

Oxidative status and fertility of ewes supplemented coffee pulp during estrous synchronization and early pregnancy[□]

Estado oxidativo y fertilidad en ovejas suplementadas con pulpa de café durante la sincronización estral y gestación temprana

Estado oxidativo e fertilidade nas ovelhas suplementadas com a polpa de café durante sincronização do estro e gestação inicial

Teodulo Salinas-Rios¹, IAZ, Dr; María Teresa Sánchez-Torres-Esqueda^{2*}, IAZ, Dra; Antonio Díaz-Cruz³, MVZ, Dr; José Luis Cordero-Mora², MVZ; Mario Cárdenas León⁴, Biol; Jorge Hernández-Bautista¹, IAZ, Dr; Cuauhtémoc Nava-Cuellar³, MVZ, MSc; Rafael Nieto Aquino⁵, IAZ, Dr.

¹Universidad Autónoma Benito Juárez de Oaxaca, FMVZ, Oaxaca, México.

²Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, México.

³Departamento de Bioquímica, FMVZ Universidad Nacional Autónoma de México, Distrito Federal, México.

⁴Laboratorio de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición, Tlalpan, México D.F.

⁵Universidad Politécnica de Francisco I Madero. Tepatepec, Hidalgo, México.

(Received: November 12, 2015; accepted: May 10, 2016)

doi: 10.17533/idea.rccp.v29n4a02

Summary

Background: it is known that coffee pulp can modify the oxidative status and fertility in dairy cows. **Objective:** to evaluate the effect of dietary supplementation with coffee pulp on the antioxidant capacity, lipid oxidation and reproductive characteristics of ewes during estrous synchronization and early gestation. **Methods:** forty Dorset-Suffolk crossbred ewes with 3 or 4 parturitions were allocated to two treatments: T0 (n = 21), ewes supplemented with 450 g of a control feed; and T1 (n = 19), ewes supplemented with 450 g of the feed with 25% coffee pulp. Supplementation began 14 days before estrous synchronization and ended 25 days after breeding. During estrous synchronization, progestogen (CIDR, Controlled Internal Drug Release)

□ To cite this article: Salinas T, Sánchez MT, Díaz A, Cordero JL, Cárdenas M, Hernández J, Nava C, Nieto R. Oxidative status and fertility of ewes supplemented coffee pulp during estrous synchronization and early pregnancy. Rev Colomb Cienc Pecu 2016; 29:255-263.

* Corresponding author: María Teresa Sánchez-Torres-Esqueda. Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, México. Tel.: 015959520200 ext. 1705. E-mail: teresa@colpos.mx

was inserted and left *in situ* for 11 days. Eighteen hours later, estrous detection began with the aid of rams. Blood samples were collected at different times of synchronization and during early pregnancy to determine antioxidant capacity, lipid oxidation and blood progesterone concentration. Pregnancy diagnosis was performed 30 and 60 days after CIDR removal. **Results:** supplementation with coffee pulp did not affect estrous onset, estrous response or progesterone concentration, but fertility decreased from 100 to 78.95%. The antioxidant capacity measured using the FRAP technique was greater in coffee pulp supplemented ewes only before progestogen insertion. Coffee pulp did not modify lipid oxidation; however, this variable was affected by sampling time, decreasing after progestogen removal to its lowest values at 22 days into pregnancy. **Conclusion:** although supplementation with coffee pulp at 25% in the concentrate increased antioxidant capacity of ewes before insertion of progestogen, it is not recommended to use this percentage during synchronization or early pregnancy since it can negatively affect gestation rate.

