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EFFECTS OF BLACK WATTLE (*Acacia mearnsii*) SUPPLEMENTATION ON OVARIAN ACTIVITY, UTERINE INVOLUTION, AND HORMONE PROFILES IN DAIRY COWS AT HIGH-ALTITUDE CONDITIONS

Efectos de la suplementación con acacia negra (*Acacia mearnsii*) sobre la actividad ovárica, la involución uterina y los perfiles hormonales en vacas lecheras en condiciones de altura

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ABSTRACT

The feeding program carried out during the pre-partum and post-partum period in dairy cows is crucial to preserve their reproductive performance. The goal of the present study was to assess the effect of black wattle (*Acacia mearnsii*) supplementation on ovarian activity, uterine involution, and hormone profiles in dairy cows maintained at high-altitude conditions. A total of 10 Holstein dairy cows were divided into 2 groups: control (C; n=5) and supplemented group (AM; n=5). A dehydrated black wattle (AM; *Acacia mearnsii*) supplement was administered to AM group and adjusted to 22% crude protein per animal (Dehydrated Supplement; MS= 2.7 kg AM/day per cow, ~20% of total DM). Body weight (BW) was recorded individually in different periods (day 15 pre-partum, and day 19, 34, and 49 post-partum). Ovarian [follicles (FL), corpora lutea (CL)] and ovarian diameter (OD; mm)] and uterine structures [uterine horn thickness (HT; mm), cervix length (CrL; cm) and diameter (CD; cm), and uterine involution %] were assessed till day 34 postpartum. Moreover, hormone profiles such as thyroid-stimulating hormone (TSH; IU/mL), follicle-stimulating hormone (FSH; IU/mL), luteinizing hormone (LH; IU/mL), and progesterone (P4; ng/mL) were measured till day 49. Statistical differences were observed in BW when day 15 prepartum was compared to day 19 postpartum ($p=0.05$). However, no differences were observed between C and AM group regarding BW within each time-point ($p>0.05$). No changes were observed in FL over time irrespective of the group studied ($p< 0.05$). However, FL was significantly different when C and AM group were compared within each time-point ($p< 0.05$). Although no significant differences were observed on day 5 post- partum regarding the number of CL, significant differences were observed on day 25 and day 34 post-partum between groups ($p> 0.05$). OD increased from day 5 to Day 35 post-partum irrespective of the group assessed and differ between groups within time-points ($p< 0.05$). Differences were observed in TSH within day 19 and day 49 post-partum ($p< 0.05$). FSH was statistically different between groups on day 19 post-partum ($p< 0.05$). LH was more variable than TSH and FSH among and within time-points and groups ($p< 0.05$). Differences were observed between groups regarding P4 within day 19 and 49 post-partum ($p< 0.05$). In conclusion, overall although no differences were observed in BW between groups or time-points, the supplementation with AM showed differential patterns in reproductive tract structures/dimensions and hormone levels in dairy cows maintained at high-altitude conditions.

Keywords: ovarian activity, uterine involution, hormone levels, Acacia spp., dairy cows

RESUMEN

La alimentación que se lleva a cabo durante el periodo pre y postparto en vacas lecheras es crucial para preservar su rendimiento reproductivo. El objetivo del presente estudio fue evaluar el efecto de la suplementación con Acacia negra (*Acacia mearnsii*) en la actividad ovárica, la involución uterina y los perfiles hormonales en vacas lecheras mantenidas en condiciones de altitud. Un total de 10 vacas lecheras Holstein fueron divididas en 2 grupos: control (C; n=5) y suplementado (AM; n=5). Al grupo AM se le administró un suplemento de Acacia negra deshidratada ajustado al 22% de proteína bruta por animal (Suplemento Deshidratado; MS= 2,7 kg de AM/día por vaca, ~20% de la MS total). El peso corporal (PC) fue registrado individualmente en cada periodo (día 15 pre-parto y días 19, 34 y 49 post-parto). Se evaluaron las estructuras ováricas [folículos (FL), cuerpos lúteos

