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Aqueous extract of yellow maca (*Lepidium meyenii*) improves sperm count in experimental animals but response depends on hypocotyl size, pH and routes of administration

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Summary

Lepidium meyenii, a Peruvian plant growing over 4000 m.a.s.l., has effects on nutrition and fertility. The purpose of this study was to evaluate the sperm count in 105 male mice receiving boiled aqueous extract of yellow maca hypocotyls from different sizes, under different pH conditions and using two different routes of administration. Five mice per group were treated daily for 3 days with vehicle (oral and intraperitoneal) or maca aqueous extracts (5 mg/0.5 ml/day) belonging to the first, second, third and fourth categories, according to their hypocotyl size. On day four, sperm count was evaluated at testis, epididymis and vas deferens. Sperm count was higher in mice receiving maca from the larger sizes (first and second categories). Reduction in maca extract pH increased sperm count, whereas an increase in the pH resulted in a reduction in sperm count. The effect of pH reduction is observed only in maca from the first and second categories. Aqueous extract of maca was effective only after oral administration. In conclusion, the larger size of hypocotyls presented the best biological effect, and the low pH in the extract and the transformation after gastrointestinal passage are both important for its biological action.

KEYWORDS
Central Andes, fertility, Huallanca, maca phenotypes, nutraceutics

1 | INTRODUCTION

Lepidium meyenii (Maca) is a plant of the Brassicaceae family that grows between 4,000 and 4,500 metres above sea level (m.a.s.l.) in the Peruvian Central Andes, particularly in Junin and Pasco. Maca grows in these places in the range of temperatures from a maximum of 12°C to a minimum of 1.5°C. This range of temperatures occurs in the Central Andes because of its proximity to the equator. For such reason, maca does not grow well in the Peruvian Southern Andes where temperature is lower with frequent frosts.

In the central Andes, maca is present in different phenotypes characterised by the colour of the hypocotyls (Valerio & Gonzales, 2005). Interestingly, each phenotype has different biological properties, in which black maca and yellow maca increase sperm count after 3 days of treatment, an effect not observed with red maca (Gonzales, Nieto, Rubio, & Gasco, 2006).

Maca has great interest in the international markets due to both its nutritional and fertility-enhancing properties (Cobo, 1956; Gonzales, Villaorduña, Gasco, Rubio, & Gonzales, 2014; Zheng et al., 2000). With regard to the demand for maca worldwide, producers sell hypocotyls of maca according to the size defined in categories (Figure 1), being the first category, the one which represents the biggest hypocotyl, while the fourth category represents the smallest hypocotyl. At this time, it is unknown whether these different categories of maca, defined by different sizes, have similar biological effect increasing sperm count. In Andrology, the first outcome for treatment should be to increase sperm quality.

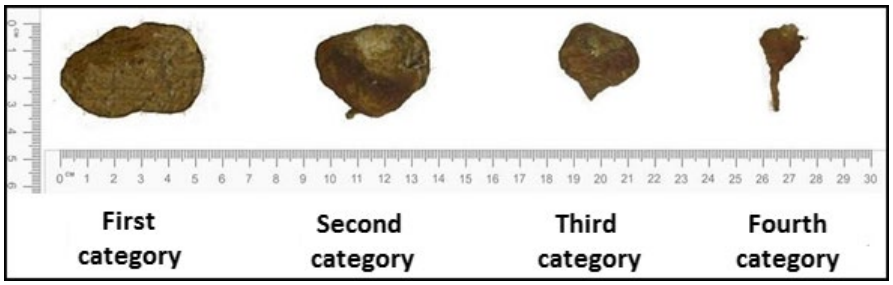

FIGURE 1 Different categories of Yellow Maca

If demand for maca is increased, then, the assessment of biological activity in response of maca administered under different conditions is important. It is known that maca grows in acid soils and this may explain why maca hypocotyls are also acids (Gonzales, 2006). Previous studies in our laboratory showed that administration of maca with lower pH has a better biological response than administration of maca with higher pH (Gonzales, 2006). It is unknown whether pH modification in an extract of maca may modify its biological properties.

Maca is used as food, and all studies with maca looking for biological activity have been performed through the oral route (Gasco, Aguilar, & Gonzales, 2007; Rubio et al., 2006; Yucra, Gasco, Rubio, Nieto, & Gonzales, 2008). This route in human has demonstrated to have high acceptability, it is safety and it has efficacy (Gonzales et al., 2014; Gonzalez-Arimborgo, 2016). It is clear that in different investigations assessing products with systemic effects, the intraperitoneal route is preferred because it is an easy route of administration in experimental animals and this procedure avoids hepatic metabolism and a small amount of intraperitoneal injection may pass directly across the diaphragm and into the thoracic lymph (Abu-Hijleh, Habbal, & Moqattash, 1995). It is still unknown whether parenteral administration of maca may be successful increasing sperm count after administered.

