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Effect of Supplementation with Dehydrated *Cucumis melo* and Copper on Reproductive Performance of Goats on Rangeland

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ABSTRACT

Background: Microelements are essential for the reproduction of goats; short-term energy supplementation also improves the estrus and ovulatory response to estrus synchronization. The influence of supplementation of cantaloupe leftover and/or copper sulphate on the reproductive response of anovulatory adult goats on rangeland was evaluated.

Methods: Sixty-seven mixed-breed adult goats were divided into four experimental groups: Control (C, n=16), without cantaloupe or Cu supplementation; Copper group (Cu, n=17), supplemented with 1 mL of copper sulphate at 2.5%; Cantaloupe group (Ca, n=17), supplemented with 200 g of *Cucumis melo* and Cu and cantaloupe group (Ca-Cu, n=17), supplemented with 1 mL of copper sulphate+200 g of *Cucumis melo*. Estrus of goats was synchronized and fixed-time artificial insemination (FTAI) was applied.

Result: There was an increase in ovulation rate for the Ca-Cu goats when compared to C (1.93 vs 1.46, respectively). Also, embryo losses decreased for Ca-Cu goats compared to C, Cu and Ca (13 vs. 42.33%, $p<0.05$). It was concluded that the combined supplementation of cantaloupe leftovers and Cu could be an alternative to increase the ovulation rate and decrease embryo losses, a frequent reproductive problem in goats on rangeland.

Key words: Copper, Cucumis melo, Goats, Reproductive response, Supplementation.

INTRODUCTION

Goats are a central part of the livestock industry in the world (Azrul-Lokman *et al*. 2018; Karnani *et al.* 2021), with most of the goats located in arid and semiarid zones (Mellado *et al*. 2012). In the semi-arid areas of northern Mexico, goats are exploited extensively they have seasonal anestrus, which coincides with the dry season (spring), where forage's availability and nutritional value decreases (Urrutia-Morales *et al.* 2012). During this season, forages of rangelands have high levels of fiber, which does not provide enough nutrients to satisfy the reproductive function of goats (Alexandre and Mandonnet, 2005; Mahfuz *et al.* 2018). This low nutrient ingestion is reflected in decreased body energy reserves, which significantly affect the reproductive function of female goats (Urrutia-Morales *et al.* 2012). A strategy to mitigate low nutrient intake is to offer feed supplementation during the dry season (Kawas *et al.* 2010). Feed supplementation can enhance the response of goats to estrus synchronization (Nogueira *et al*. 2016). It has also been reported that short-term nutritional supplementation increases the ovulatory rate (Scaramuzzi *et al*. 2010). That energy supplementation could be administered using agricultural byproducts such as fruit leftovers, like cantaloupe, which has a high level of energy (Liu *et al*. 2009).

Besides energy, other essential nutrients are microelements necessary for growth, production (Yatoo *et al*. 2013) and reproduction (Chester-Jones *et al*. 2013). It has been reported that the ovarian activity in ruminants is affected by mineral deficiency (Yatoo *et al*. 2013), particularly Cu, for its role in reproduction. This element has been reported as the second most widespread mineral deficiency that affects grazing globally (Anchordoquy *et al*. 2021). Pregnancy is associated with an increase of plasma Cu as serum ceruloplasmin. Also, it has been demonstrated that embryonic death and fetal losses happen with Cu deficiencies (Hill and Shanon, 2019). Considering those above, this study aimed to evaluate the influence of an energetic supplementation (cantaloupe crop leftovers) and copper upon the reproductive response of adult goats during seasonal anestrous and kept on a semi-arid rangeland of northern Mexico.

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MATERIALS AND METHODS

Ethics statement

All methods and procedures used in this study regarding animal use and care were performed in strict conformity of

international (FASS, 2010) and national (NAM, 2002) guidelines for the use and care of animals with institutional approval number UAAAN-UL-38111-425503002-2867.

Location and environmental conditions

This investigation was carried out in Ejido Petronilas in Northern Mexico from April to June 2018 (25°N, 103°W). This site is located at 1111 meters above sea level, has a semi-arid climate, an average annual rainfall of 230 mm and an average yearly temperature of 22.5°C.