(CL)] y diámetro ovárico (OD; mm)] y uterinas [grosor del cuerno uterino (HT; mm), longitud y diámetro del cérvix (CrL; cm), y % de involución uterina] hasta el día 34 post-parto. Además, se midieron los perfiles de la hormona estimulante de la tiroides (TSH; UI/mL), folículo estimulante (FSH; UI/mL), luteinizante (LH; UI/mL) y progesterona (P4; ng/mL) hasta el día 49. Se observaron diferencias significativas en PC cuando se comparó el día 15 pre-parto con el día 19 post-parto ($p=0,05$). Sin embargo, no se observaron diferencias entre los grupos C y AM en lo que respecta al PC en cada tiempo evaluado ($p>0,05$). FL fue significativamente diferente cuando se compararon los grupos C y AM dentro de cada punto de tiempo evaluado ($p< 0,05$). Aunque no se observaron diferencias significativas en el día 5 post-parto con respecto a CL, se observaron diferencias significativas en el día 25 y en el día 34 post-parto entre los grupos ($p> 0,05$). DO aumentó desde el día 5 hasta el día 35 post-parto independientemente del grupo evaluado y difirió entre grupos en cada punto de tiempo evaluado ($p< 0,05$). Se observaron diferencias en la TSH en el día 19 y 49 post-parto ($p< 0,05$). La FSH fue estadísticamente diferente entre los grupos en el día 19 post-parto ($p< 0,05$). La LH fue más variable que la TSH y la FSH entre y dentro de los puntos de tiempo y los grupos evaluados ($p< 0,05$). Se observaron diferencias entre grupos con respecto a la P4 en el día 19 y 49 post-parto ($p< 0,05$). En conclusión, aunque en general no se observaron diferencias en PC entre grupos o tiempos, la suplementación con AM mostró patrones diferenciales en las estructuras y dimensiones del tracto reproductivo así como en los niveles hormonales en vacas lecheras mantenidas en condiciones de altitud elevada.

Palabras clave: Actividad ovárica, involución uterina, niveles hormonales, Acacia spp., vacas lecheras

INTRODUCTION

The genus Acacia is the largest genus of flowering plants in Australia, occurring in all terrestrial habitats from tropical rainforests to alpine regions (Maslin, 2001). The black wattle (*Acacia mearnsii*) has been considered an invasive tree species in several parts of the world; however, it has an interesting potential as it adapts to different climatic conditions (Monteiro et al., 2019). Currently, due to the high demand for meat and milk consumption, new feeding alternatives have been implemented in ruminants that are environmentally sustainable and improve low and medium-scale livestock farming (Junior et al., 2017). The black wattle can be used as fodder for dairy ruminants due to its high protein nutritional value and can be used to obtain low-cost diets with significant production yields (Uushona et al., 2021). There is currently a growing interest in the use of secondary metabolites produced by plants (Jouany and Morgavi, 2007). The black wattle has a high content of condensed tannins that when supplied in the diet bind to proteins in the rumen and reduce their degradation (Noreljaleel et al., 2019). The protein balance (crude protein) needed in ruminants increases milk yield, the opposite affects negatively causing detrimental effects in ruminants (Waghorn, 2008). Plants rich in tannins and saponins, such as the black wattle, have the potential to improve rumen microbial protein flow by increasing its efficiency of diet utilization and decreasing gas production (Barros-Rodríguez et al., 2015; Gerlach et al., 2018). Condensed tannins reduce rumen methanogenesis by decreasing hydrogen formation and inhibiting methanogenic compounds (Eckard et al., 2010; Patra and Saxena, 2011; Vélez-Terranova, 2014).