For this reason, this study was designed to comparatively assess the effects on sperm count 1) with different sizes of yellow maca 2) under acidifying or alkalinising maca extracts and 3) by oral or intraperitoneal route in adult mice.

2 | MATERIAL AND METHODS

2.1 | Animals

A total of 105 adult male mice of Swiss strain were obtained from the animal house at the Universidad Peruana Cayetano Heredia. They were moved to the laboratory and maintained in a standardised environment with 12:12 hr light/dark cycle and 22°C temperature. Mice were fed ad libitum with food (Papeadito®) and water. The ethical Committee at the Universidad Peruana Cayetano Heredia approved the study (SIDISI number 65552) protocol.

2.2 | Maca

The dry hypocotyls of yellow maca were collected in Huallanca District of Bolognesi Province, Department of Ancash, from MGAT1P1530 area of Torres Parcela Regalado farm, at approximately 4250 m.a.s.l., with an average annual temperature of 9.2°C and a soil pH range of 4.19–5.16. Four categories were used according to the hypocotyl size (from highest to lowest size): first, second, third and fourth categories (Figure 1).

2.3 | Preparation of maca aqueous extract

The aqueous extracts of the hypocotyls were prepared according to the traditional method. In brief, dried hypocotyls (100 g) were milled and then placed in a recipient with 2 litres of water and boiled at 100°C for 2 hr. Longer boiling time may affect functional chemical compounds as observed in the IR profile (Figure 2) (Gonzales, 2006). The final preparation was left to cool, filtered and solutions were placed in small vials and kept at 4°C until use.

The recovered liquid was analysed to determine the pH value, glucose content, total polyphenols content and antioxidant activity using the DPPH test. pH was measured using a calibrated *Hanna*™ pH metre in the pH range of 1–14. Glucose was measured using an Accu-Chek® glucometer, and values were expressed as mg/g maca.

Polyphenol was measured using the method of Folin–Ciocalteu as described by Kähkönen, Hopia, and Vuorela (1999), and results were expressed as g Gallic acid/100 g maca. DPPH test was performed using the method described by De Martino et al. (2012).

2.4 | Experiments

Three independent experiments were performed to determine the effect of aqueous extract of yellow maca on sperm count. In each experiment, five mice were studied per group.

2.5 | Effect of size of the hypocotyls

Aqueous extracts of maca obtained from each of the four categories defined by size were administered in a concentration of 5 mg0.5 ml for 3 days to each of six groups of animals. The first group received vehicle (distilled water) in an amount of 0.5 ml/day, the second group received a positive control (spray-dried hydroalcoholic extract of black maca), whereas the other four groups received aqueous maca extract from the first, second, third and fourth categories. After treatments, mice were sacrificed at day four for the assessment of daily sperm production (DSP), and the sperm count in the epididymis and vas deferens.