Animals and experimental treatments

Sixty-seven mixed breed multiparous goats, managed under a semi-intensive system, were used. Goats grazed during the day (11:00 to 16:00 h) and were penned at night, with free access to clean water. Goats were randomly divided into four homogenous groups considering body weight (BW, 38±2.7 kg) and body condition score (BCS, 2.13±0.2 units) and assigned to the following treatments: Control (C, n=16), without food or mineral supplementation; Copper group (Cu, n=17), which received 1 mL of copper sulphate at 1% ($CuSO_4$, distilled water plus 1% copper sulfate) s.c. one dose per animal at -30 days (FTAI, 0 day); cantaloupe group (Ca, n=17) which was supplemented with 200 g of dehydrated cantaloupe (*Cucumis melo*) per animal at 8:00 h, before goats were led out to graze, during 14 days, starting at day-7 (FTAI); goats in the copper and cantaloupe group (Ca-Cu, n=17) were supplemented with copper sulphate and cantaloupe in the same amounts and frequency as the Ca and Cu groups. A schematic representation with the main activities performed during the experimental protocol is shown in Fig 1.

Supplementation with *C. melo*

To supplement the Ca and Ca-Cu, the leftovers of the cantaloupe harvest were used for goats due to their unsuitable quality for human consumption. This material was harvested, crushed and left to dry at ambient temperature until dehydrated. Afterward it was ground and stored in a fresh and dry place until used. The chemical composition of the processed cantaloupe (Table 1).

Table 1: Chemical composition of dehydrated cantaloupe leftover (*C. melo*) used as a food supplement in mixed breed adult goats during the dry season (anestrus).

Humidity	Crude protein (PC)	Fat	Crude fiber	Ash
10%	18%	5.5%	20%	17%

Synchronization and FTAI

Goats from the four groups were subjected to estrus synchronization administering 20 mg of progesterone (0.4 mL per goat, i.m.; Progesterona®, Zoetis, México) four days before insemination. 24 h after the progesterone administration, 50 IU of human chorionic gonadotrophin (hCG) were administered in the vulvar submucosa (Chorulon®, Intervet). 72 h after signs of estrus, fixed-time artificial insemination (FTAI; day 0) was performed in the cervical area with fresh semen (Fig 1).

Measurements and response variables

Bodyweight (BW), body condition score (BCS) and serum glucose concentrations

Each goat was weighted before the beginning of the feed supplementation and at the end of the study, using a weighing scale (WeiHeng, Model: WH-C100, China). The BCS was determined by dorsal and costal palpation on a 1-5 scale, with one being very thin and five being overly fat, with intermediate points. A blood sample was extracted by jugular venipuncture to quantify the serum glucose levels (Accu-Check® Sensor Comfort, Roche, Mexico). BW, glucose levels and BCS were recorded before the beginning of the treatments, seven days before FTAI, the day of FTAI and seven days after FTAI (-30 -7, 0 y 7 d, respectively).

Ovulatory response, ovulatory and embryonic rate and pregnancy diagnosis

Ten days after FTAI, the percentage of females ovulating was determined by observing the presence of corpus luteums. For this, a transrectal ultrasound was used with a 7.5 MHz transducer (Aloka SSD 500 echo camera, Overseas Monitor Corp. Ltd., Richmond, BC, Canada) and afterward, the ovulatory