In addition, condensed tannins possess health benefits due to their bacteriostatic/bactericidal activity (Okuda, 2005), as well as anticarcinogenic (Uushona et al., 2021) and lipid peroxidation inhibitors (Okuda, 2005). The study of the antioxidant activity of condensed tannins in vitro (inhibiting hydroxyl radicals) and in vivo (inhibiting the enzyme xanthine oxidase) shows that they are free radical scavengers and inhibit tissue oxidation better than vitamins A, C, and E (Fine, 2000). Regarding the effect of diets based on grass legumes on reproductive parameters, the use of lucerne (Medicago sativa) and common sainfoin (*Onobrychis viciifolia*) showed that there is no clear evidence that the presence of condensed tannins in low-moderate concentration contained in these species has a significant effect on reproductive parameters (Mueller-Harvey et al., 2019). However, previous studies observed a beneficial effect of the presence of condensed tannins on reproductive parameters in small ruminants (Blache et al., 2008). Several studies analyzing the effect of grazing pastures sown with common bird's-foot trefoil (*Lotus corniculatus*) compared to others sown with perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) during mating showed a higher ovulation rate and prolificacy for the former type of pasture (Barry et al., 1999; Ramírez-Restrepo and Barry, 2005; Viñoles et al., 2009). It is likely that also the structure of the condensed tannins, in addition to the amount of condensed tannins ingested, positively influences the effect they have on the reproductive tract in cattle (Cheng et al., 2019). On the other hand, supplementation of cows with diets up to 33% of daily dry matter from white leadtree (*Leucaena leucocephala*) as a forage supplement in silvopastoral livestock systems showed no differences between treatments with respect to follicle count, number of follicular waves, and the lifespan of the first pre-ovulatory follicle (Bottini-Luzardo et al., 2015). However, active principles present in white lead tree, such as mimosine or 2,3-DHP, are chelating agents of minerals such as iron, iodine, and zinc being essential for the synthesis and transport of steroid hormones in the follicle and oocyte maturation (Halliday et al., 2013; Shelton et al., 2019). Inhibition or decreased levels of these minerals can decrease ovulation and corpus luteum development in ruminants (Akhtar et al., 2015). In addition, there are antimitotic and anti-apoptotic effects on granulosa cells capable of blocking luteinizing hormone (LH) receptors at the ovarian level (Sadeghian-Nodoushan et al., 2014). Such effects are important because apoptosis in granulosa cells is delayed or even inhibited when exposed to a medium containing active principles such as mimosine which would give a longer lifespan to the corpus luteum (Quirk et al., 2004; Bottini-Luzardo et al., 2015).

Thus, the main objective of the present study was to determine the effects of dietary supplementation with black wattle (*Acacia mearnsii*) on ovarian activity, uterine involution, and hormonal profiles in dairy cows maintained at high-altitude conditions.

MATERIAL AND METHODS

Reagents

All reagents were purchased from Human DW (Wiesbaden, Germany) and from Monobind Inc. (CA, USA).

Animals and experiment location

A total of 10 Holstein breed dairy cows (2nd to 4th lactation; BW: ~580 ± 15 kg; BCS: 3-3.5) were randomly divided into 2 experimental groups; control group (C; n=5) and supplemented group (AM; n=5). Both experimental groups were supplemented with mineral blocks and water ad libitum and maintained under the same climate conditions (Cotopaxi Province, Ecuador; Coord.: S~01° 25´ 00´´; W~78° 36´ 00´´; Precip.:~520 mm; R.H.:~90%; M.T.:~13.2 °C; Alt.: ~3,500 m.a.s.l.).

Diet and *Acacia mearnsii* supplementation

A dehydrated black wattle (*Acacia mearnsii*) supplement was administered (individually) to AM group and adjusted to 22% crude protein per animal (Dehydrated Supplement; MS= 2.7 kg AM/day per cow, ~20% of total DM). The chemical composition of both diets (control vs. supplemented) are shown in Table 1.

Table 1. Chemical composition of diets for each experimental group.