Keywords: *antioxidant capacity, CIDR, oxidation, progestagen.*

Resumen

Antecedentes: se sabe que la pulpa de café puede modificar el estado oxidativo y la fertilidad en vacas lecheras. **Objetivo:** evaluar los efectos del suministro dietario de pulpa de café y su capacidad antioxidante, oxidación lipídica, y características reproductivas en ovejas durante la sincronización del estro y la gestación temprana. **Métodos:** cuarenta ovejas cruzadas Suffolk x Dorset de 3 y 4 partos fueron asignadas a dos grupos: T0 (n = 21), suplementación con 450 g de alimento (grupo testigo); y T1 (n = 19), suplementación con 450 g de alimento con 25% de pulpa de café. La suplementación inició 14 días antes de la sincronización del estro y terminó 25 días después del apareamiento. El progestágeno (CIDR, Dispositivo Intravaginal de Liberación Controlada) fue insertado en los animales por 11 días. Dieciocho horas después de su retiro se inició la detección de estros. Se muestreó a diferentes tiempos después de la sincronización y durante la gestación temprana para determinar capacidad antioxidante, oxidación lipídica y concentración de progesterona. El diagnóstico de preñez se realizó 30 y 60 días después de la remoción del CIDR. **Resultados:** la suplementación con pulpa de café no afectó el inicio del estro, la respuesta al estro ni la concentración de progesterona. Sin embargo, la fertilidad disminuyó de 100 a 78,95%. La capacidad antioxidante, medida mediante la técnica FRAP, fue mayor en ovejas suplementadas con pulpa de café, pero solo antes de la inserción del progestágeno. La pulpa de café no modificó la oxidación lipídica; sin embargo, si fue modificada por el tiempo de muestreo, decreciendo desde la remoción del progestágeno hasta los 22 días de preñez. **Conclusión:** aunque la suplementación del concentrado con 25% de pulpa de café incrementó la capacidad antioxidante antes de la inserción del progestágeno, no se recomienda ese porcentaje durante la sincronización y gestación temprana, ya que redujo el porcentaje de gestación de las ovejas.

Palabras claves: *capacidad antioxidante, CIDR, oxidación, progestágeno.*

Resumo

Antecedentes: a polpa de café pode modificar o estado oxidativo e a fertilidade em vacas leiteiras. **Objetivo:** avaliar a polpa de café na capacidade antioxidante, oxidação da gordura e nas características reprodutivas das ovelhas durante sincronização do estro e gestação inicial. **Métodos:** quarenta ovelhas cruza Suffolk e Dorset de 3 e 4 nascimentos foram agrupadas no T0 (n = 21), suplementação com 450 g de alimento controle e T1 (n = 19), suplementação com 450g de alimento com 25% de polpa de café. A suplementação iniciou 14 dias antes da sincronização do estro e terminou 25 dias depois do acasalamento. O hormônio progestina (CIDR, dispositivo intravaginal de liberação controlada de fármaco) foi inserido por 11 dias. Dezoito horas depois da retirada iniciou-se a detecção do estro. Fizeram-se amostras de diferentes tempos do período e da gestação inicial para determinar a capacidade antioxidante, oxidação dos lipídeos e concentração de progesterona. Realizou-se o diagnóstico de gestação 30 e 60 dias depois de remover o CIDR. **Resultados:** a suplementação com a polpa de café não afetou o início do estro, a resposta ao estro e a concentração de progesterona, mas a fertilidade decresceu de 100 a 78,95%. A capacidade antioxidante que foi medida pela técnica de FRAP foi maior nas ovelhas suplementadas com a polpa de café somente antes da inserção do progestágeno. A polpa de café não modificou a oxidação dos lipídeos; no entanto, estes foram modificados pelo tempo de amostra, decrescendo depois de remover o progestágeno até 22 dias de gestação. **Conclusão:** ainda que a polpa de café a 25% de concentração incrementa a capacidade antioxidante antes da inserção do progestágeno, não é recomendado este percentual para as ovelhas durante a sincronização do estro e a gestação inicial, já que decresce a porcentagem de gestação.

Palavra chave: *capacidade antioxidante, CIDR, oxidação, progesterona.*

Introduction

Synchronization has been widely used to program lambing, increasing the number of lambs, and to introduce semen of high genetic merit into the herd (Cleef *et al.*, 1998; Godfrey *et al.*, 1999). Progesterone is the most widely used hormone in these controlled breeding protocols (Fleisch *et al.*, 2006; Hashemi *et al.*, 2006; Seekallu *et al.*, 2010). Several researchers have reported that maternal recognition and implantation, during the onset of gestation, is when most fertility failures occur (Fthenakis *et al.*, 2012).