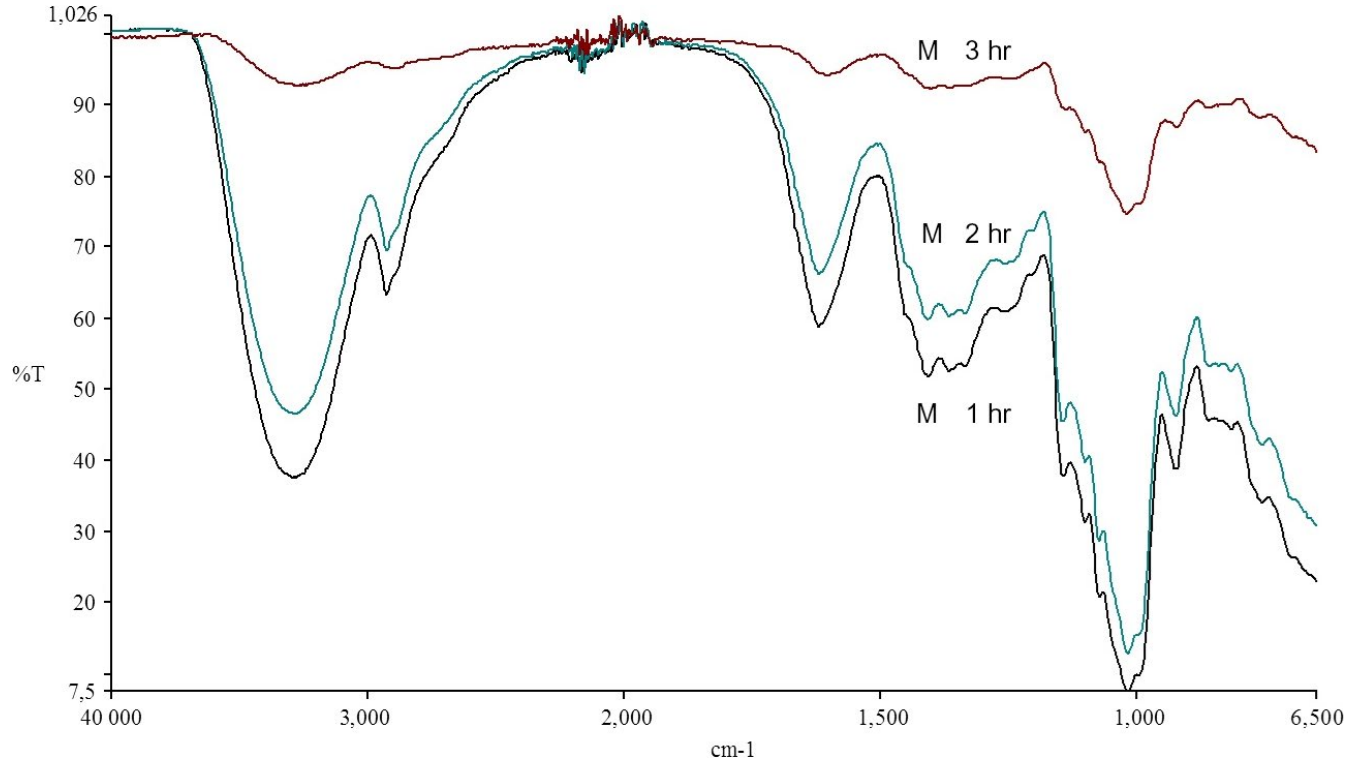

FIGURE 2 Infrared Profile of *Lepidium meyenii* (maca, M). Maca was boiled for 1, 2 or 3 hr. After 3 hr of boiling, maca lost main functional chemical groups (Source: Gonzales, 2006)

2.6 | Effect of pH of aqueous extract of maca

In this experiment, we compared the effect of three different pH levels of the hypocotyls (pH = 4, 6 and 8) on sperm count in mice. At each pH, six groups were studied (vehicle, positive control and maca of each of the four categories according to the size of the hypocotyls. Each group included five mice. For this, pH measured in the maca aqueous extract is considered as normal pH and a group of animals received this extract as treatment for 3 days. A second group received maca aqueous extract in which pH was acidified with hydrochloric acid (0.1 N) to obtain a pH = 4. Another group of mice received maca aqueous extract alkalinised with sodium hydroxide (0.1 N) to obtain a pH = 8.

2.7 | Effect of administration route of aqueous maca extract

In the third experiment, we compared the effect of two routes of administration (orogastric vs intraperitoneal) on sperm count in mice. For this, three groups of mice received vehicle (oral) or aqueous extract from the first category by orogastric route or intraperitoneal route (5 mg/0.5 ml/day) during 3 days. Each group included five mice.

2.8 | Daily sperm production (DSP)

Left testis was used for standardisation, and the tunica albuginea was carefully removed. Testis was homogenised in 1 ml of 0.9% saline-0.05% (v/v) TRITON™ X-100 solution for 1 min by a homogeniser. After a dilution 1/10, the number of homogenisation-resistant elongated spermatids nuclei per testis was counted with a haemocytometer. Measurement of spermatids in testis was performed four times using an improved Neubauer ®Marienfeld Superior counting chamber (Gonzales et al., 2006). The PDE and its efficiency (sperm × 10^6/gram testis) were determined by dividing the number of spermatids elongated by testis and spermatocytes per gram of testis by 4.84 days in steps 14–16 during spermatogenesis for mice (Thayer et al., 2001).

2.9 | Epididymal sperm count

The epididymis was removed and weighed when dry. Then, they were divided into two parts, head-body and tail. Epididymal sperm count resistant to homogenisation of nonperfused mouse was performed as described by Gonzales et al. (2006). The homogenate was performed and placed in 1 mL of physiological solution (0.9% NaCl) in a tube. The homogenates were kept refrigerated at 4°C for 24 hr, to facilitate lysis of the tissue and obtain the total sperm release.

Then, the refrigerated homogenate was added to 1 ml of eosin (2%). A further dilution of 1:4 with eosin was performed. A 10 µl sample of this dilution was placed into an improved Neubauer counting chamber branded Marienfeld® Superior. Head spermatozoa resistant to homogenisation were counted in 25 squares of the chamber for four times. The sperm count obtained by each animal was multiplied by 0.04 and then by 2 mL to obtain values expressed in sperm ×10^6/epididymis as described by Inoue, Farfan, and Gonzales (2016).

2.10 | Sperm count in vas deferens

Vas deferens was placed in a small Petri dish with 1 ml of saline solution and fractioned in several parts, allowing the sperm release from the walls. In addition, 1 ml of eosin (2%) was added in the Petri dish. The sperm heads were counted in 25 squares of the improved Neubauer chamber ©Marienfeld-Superior. Four chambers were measured in each sample, and an average was calculated. The results were multiplied by 0.01 and defined as sperm × 10^6 by vas deferens. Data were expressed as the total amount of sperm in vas deferens.