Experimental Group	Control (C)	Supplemented (AM)
Ingredients (%)		
Ground Acacia mearnsii forage (GAMF)	0.00	20.00
Ground Lolium perenne forage (GLPF)	66.45	54.28
Ground Medicago sativa forage (GMSF)	33.55	25.72
Chemical composition (g / 100 g DM)		
Dry Matter (DM)	88.67	89.37
Organic Matter (OM)	90.39	91.21
Crude Protein (CP)	20.00	20.00
Ether Extract (EE)	3.51	3.35
Crude Fibre (CF)	23.06	23.10
Neutral Detergent Fibre (NDF)	43.77	42.27
Acid Detergent Fibre (ADF)	23.54	23.94
Net Energy (NE; MJ/ kg DM)	9.21	9.20
Condensed tannins (CT; g / kg DM)	0	3.56

Body weight (BW) score in each period (partials recorded individually before supplementation on day 15 pre-partum, and on day 19, 34, and 49 post-partum. The general health status of the animals was determined at the beginning and during the study, recording all events related to parturition and puerperium. The study was conducted between August 2019 and March 2021.

Reproductive tract ultrasonography: ovarian activity and uterine involution

The ultrasonographic assessment was performed every 6 days using an ultrasound device (KAIXIN-RKU10, SIUI, China). The development of uterine involution was evaluated in cows on day 15 pre-partum, and on day 19, 34, and 49 post-partum, by determining the degree of uterine involution in relation to the chronology of days during the post-partum period. Reproductive tract structures such as uterine horn thickness (HT; mm), cervix length (CrL; cm) and diameter (CD; cm), and uterine involution %] were assessed till day 34 postpartum. The ovarian activity was determined to identify the appearance of the dominant follicle (FD; diameter ≥ 5 mm). Chronological measurements were taken till ovulation or follicular atresia or even when showing P4 values above 1 ng/mL. The first ovulation was determined with the elimination of the pre-ovulatory follicle and the development of the corpus luteum (CL), as well as when P4 was above 1 ng/mL (Shrestha et al., 2005). The timing of luteal activity was determined by assessing serum P4 concentrations above 1 ng/mL (Hannan et al., 2010). The diameter of the CLs was also measured during the study. Ovarian structures [follicles (FL), corpora lutea (CL)] and ovarian diameter (OD; mm) were assessed till day 34 post-partum.

Blood samples and hormone analyses

Blood was collected by coccygeal vein puncture and deposited in IDEXX VetTube™ tubes (IDEXX, Maine, USA). For haematological analyses, 5 mL of blood was collected with EDTA (Ethylenediamine tetraacetic acid: 1 mg/mL) and 10 mL for biochemical blood parameters without EDTA. Samples were centrifuged at 3,500 g for 15 minutes to obtain blood serum. Then, serum samples were frozen at -10 °C for further analysis. Blood analysis was performed by using the haematological capillary technique, and for haematochemical analysis of blood parameters, spectrophotometry combined with chemical colorimetry (semi-automatic biochemistry analyzer, Mindray BA-88A, China). The determination of progesterone levels (P4; ng/mL) was performed till day 49 by using the Enzyme-Linked Immunosorbent Assay (ELISA) technique and a semi-automated ELISA programmable strip reader (Stat Fax 4700, MA, USA), according to the manufacturer indications. In addition, hormone profiles such as thyroid stimulating hormone (TSH; IU/mL), follicle stimulating hormone (FSH; IU/mL), and luteinizing hormone (LH; IU/mL) were measured till day 49.

Statistical analysis

The statistical analyses between the control and the treated group were carried out by using repeated measures of variance (ANOVA) tests followed by Bonferroni's test (SPSS v.25, USA). The plasma concentrations analyses of different hormones were performed by using a repeated measure design approach with treatments (groups) and time of sample collection (pre-partum and post-partum monitorization) being fixed effects and with all interactions included. All data are presented as the means ± SEM and significancy was set at $p \leq 0.05$.