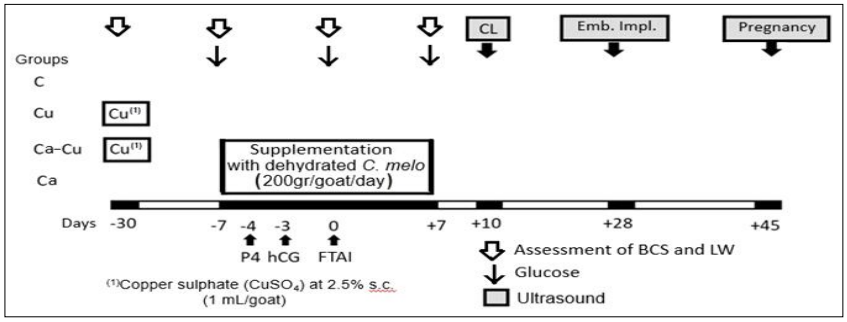

Fig 1: Schematic representation of the experimental protocol of feed supplementation with dehydrated leftover of *C. melo* and/or treated with 2.5% of copper sulphate upon the reproductive response of adult mixed-breed goats during the dry season (anestrous). US-CL= Ultrasound to determine the presence of *Corpus luteums*. Cu= Copper, Hormonal, Tx= Ultrashort hormonal treatment. P4= progesterone, hCG= Human chorionic gonadotropin, FTAI= Fixed time artificial insemination.

rate was calculated (total number of corpus luteums divided by the number of goats in each group). Also, a transrectal ultrasound exam was carried out 28 days after FTAI to quantify the number of embryos (embryonic rate) and at 45 days post-FTAI, the pregnancy diagnosis was performed. All evaluations were performed at 08:00 h by the same expert technician.

Statistical analysis

The statistical model used for the data obtained for the BW, BCS and serum glucose level variables was an analysis of variance (GLM procedure of SAS) for comparing independent groups. When statistical differences were found, an LSD test was performed to establish differences between treatment means. The percentage of females that ovulated and the ovulatory, embryonic and pregnancy rates were compared by SAS's GENMOD procedure (9.1 version, Cary, NC, EE. UU.). The results are presented as means (±SE) and differences were considered significant with $p \leq 0.05$.

RESULTS AND DISCUSSION

The present research hypothesized that supplementation with dehydrated cantaloupe and/or copper sulphate before and after insemination would enhance the metabolic state and the reproductive response of goats on rangeland. There were no significant changes for BW due to treatments. Likewise, BCS did not vary with treatments ($p<0.05$; Table 2). However, some relevant changes were detected for the reproductive response. The ovulatory rate was higher for the Ca-Cu than C ($p<0.05$). In this matter, it has been demonstrated that a short-term energetic supplementation increases in the ovulatory rate and prolificity in small ruminants (Scaramuzzi *et al*. 2006). Goats in the Ca-Cu group showed fewer embryo losses than C, Cu and Ca goats (13 vs 42.33%, $p<0.05$), probably due to the interaction or the feed supplementation with cantaloupe and Cu, because of the antioxidant properties of cantaloupe (Bianchi *et al*. 2016), which improves blood flow in the endometrium and has less hyperandrogenism, which helps to have higher fertility and a better embryos implantation (Agarwal *et al*. 2012). Also, Cu probably potentiated such antioxidant effects because it plays an important role in eliminating the excessive production of superoxide radicals, improving embryo survival by increasing implantation and cellular division (Narasimhaiah *et al*. 2018; Kalmath and Narayana, 2019). Considering that

Table 2: Means±SE of body weight (BW, kg), body condition score (BCS, 1-5 units) and serum glucose levels (mg/dL) for days -30, -7, 0 (FTAI) and 7, from adult mixed-breed goats on rangeland supplemented with dehydrated cantaloupe (*C. melo*) and/or copper sulphate (Cu) during seasonal anestrus.

Treatment	Time				
	Day-30	Day-7	Day 0 (FTAI)	Day+7	SE
	Body weight (BW, kg)				
Control	37.9	37.2	36.9	38.6	0.91
Copper (Cu)	37.8	37.3	36.7	37.2	0.99
Cantaloupe-Cu	39.0	38.6	38.2	38.5	0.91
Cantaloupe	39.2	38.4	37.7	39.3	0.95
	Body condition score (BCS, 1-5 units)				
Control	2.18^{abcd}	2.06^{abcd}	2.00^{cd}	2.18^{abcd}	0.04
Copper (Cu)	2.20^{abc}	2.20^{abc}	1.97^{d}	2.20^{abc}	0.03
Cantaloupe-Cu	2.23^{ab}	2.26^{a}	2.11^{abcd}	2.23^{ab}	0.04
Cantaloupe	2.20^{abc}	2.17^{abcd}	2.20^{abc}	2.20^{abc}	0.04
	Serum glucose levels (mg/dL)				
Control	52.4^{cd}	39.1^{e}	57.8^{ab}	61.1^{a}	0.87
Copper (Cu)	51.7^{cd}	37.1^{e}	58.0^{ab}	55.4^{bc}	0.81
Cantaloupe-Cu	49.2^{d}	38.2^{e}	59.8^{a}	57.0^{ab}	0.83
Cantaloupe	52.5^{cd}	40.8^{e}	58.5^{ab}	58.3^{ab}	0.94