2.11 | Statistical analysis

All statistical analyses were performed in the statistical package STATA version 12. Data are presented as mean ± *SEM*.

If the data followed a normal distribution curve, then the one-way analysis of variance (ANOVA) was performed. If *F*-value in the ANOVA test was statistically significant, then the differences between pair of means were assessed applying the Scheffé test. A value of $p < .05$ was considered as statistically significant.

3 | RESULTS

3.1 | Sizes of the maca hypocotyls

In Table 1, data are presented related to the sizes of the maca hypocotyls from the four categories. Mean hypocotyl weight in the first category was 11.88 times higher than the size in the fourth category. The mean width of hypocotyls was four times higher in the first category than in the fourth category.

3.2 | pH, glucose and polyphenol content and antioxidant activity of aqueous extract of yellow maca

Maca extract from first category showed the lowest pH value, whereas the fourth category showed the highest pH value. Similarly, extract obtained from maca of the first category had the highest glucose levels, and glucose values were low in the lowest hypocotyl size. With respect to polyphenol content, highest values were observed in the extract produced with maca from the first category. Antioxidant activity assessed by DPHH test showed a low activity for maca without differences between different categories (Table 2). Maca from first and third categories presented the highest antioxidant percentage (Table 2).

TABLE 1 Weights and diameters of four categories of yellow maca from Huallanca

Categories	Weights (g)*	Diameters (cm)	
		Width*	Long*
First	20.2 ± 1.7	4.0 ± 0.2	3.4 ± 0.2
Second	12.7 ± 0.9	3.0 ± 0.1	2.8 ± 0.1
Third	6.3 ± 0.6	2.3 ± 0.1	2.0 ± 0.1
Fourth	1.7 ± 0.6	1.0 ± 0.1	1.1 ± 0.1

All values are shown as mean ± *SEM* of ten replicates. ANOVA *$p < .001$ There are differences between all groups.

3.3 | Effect of aqueous extract of maca from different sizes on sperm count

Figure 3 shows that maca aqueous extract had better biological effects when hypocotyls from the highest size (first and second categories) were used. DSP and sperm counts in epididymis and vas deferens were similar in the group receiving maca extract obtained from hypocotyls of the third and fourth categories and the group receiving vehicle. These data indicate that maca of a shorter size has no biological effect in the bioassay used in this experiment.

3.4 | Effect of pH on DSP and sperm count in epididymis and vas deferens

Modification of the pH of the maca extract resulted in changes in DSP and sperm counts but only in the groups using maca from first and second categories. The positive control (spray-dried from black maca) showed the same pattern: increasing sperm count with a reduction in pH and reducing sperm count with an increase in pH (Figure 4).

3.5 | Effect of routes of administration

Figure 5 showed the differences in DSP and sperm counts in epididymis and vas deferens after 3 days of treatment with aqueous extracts of yellow maca administered by orograstric and intraperitoneal routes. In both cases, the maca hypocotyl used was that of the first category (highest size). According to Figure 5, DSP and sperm counts in epididymis and vas deferens were higher in the group receiving maca administered by oral route compared to vehicle, whereas no effect was observed when maca was administered by an intraperitoneal route.

4 | DISCUSSION

Maca is present in nature in different sizes under the same cultivation conditions. For such reason, this study was performed to determine whether maca from different sizes has a different biological response and whether changes in pH of the hypocotyl or an intraperitoneal route of administration resulted in different biological responses than that observed under classical conditions previously published (Gonzales et al., 2006, 2014).

Comparing four different categories depending on maca size, this study demonstrated that hypocotyls from the first category had the highest content of polyphenols and glucose levels but low pH without changes in antioxidant activity by DPPH test.

TABLE 2 pH, glucose, polyphenols and DPPH values for four categories of yellow maca

	First	Second	Third	Fourth
pH	5.8 ± 0.15*	6 ± 0.01	6.1 ± 0.01	6.3 ± 0.02
Glucose (mg glucose x g maca)	50.0 ± 0.7**	20.7 ± 1.2	15.6 ± 20	18.9 ± 0.9
Polyphenols (g Gallic acid/100 g maca)	0.51 ± 0.01***	0.38 ± 0.01	0.33 ± 0.02	0.36 ± 0.01
DPPH (%Act.Antiox.)	18.4 ± 0.13****	16.2 ± 0.22	18.9 ± 0.22****	17.8 ± 0.40

All values are shown as mean ± *SEM*. ANOVA *$p < .01$ for pH when compared first vs second categories. ANOVA **$p < .0.01$ for glucose when compared first vs second, third and fourth. ANOVA ***$p < .05$ for polyphenols when compared first vs second, third and fourth. ANOVA ****$p < .02$ for DPPH when compared first vs second and third and ANOVA ****$p < .02$ for DPPH when compared third vs second and fourth.