RESULTS

The body weight scores in dairy cows supplemented with black wattle (*Acacia mearnsii*) vs. control are shown in Table 2. Overall, cow BW was unaffected by treatment × day or treatment alone ($p > 0.05$). However, significant differences were observed in AM group when day 15 pre-partum was compared to day 19 post-partum ($p < 0.05$; Table 2). Greater weight gains were observed for AM group compared to control group, however no significant differences were detected between groups within each day or among days ($p > 0.05$; Table 2). Overall, these data demonstrate that cows receiving control diet (C) or *Acacia mearsii* diet (AM) had greater BW pre-partum and lower BW post-partum, however, although non-significant the weight gains in AM group were greater when compared to cows feed with the control diet over time (Table 2).

Table 2. Body weight scores in dairy cows supplemented with black wattle (*Acacia mearnsii*) on day 15 pre-partum, and on day 19, 34, and 49 post-partum at high altitude conditions.

	Body Weight (kg)			
Groups	Day 15 pre-partum	Day 19 post-partum	Day 34 post-partum	Day 49 post-partum
Control (C)	564.80±19.82 A	526.40±13.59 A	526.80±14.01 A	534.80±19.44 A
Supplemented (AM)	580.69±12.24 A	535.60±13.01B	561.40±17.91 A	564.00±17.57 A

Mean ± S.E.M values. Different superscripts within a row (A-B) show statistical differences among days within each group ($p \leq 0.05$). No differences were observed between groups (C vs. AM) within each time-point ($p > 0.05$).

The number of ovarian structures regarding each experimental group are shown in Table 3. The number of follicles remained constant over time irrespective of group or time-point assessed; thus, no significant changes were observed in the number of follicles over time irrespective of the group studied ($p < 0.05$; Figure 3). However, the total number of follicles was significantly different when the control and supplemented group were compared within each time-point ($p < 0.05$; Table 3). Although no significant differences were observed on day 5 post- partum with regard to the number of corpora lutea significant differences were observed on day 25 and day 34 post-partum between both groups ($p > 0.05$; Table 3). Ovarian dimensions (diameter) increased from day 5 to Day 35 post-partum irrespective of the group assessed and differ between groups within time-points ($p < 0.05$).

The uterine parameters assessed regarding each experimental group are shown in Table 3. There was no effect on uterine horn thickness within each time-point between groups ($p > 0.05$). However, on day 25 and day 34 post-partum significant differences were observed regarding horn thickness when compared to day 5 post-partum in both groups ($p < 0.05$). Similarly, regarding the uterine involution no statistical differences were observed within each time-point between both groups ($p > 0.05$). However, on day 25 and day 34 post-partum significant differences were observed regarding uterine involution when compared to day 5 post-partum in both groups ($p < 0.05$; Table 3). Regarding cervical structures, there was no effect on both diameter and length parameters within each time-point between groups ($p > 0.05$). However, on day 34 post-partum significant differences were observed regarding cervical length and diameter when compared to day 5 and day 25 post-partum in both groups ($p < 0.05$). No statistical differences were observed between day 5 and day 25 post-partum regarding cervical length and diameter irrespective of the group or time-point assessed ($p > 0.05$; Table 3).

Table 3. Ovarian and uterine structures in dairy cows supplemented with black wattle (*Acacia mearnsii*) on day 5, 25, and 34 post-partum at high-altitude conditions.

