1.47 b	17.70 ± 3.27 b
Number of ejaculations	2.30 ± 0.11 b	3.10 ± 0.22 a	3.40 ± 0.25 a
Ram efficiency (mounts/ejaculations)	3.52	3.96	5.11
Scrotal circumference (cm)	30.63 ± 0.56	31.44 ± 0.62	30.75 ± 0.68
Semen volume (ml)	0.96 ± 0.13	1.09 ± 0.05	1.12 ± 0.12
Sperm concentration (sperm/10⁹/mL)	3.04 ± 0.54	2.99 ± 0.24	3.55 ± 0.18

Within a row, values marked with unequal letters, indicate significant differences ($P < 0.05$).

Our results showed that maca supplementation enhanced mating activity in Saint croix rams after 8 weeks of treatment. These findings are in accord with previous studies in which oral administration of maca improved the sexual function of mice, rats (Zheng *et al.*, 2000; Cicero *et al.*, 2001; 2002) and men (Stone *et al.*, 2009), as evidenced by an increase in the number of complete intromissions and improvement of self-

rated sexual desire, respectively. However, differ from those that found an increase in sperm count in men (Gonzales *et al.*, 2001), rats (Gonzales *et al.*, 2006a; Gonzales *et al.*, 2006b; Gasco *et al.*, 2007), mice (De la Cruz and Arroyo, 2012) and cattle (Clément *et al.*, 2010a). However, this apparent disagreement could be due to the different methodologies used to quantify this variable. Most of the studies reporting an increase

in sperm count, have estimated the amount of cells from different stages during spermatogenesis in seminiferous tubules, vas deferens or epididymal reservoirs. To our knowledge, only two studies have measured the amount of sperm/ml/ejaculation; Clément *et al.* (2010a) who believed that the effect was mostly due to the particularly low value found in the control bulls at the beginning of the experiment, and Gonzales *et al.* (2001) who found an effect only in the total sperm count. Similarly, in the present experiment maca supplementation did not affect the number of spermatozooids per ejaculate, but the total sperm count was increased by 47% due to the increase in the number of ejaculates.

Thus, different results might be due to species variation in the response to maca supplementation, different maca ecotype (Gonzales *et al.*, 2006b), doses used (Chung *et al.*, 2005) or methodologies. For example, increase in mating behavior has been observed in mice and rats (Zheng *et al.*, 2000) but not in bulls (Clément *et al.*, 2010a). In the present study, the interval between 1st and 2nd ejaculation was reduced in the treated animals, but no effect was found in the time required to accomplish the 1st mount or ejaculation. On the contrary, Zheng *et al.* (2006) found a decrease in the latent period of erection in male rats with erectile dysfunction. However, neurophysiological mechanisms of erection are different in these species, enhanced by erection problems.

In the other hand, only black maca has been reported to affect sperm count as early as one day after beginning treatment (Gonzales *et al.*, 2006a), while red maca has no effect in sperm counts, but reduces prostate weight (Gonzales *et al.*, 2006b). Furthermore, early studies demonstrated that the epididymal sperm count increases in a dose-dependent manner in maca-treated rats (Gasco *et al.*, 2004), whereas higher dosages have the opposite effect (Gonzales *et al.*, 2003b). These physiological processes do not seem to be regulated by changes in serum hormone levels (Gonzales *et al.*, 2003). However, the specific mechanism is not yet known (Bogani *et al.*, 2006). Thus, further chemical and molecular research is required to identify which of the many components of maca accounts for the effects observed (Gonzales, 2012). The fact that the number of mounts and ejaculations remained higher for at least 8 weeks after maca supplementation, suggests a residual effect of some secondary metabolites, or distinct combinations of them (Clément *et al.*, 2010b and c).

Future research is needed to clarify the physiological basis supporting these findings and to

address potential differences between species in their response in mating behavior, sperm production and quality of the maca supplement.

The increase in number of ejaculations and total sperm count by maca supplemented rams could be an important achievement to the Artificial Insemination industry, and could also lead to more ewes served during natural mating. In sheep a higher proportion of ewes is served and receives more ejaculations by high sexual performance rams (Perkins *et al.*, 1992). In addition, Zheng *et al.* (2000) found an increase in the number of sperm-positive females exposed to maca oral-treated normal mice.

It was concluded that maca consumption improves some mating behaviors of hair sheep rams, while semen characteristics appeared unaffected.

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