It is possible that higher glucose levels in maca from the first and second categories could be associated with higher levels of secondary metabolites. This is based on the fact that the first and second categories of maca have the highest amount of polyphenols. However, recent studies have demonstrated a role for maca polysaccharide as an antifatigue agent (Tang et al., 2017) or as having a protective effect against oxidative stress (Zhang, Zhao, Wang, Zhao, & Zhao, 2017). However, the role of different concentrations of glucose on maca hypocotyls on sperm count requires further investigation.

It is suggested that biological activity of maca could be associated with the antioxidant activity and the amount of total polyphenols. (Castillo & Lock, 2005; Lee, Dabrowski, & Rinchard, 2004). Our results demonstrate that different biological effects on sperm count were not associated with the antioxidant activity. In fact, sperm count may change as effect of hypocotyl size, whereas antioxidant activity was low and it is not modified by the effect of hypocotyl size. However, it is possible that higher biological activity in maca with a larger size could be correlated with higher amounts of polyphenols.

Auxins, gibberellins and cytokines are phytohormones produced naturally by the plant, responsible for growth and development phenomena (Rastogi et al., 2013). In *Arabidopsis thaliana*, an enhancement of hypocotyl elongation mediated by auxin and phytochrome-interacting factors (Miyazaki et al., 2016) has been demonstrated. As phytohormones increase in concentration they may increase hypocotyls' growth. Therefore, the larger size of maca hypocotyls could be due to a higher concentration of phytohormones that modulate growth.

In this sense, it is possible that seed size was larger in hypocotyls with bigger sizes. Several studies show that a larger seed with large reserves produces a vigorous seedling, with a better development and size than a small seed (Jiménez, Rangel, Mendoza, Cervantes, & Rivera, 2014; Rubio, Romero, Rojas, Durán, & Gutiérrez, 2011). A study with *Allium sativum L* (Garlic) showed that the shorter the germination time of the seed, the silver will be more likely to capture more nutrients due to the low competition for resources in the microenvironment, generating larger bulb size and leave size (Karaye & Yakubu, 2006). We have not studied these parameters and it is a matter of future research for agriculture scientists.

In the case of larger maca, the nutrition that they had during growing time was higher in the reserving parenchyma, concentrating a greater amount of primary and/or secondary metabolites, whereas smaller hypocotyls would capture the least amount of nutrients (minerals, vitamins, secondary metabolites responsible for the biological effect), accumulating nonuseful residues that would not give the expected biological effect. These results are in agreement with Burba (1997), indicating that the greater weight of the large bulb of garlic is due to the greater amount of reserves that the garlic plant accumulates during its vegetative growth.

For the study of biological response, we have assessed the response to a three-day administration intervention with maca extracts obtained from hypocotyls of different sizes. This is a bioassay previously assessed in our laboratory (Gonzales et al., 2006; Inoue et al., 2016). We have validated this method in rats (Gonzales et al., 2006) and mice (Inoue et al., 2016). Spermatogenesis is a large process and it cannot be studied in a three-day period. Spermatogenesis lasts 4.5 times the spermatogenic cycle length. In rats, the spermatogenic cycle lasts 12.5 days (Aslam et al., 1999) and in mice it lasts 8.9 days (Johnson, 1995). In general terms, it is estimated that the length of spermatogenesis is 56 days in rats and 40 days in mice.

Maca has been demonstrated to increase epididymal sperm count at 3 days and after a spermatogenic cycle (Johnson, 1995), at 42 days (Gonzales et al., 2006) and after 84 days (Gasco et al., 2007). This suggests that assessment of sperm count at day three relates with the effect of maca on the whole spermatogenesis. Then, sperm count after three-day intervention with maca is useful to determine the biological effect of this plant.

The treatment with boiled aqueous extract of first category maca had the highest sperm count in testis, epididymis and vas deferens, and it was concluded that the size of the hypocotyls plays a very important (but not the only) role in biological activity. To our knowledge, there is no study assessing the biological effect of administration to experimental animals or human being with plant extracts obtained from parts with different sizes (fruits, hypocotyls, tubers, leaves, etc.).

Another parameter evaluated was the effect of pH on maca extract on its biological response when it is administered to mice. Previous studies have shown that the lower pH of the maca extract collected from the Peruvian central Andes has a higher biological activity