Timepoints	Day 5 post-partum		Day 25 post-partum		Day 34 post-partum	
Groups	Control (C)	Supplement. (AM)	Control (C)	Supplement. (AM)	Control (C)	Supplement. (AM)
Ovarian structures						
follicles	1.40±0.67Aa	2.00±0.44Ba	2.00±0.54Aa	2.40±0.24Ba	2.20±0.20Aa	2.00±0.44Aa
corpora lutea	0.00±0.00Aa	0.00±0.00Aa	0.00±0.00Aa	0.20±0.20Bb	0.00±0.00Aa	0.40±0.24Bb
diameter	11.20±0.73Aa	15.20±1.62Bb	14.20±2.01Aab	18.00±0.83Bbc	15.40±1.80Ab	19.80±3.51Bc
Uterine structures						
horn thickness	21.00±1.78Aa	20.80±0.97Aa	15.60±0.81Ab	17.20±0.37Ab	14.60±0.24Ab	16.60±0.51Ab
uterine involution	24.20±1.80Aa	25.60±1.69Aa	85.00±4.47Ab	86.00±2.91Ab	99.00±1.00Ab	100±0.00Ab
cervix length	15.20±0.66Aa	14.60±0.40Aa	13.40±0.67Aab	13.20±0.20Aab	12.20±0.37Ab	12.40±0.24Ab
cervix diameter	7.60±040Aa	7.80±0.20Aa	6.60±0.24Aab	6.80±0.37Aab	5.60±0.24Ab	5.70±0.25Ab

Control (C) vs. Supplemented (AM) groups. Follicles (FL; number), corpora lutea (CL; number) ovary diameter (OD; mm), uterine horn thickness (HT; mm), cervix length (CrL; cm) and diameter (CD; cm), and uterine involution (%). Mean ± S.E.M values. Different superscripts within a row (A-B) show statistical differences within the same day and within each reproductive tract structure ($p \leq 0.05$). Different superscripts within a row (a-c) show statistical differences between days, among groups, and within each reproductive tract parameter ($p \leq 0.05$).

The hormone level profiles analyzed regarding each experimental group are shown in Table 4. Before calving (day 15 pre-partum), the plasma concentration of TSH did not differ within the time-point ($p > 0.05$). However, significant differences were observed regarding TSH concentration within day 19 and day 49 post-partum ($p < 0.05$). On the other hand, FSH concentration was statistically different between groups only within day 19 post-partum ($p < 0.05$).

Table 4. Hormone profiles in dairy cows supplemented with black wattle (Acacia mearnsii) on day 15 pre-partum, and on day 19, 34, and 49 post-partum at high altitude conditions.

Timepoints	Day 5 post-partum		Day 25 post-partum		Day 34 post-partum		Day 49 post-partum	
Groups	Control (C)	Supplement. (AM)	Control (C)	Supplement. (AM)	Control (C)	Supplement. (AM)	Control (C)	Supplement. (AM)
TSH	0.73±0.14Aa	0.86±0.27Aa	0.38±0.39Ab	0.67±0.13Ba	0.39±0.56Ab	0.51±0.05Aab	0.39±0.08Ab	0.59±0.06Bab
FSH	-	-	14.07±4.02Aa	7.00±2.92Bb	11.78±0.45Aab	6.80±1.74Ab	10.02±2.68Aab	13.44±3.60Aa
LH	-	-	0.47±0.19Aa	1.99±1.58Bb	0.45±0.14Aa	1.33±0.07Bb	0.58±0.17Aa	1.12±1.09Bab
P4	-	-	0.81±0.14Aa	2.95±1.16Bb	2.04±0.54Ab	2.68±0.67Ab	2.84±0.96Ab	6.23±1.70Bc

Control (C) vs. Supplemented (AM) groups. Thyroid-stimulating hormone (TSH; IU/mL), follicle-stimulating hormone (FSH; IU/mL), luteinizing hormone (LH; IU/mL), and progesterone (P4; ng/mL). Mean ± S.E.M values. Different superscripts within a row (A-B) show statistical differences within the same day and within each hormone ($p \leq 0.05$). Different superscripts within a row (a-b) show statistical differences between days, among groups, and within each hormone parameter ($p \leq 0.05$).