^{a,b,c,d} Within columns and variables, means different superscript ($p<0.05$).

Table 3: Reproductive response of adult mixed-breed goats on rangeland supplemented with dehydrated cantaloupe (*C. melo*) and/or copper sulphate (Cu) during anestrous during days -30, -7, 0 (FTAI) and 7. Estrus of goats was synchronized with progesterone and human chorionic gonadotrophin and were inseminated using fixed-time artificial insemination (fresh semen).

	Ovulation rate	Numbers of embryos	Embryos losses	Pregnancy rate
Control	1.46 ± 0.13^{b}	1.44 ± 0.13^{a}	$44\ (4/9)^{a}$	$56\ (9/16)^{a}$
Copper (Cu)	1.54 ± 0.13^{b}	1.00 ± 0.0^{a}	$40\ (2/5)^{a}$	$29\ (5/17)^{c}$
Cantaloupe-Cu	1.93 ± 0.22^{a}	1.50 ± 0.13^{a}	$13\ (1/8)^{b}$	$47\ (8/17)^{ab}$
Cantaloupe	1.60 ± 0.18^{ab}	1.14 ± 0.09^{a}	$43\ (3/7)^{a}$	$41\ (7/17)^{bc}$

^{a,b,c,d} Within columns and variables, means different superscript ($p<0.05$).

groups treated separately with Cu and cantaloupe showed no differences compared with C, we assume a synergism between cantaloupe and Cu, benefiting the ovulatory rate and decreasing the embryo losses.

Additional energy intake has been shown to increase body energy stores, essentials for reproductive performance (Widiyono *et al.* 2020). However, BCS in our experimental animals did change with the feed supplementation (average 2.1±0.1, $p>0.05$). So, we consider that grazing in grasslands was sufficient to satisfy their metabolic maintenance needs, which was also reflected in blood glucose levels since very similar results were observed between the treated groups ($p>0.05$).

Even though in previous studies it has been proposed that goats can select a diet that reflects their needs of protein by their physiologic state (Mellado *et al.* 2006), results of the present study show that grazing goats were not able to obtain the total recommended amount of protein for pregnancy, because only about 50% of the goats from the supplemented groups with cantaloupe and the control group got pregnant (C, 56 vs Ca-Cu, 47%; $p>0.05$). Although Cu has been related to an essential role in the metabolism of proteins, lipids and carbohydrates (Underwood and Suttle, 2003), the Cu group had the lowest number of embryos and pregnancy percentage of all groups (Table 3). On the other hand, even though no significant differences were observed, the ovulatory rate slightly increased compared to the control group. These results are consistent with Peacey *et al.* (2020) who reported that exogenously added Cu can interact with receptors and other extracellular components to promote the release of GnRH-I and influence the action of LH/FSH. Using a greater number of animals in our experiment, we could have a significant difference between the goats supplemented with copper and the control group in terms of ovulatory rate.

We consider is essential to conduct additional research to elucidate the interaction of copper and cantaloupe with molecular measurements to understand better its effect upon reproductive parameters in goats on rangeland.

CONCLUSION

Cantaloupe byproduct, which an agricultural waste, in combination with copper, can be supplemented to the diet of goats on rangeland to compensate for nutrient deficiency. This combination markedly improved the reproductive performance of mixed-breed goats during the dry season.

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Conflict of interest: None.

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