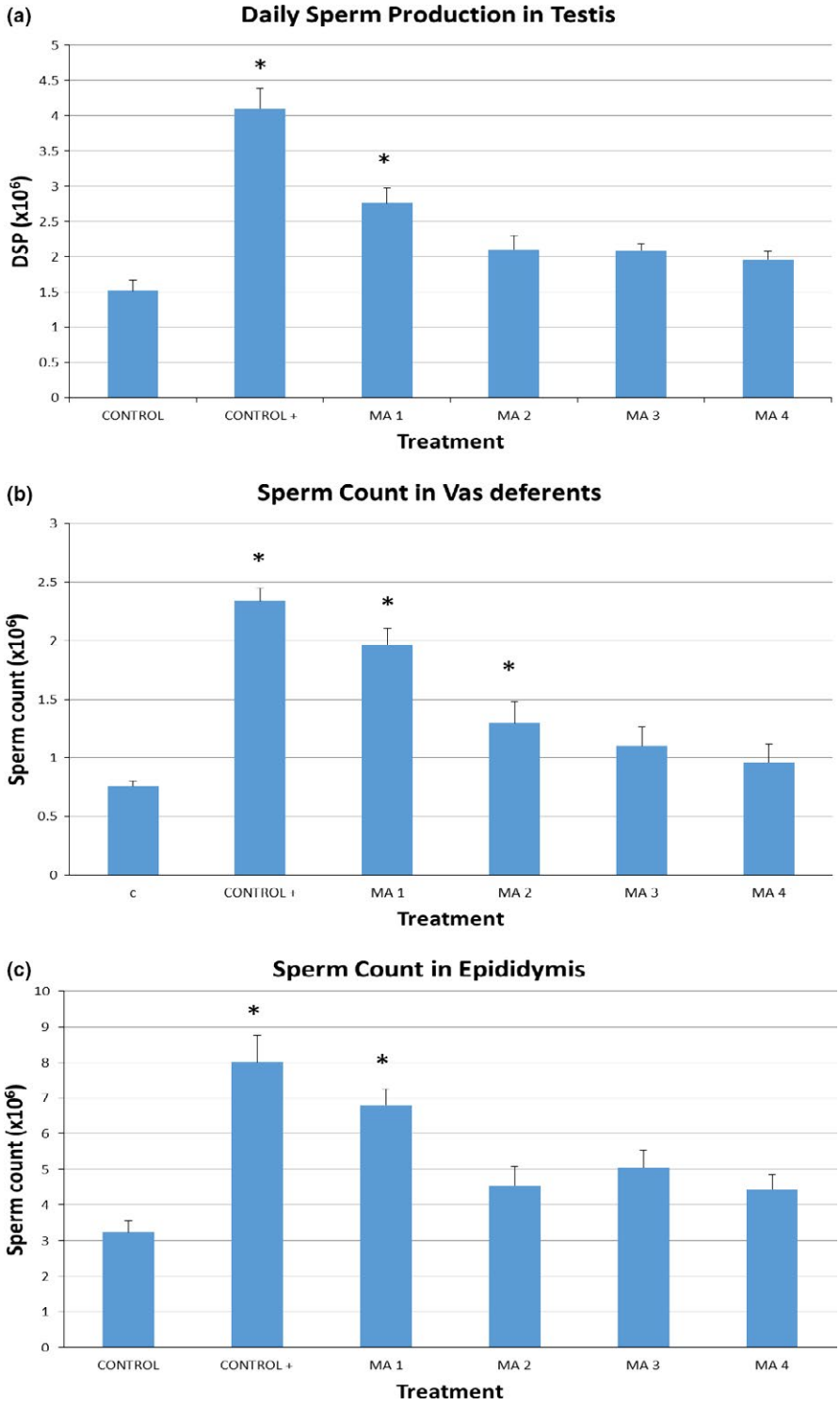

FIGURE 3 Sperm count in testis (a), vas deferens (b) and epididymis (c) of mice treated with four categories of yellow. $^{*}p < .05$ respect to control group. Bars indicate standard error of the mean (*SEM*). CONTROL is the treatment with distilled water, CONTROL + is the treatment with black maca, MI1 is the treatment with first category of maca, MA2 is the treatment with second category of maca, MA3 is the treatment with third category of maca and MA4 is the treatment with fourth category of maca

when administered to rats (Gonzales, 2006). The present study confirms these findings using maca obtained from another place named Huallanca.

The soil where the maca grows is acidic in nature (Quirós, Epperson, Hu, & Holle, 1996), and then, this characteristic could be responsible for the acidic pH of the hypocotyls. In our study, we evaluated whether the acidic pH of the extracts influenced the sperm count, so we compared the effect of extracts with different pH, the initial pH of the four categories was found in the range of 5.8–6.3. These values could be caused by the compounds present in these extracts, together with the acid pH of the substrate where the root grows giving it the necessary resistance to hostile environments, better alimentary properties and therefore a better biological effect after administration in animals and humans. In our study, a reduction in the pH of the maca extract improves the activity of the secondary metabolites, thus giving a better effect as sperm count was higher. Meanwhile, treatment