Regarding LH levels, these were more variable than those of TSH and FSH among and within time-points and groups ($p < 0.05$; Table 4). There was a discharge of LH; however, the discharge was carried mainly in the supplemented group irrespective of the time-point assessed ($p < 0.05$). Finally, significant differences were observed between groups regarding P4 concentration within day 19 and 49 post-partum ($p < 0.05$; Table 4).

DISCUSSION

The present study was carried out to determine the effects of dietary supplementation with black wattle (Acacia mearnsii) on ovarian activity, uterine involution, and hormonal profiles in dairy cows maintained at high-altitude conditions. Regarding the body weight parameter, only significant differences were obseved when comparing day 15 pre-partum weight with day 19 post-partum in the supplemented group. The results observed in the supplemented group could be due to the fact that feed sources with degradation-resistant proteins such as tannin-rich plants, could alter the proportions of available amino acids that pass into the small intestine influencing the total nitrogen absorption carried out. It has been described that supplementation with tannin extract should not exceed 0.3 g/kg body weight/day (g/kg BW/d) as higher levels may decrease feed intake and digestibility (Kozloski et al., 2012). This may occur concomitantly with the increase of the negative energy balance during the first weeks post-partum, with the consequent weight loss in the supplemented group (AM). However, the polyphenols of the black wattle could, at least in part, protect its degradation in the rumen, and therefore, the black wattle could be considered a protein source of low rumen degradability with good intestinal absorption. This would explain, at least in part, the increase in weight gain in the supplemented group in the long term. According to the literature, the antimicrobial activity produced by tannins from Acacia spp. could inhibit the growth of harmful bacteria, maintaining the production of beneficial bacteria for the digestion process (Ogawa and Yazaki, 2018), which could improve the fibre degradation process in the rumen. In addition, the weight could also be affected by the high degradability of the forage provided which in turn may result in inefficient protein digestion process, especially when there is no synchrony with the energy level provided by the diet (Patra and Saxena, 2011). Arboreal-origin legumes have been described as having anti-nutritional effects due to metabolites that influence ruminal digestión process, as well as the carbohydrate digestion, minerals, and the vitamin availability (Monagas et al., 2010). According to other study, a prior ammonium detoxification process is advisable and requires an extra energy intake, which would negatively affect dairy cows during late gestation and early lactation (Dias et al., 2010). Furthermore, in cases where there is an excess of crude protein in the diet, it would be advisable to supplement the animals with high-energy diets to prevent the energy demand caused by an excess of protein in the diet avoiding marked body weight losses (Butler, 1998; Katongole and Yan, 2020). In addition, the negative energy balance in dairy cows is accentuated due to the high energy requirements derived from the progressive increase in milk production during the first weeks post-partum together with the decrease in dry matter intake during the puerperal period (van Knegsel et al., 2005; Wankhade et al., 2017).

The loss of ≥1 unit of body condition in post-partum cows has been related to a longer interval to the onset of luteal activity, and also with a higher risk of a first ovulation delay. Moreover, the poor post-partum nutritional status has been associated with a delay in the first ovulation post-partum. Therefore, the regular monitoring of body condition score before and after calving is very important for adjustments in the nutritional management during the early post-partum period (Shrestha et al., 2005). Regarding the uterine structures (follicles, corpora lutea, and ovarian diameter) on day 5, 25, and 34 post-partum, significant differences were observed between both groups (control vs. supplemented). These results agreed with other studies describing that the legume presence in the ruminant diets could improve their reproductive performance due to an increase in the ovulation rates (Waghorn and McNabb, 2003) . It has been described that the effect of tree-derived legumes on ovulation rates depended, at least in part, on the concentration of active tannins, which after the addition of polyethylene glycol in the diet (to inactivate the tannins) decreased its effect by about 9% (Barry et al., 1999). This positive effect on reproductive performance is not attributed to a reduction in the bioactivity of estrogenic plant compounds, but to a reduction in protein degradation in the rumen and an increase of amino acids supply at the small intestine (Lara and Londoño, 2008). In addition, studies conducted on ewes grazing common bird's-foot trefoil (Lotus corniculatus) compared to others grazing perennial ryegrass (Lolium perenne)/ white clover (Trifolium repens) showed that there was a 5-33% increase in the ovulation rates in ewes grazed on the former for