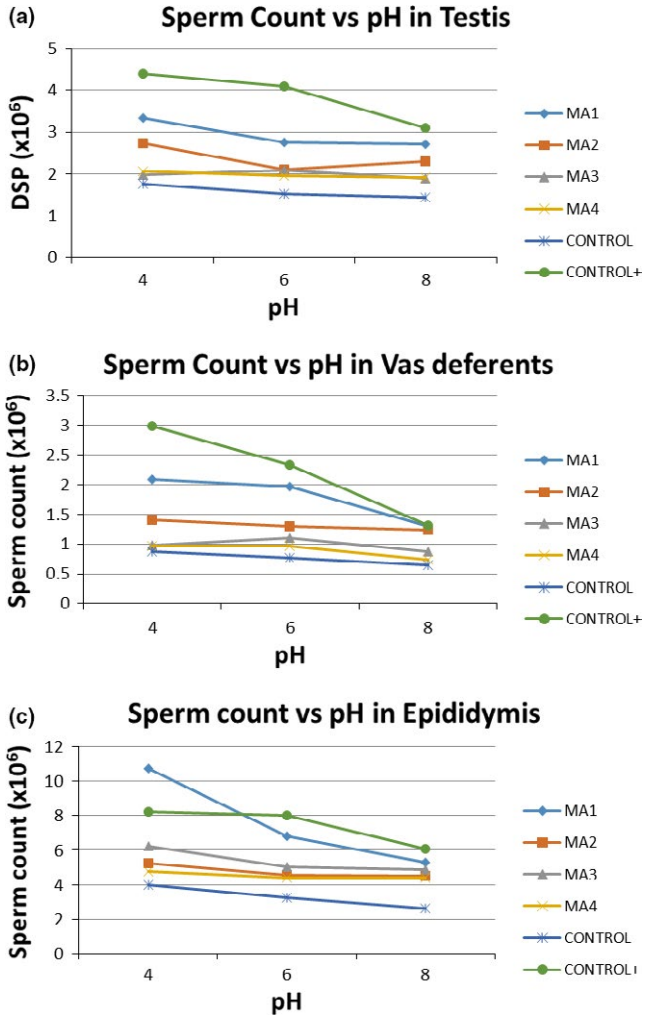

FIGURE 4 Sperm count in testis (a), vas deferens (b) and epididymis (c) at different pH

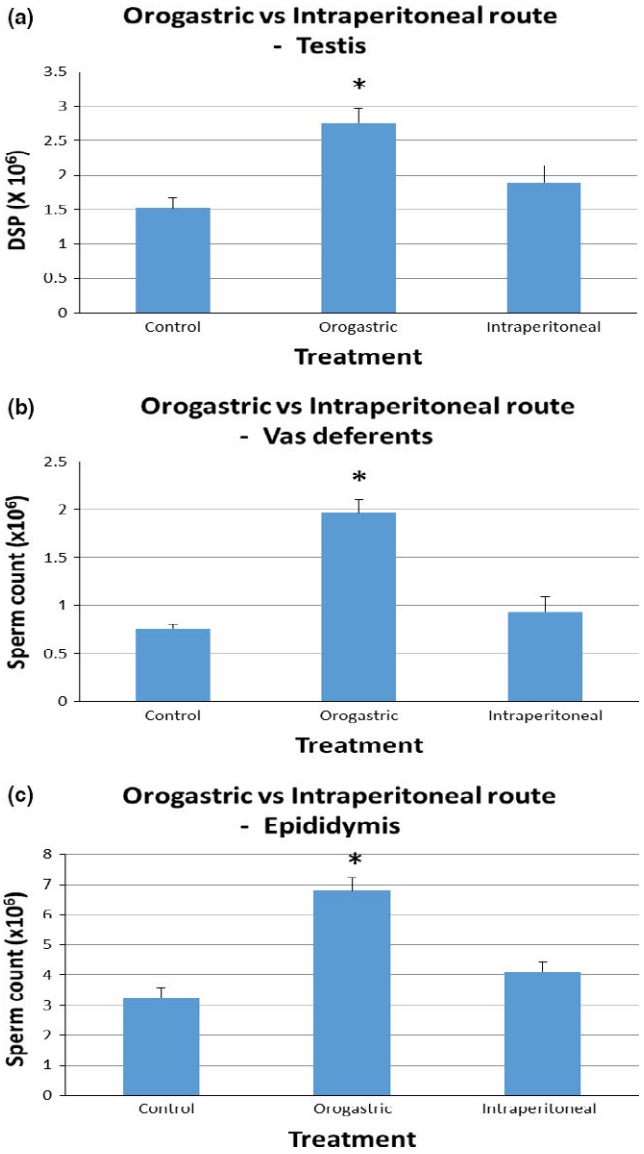

FIGURE 5 Comparison of two routes of administration: Orogastric vs Intraperitoneal in testis (a), deferent duct (b) and epididymis (c) of mice treated with four categories of yellow maca. *$p < .05$ respect to control. Bars indicate the standard mean values

with alkaline aqueous extract showed a decrease in sperm count in all groups which could indicate that the metabolites to acquire biological activity in the digestive tract require acidic pH.

Acid medium is important for activating pepsin at a gastric level. Food with acid pH may favour pepsinogen release by the Chief cells of the stomach to be transformed into pepsin (Fruton, 2002). It has been shown that the acid fermentation through *lactobacillus*, which in turn produce lactotripeptides which have functional properties such as inhibition of angiotensin-converting enzyme (ACE) and thus favour the reduction in blood pressure (Jäkälä & Vapaatalo, 2010). Maca has also been shown to inhibit ACE (Ranilla, Kwon, Apostolidis, & Shetty, 2010).

In recent years, there is a greater interest in alkaline foods; so, it is recommended to decrease the acid diet particularly in patients with chronic kidney disease (Scialla & Anderson, 2013). Diet is an important determinant of the acid load that the kidney must excrete to maintain acid–base balance.

A recent systematic review of the literature showed a lack of evidence for or against diet acid load and/or alkaline water for the initiation or treatment of cancer. Promotion of alkaline diet for cancer prevention or treatment is not justified (Fenton & Huang, 2016). Similarly, evidence that acid loading or an acidic diet produce osteoporosis is not consistent (Hanley & Whiting, 2013). Other studies showed that maca reversed osteoporosis (Gonzales et al., 2010) and treatment of rats with maca during 90 days did not affect kidney function in adult rats (Bernuy & Gonzales, Personal communication).

Our results showed that acid maca administration may improve the sperm count response suggesting the importance of pH when maca is administered to improve physiological parameters in the organisms of experimental animals or humans.

Maca from the first category obtained the largest amount of total polyphenols; so, the larger maca could have an effect on phenolic protection against oxidative degradation along the gastrointestinal tract

and the establishment of a positive antioxidant environment giving it better biological effects as the increase in sperm count.

Our study demonstrates that the best route of administration was orogastric suggesting that gastrointestinal passage promotes the production or conversion of inactive secondary metabolites to active principles.

Maca contains a large amount of aromatic glucosinolates, even more than that reported in other crucifers (Li, Ammermann, & Quiroz, 2001). Glucosinolates are nitrogen-sulphur compounds which are initially in their inactive form. After intake of maca by humans or rodents through the gastrointestinal tract, glucosinolates are converted into isothiocyanates (active metabolites) by the intestinal microflora or by the enzyme myrosinase obtained from the diet (Rouzaud, Rabot, Ratcliffe, & Duncan, 2003). In parallel, hydrolysis occurs and benzylamines, which are precursors of macamides (active metabolites), are produced. Then, the responsible for the biological effects of glucosinolates are those products obtained from its degradation.

Maca has polyphenols, with higher amount in the hypocotyls with higher size. These greater hypocotyls showed also higher biological activity. In the last year, an importance on microbiota on interaction between food and health has been acquired. In fact, metabolism of polyphenols by the intestinal microbiota is crucial for understanding the role of the metabolites of polyphenols and their impact on health (Duda-Chodak, Tarko, & Satora, 2015).

In a study conducted in 2005 by Sharma, it is shown that curcumin, despite having a low availability after oral intake, in the gastrointestinal tract favours the achievement of biologically active levels. Through the digestive tract, the stomach is a primer location for the antioxidant action of secondary metabolites as polyphenols. Effective levels of their antioxidant action would be reached after the consumption of red wine, tea, fruits and vegetables and plant-derived antioxidants (Kanner & Lapidot, 2001). They also suggest that human gastric fluid and the gastric passage may be a necessary medium for enhancing the oxidation of lipids and other dietary constituents and obtain a biological effect (Kanner & Lapidot, 2001).

Our finding is important because it indicates that compounds present in maca as such have no effect on sperm count and need their passage in gastrointestinal tract for conversion into biologically active principles.

Many pharmacological studies show that intraperitoneal route is preferred because it favours a better absorption of active principles (Chan et al., 2014). In addition, the absorption and metabolism of these through the gastrointestinal tract can affect their concentrations and structures in vivo (Memmott et al., 2010; Sharma, Gescher, & Steward, 2005). Moreover, they may also undergo liver metabolism before reaching the circulation, and an amount of the administered treatment may be lost to the thoracic lymph (Abu-Hijleh et al., 1995).

The present study is important looking for scaling up a treatment using the Peruvian traditional medicine in which maca is recognised by millennial that improves fertility (Cobo, 1956). For a better effect on sperm count, data suggest that bigger hypocotyls of maca with low pH should be used and after the hypocotyls are boiled, the preferred treatment route should be the orogastric.

In summary, results of the present study demonstrate that the treatment with the larger size of maca hypocotyl produces the best biological effect; it is confirmed that acid pH improves biological activity of maca and for its action requires a gastrointestinal route of administration.

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CONFLICT OF INTEREST

Lizet Sanchez-Salazar and Gustavo Gonzales have no conflict of interest with this manuscript.

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