2-3 estrus cycles (Lara and Londoño, 2008). Moreover, common bird's-foot trefoil grazing for 2-3 estrus cycles prior to mating, increased from 6 to 39 % the offspring rates and from 14 to 26 % the weaning rates (Ramírez-Restrepo and Barry, 2005). Thus, it has been observed that the protein increase in the diet improves the reproductive performance in ruminants by improving follicular dynamics and affecting the hormone levels (Armstrong et al., 2001). In the present study, the significant changes in TSH, LH, and P4 on day 19 post-partum may be due to the fact that legumes provide a high percentage of protein increasing a lack of degradability at the rumen level (Barry et al., 1999; Grosse Brinkhaus et al., 2018). That´s why a higher percentage of protein in the diet could lead to a higher follicular recruitment improving the modulation of gonadotropin action in the ovary (Barry et al., 1999). However, the dynamics in FSH hormone levels remained quite constant during the entire puerperal period. On the other hand, the LH concentration was significantly different between the control and supplemented groups. This could be due to the pulsatile nature of LH release, which could be influenced by the fat animal reserves as well as by blood metabolic signals (Mellouk et al., 2017). In addition, the unovulated dominant follicles during the early post-partum period which come from the ovarian follicular waves increased, maybe because of the incomplete follicular maturation. This fact could be due to the absence of appropriate LH pulses, mainly due to the depletion of LH reserves in the anterior pituitary together with the absence of GnRH pulses during the lactation (Kawashima et al., 2007). The LH reserves between day 15 and 30 post-partum are replenished as the post-partum interval increases, followed by an increasing in the frequency of GnRH, FSH, and LH pulses, the final follicular maturation, ovulation, and ovarian cyclicity (Yavas and Walton, 2000). The ovarian cyclicity may be delayed due to the inhibitory influence on the hypothalamic activity, in fact, the plasma FSH levels increased in most cows between day 5 and 10 post-partum and, thereafter, the observed random changes have a poor relation to the onset of cycles. Therefore, the recovery of FSH release occurs earlier post-calving compared to the recovery of LH release. Normally, before the first ovulation there is an increase in the frequency of LH release leading to an increase in the plasma LH concentration, the pre-ovulatory LH surge, the first ovulation, the corpus luteum appearance and, finally, the P4 release (Lamming et al., 1981). Moreover, negative feedback from 17beta oestradiol has been reported stimulating the release of hypothalamic endogenous opioid peptides as the post-partum interval increases, and as a consequence decreasing the GnRH pulses (Yavas and Walton, 2000). This occurs because GnRH pulses are also absent during the early post-partum period due to the lactation effect (Crowe, 2008).

CONCLUSIONS

In conclusion, overall although few differences were observed in BW within AM group the supplementation with AM showed differential patterns in reproductive tract structures/dimensions and several hormone levels. These differences may be related to the specific Acacia mearnsii-derived supplementation diet which may affect the energy and protein balance together with the black wattle-derived tannin content which may influence the protein digestibility and absorption during the early post-partum period in dairy cows maintained at high-altitude environments.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

All the authors listed in this paper have contributed to the preparation and execution of this research. L.M.V.-O. and M.A.G.-R.: Conceptualisation, Methodology, Data Curation, Writing—Original Draft, and Funding Acquisition; M. B.-R., R. L.-O: and V. A.-Y.: Methodology, Critical Reading and Funding Acquisition; and M.G.-H.: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing—Original Draft, Critical Reading, Funding Acquisition, and Writing—Review and Editing.

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