Using exogenous progesterone during synchronization increases lipid oxidation (Sönmez *et al.*, 2009). Moreover, it has been reported that metabolic changes modify the oxidative state of the animals, possibly causing oxidative stress (See-Ling *et al.*, 2013). According to Al-Gubory *et al.* (2010), this stress can cause failures in reproductive processes, such as damage to lipids, proteins and DNA, infertility and miscarriages. Knowledge of the oxidative status before and after estrus synchronization may help determining when to supplement antioxidants. It has been reported that antioxidants such as vitamin E before insemination increases prolificacy and reduces lipid oxidation (Sönmez *et al.*, 2009).

Coffee pulp is a byproduct freely available in coffee growing regions. It has high contents of phenolic compounds and has strong antioxidant properties (Arellano *et al.*, 2011). However, it also contains 0.87% caffeine (Ferreira *et al.*, 2001), which is not recommended in humans, since it delays fetal development during the last third of pregnancy (Vik *et al.*, 2003) and causes embryo losses in early pregnancy (Stefanidou *et al.*, 2011). Some byproducts of coffee processing have been tested in dairy cows. Findings show that up to 150 g/Kg coffee pulp can be included in cow diets without affecting production (Cipriano *et al.*, 2006), or 25% in the concentrate at a 60:40 forage:concentrate ratio (Soares *et al.*, 2007a; Soares *et al.*, 2007b). Although inclusion of coffee byproducts in animal diets has been acceptable up to given inclusion levels, it is necessary to determine its influence on reproductive variables; on one hand, natural antioxidants help to counteract lipid peroxidation, but on the other, there is controversy over the effects of caffeine in reproduction. So, we hypothesize that

changes in the oxidative state during pregnancy and lactation can be modified by 25% inclusion of coffee pulp; however this could affect reproduction. Therefore, the objective of this study was to evaluate the effect of diet supplementation with coffee pulp for 50 days as a nutritional flushing on the antioxidant capacity, lipid oxidation and reproductive characteristics of ewes during estrous synchronization and early gestation.

Materials and methods

Ethical considerations

This study was conducted observing the standards for ethics, biosafety and animal well-being of the Colegio de Postgraduados, Campus Montecillo, Mexico, according to the Official Mexican Standard NOM-033-ZOO-1995 (SAGARPA, 2015).

Experimental design and animal management

The trial was conducted from September (Autumn, reproductive season) to April (Spring, anestrus season) 2014 at the experimental farm of the Colegio de Postgraduados, Campus Montecillo. The climate is temperate subhumid C (Wo) (w)b(i)g with summer rains and 750 mm rainfall (Garcia, 1981). Forty Dorset-Suffolk crossbred ewes with 3 or 4 parturitions were fed oat hay (crude protein: 8.5) and distributed in a completely randomized design to the following two treatments: T0 (n = 21): ewes supplemented with 450 g feed concentrate without coffee pulp (control), and T1 (n = 19): ewes supplemented with 450 g of feed with 25% coffee pulp. The 25% coffee pulp inclusion level was in accordance with previous work conducted in Brazil (Soares *et al.*, 2007a; Soares *et al.*, 2007b).

Supplementation was individually done at 8:00 a.m. The chemical composition of coffee pulp was: caffeine (0.572%), crude protein (10%), ash (6.06), neutral detergent fiber (50.5), and acid detergent fiber (50.2). Ewes were confined in individual pens from 8:00 to 10:00 a.m and later regrouped with free access to oat hay. Concentrate contained 13% crude protein and 2.8 Mcal/Kg dry matter (Table 1), which was adjusted according to the NRC requirements (1985). Ewes were supplemented from the beginning of the experiment when pre-synchronization began

(14 days before insertion of progestagen) to 25 days after mating (Figure 1). Supplementation was aimed to evaluate the effect of coffee pulp, as a nutritional flushing, for up to 25 days. Pregnancy diagnosis was performed 30 and 60 days after CIDR (controlled internal drug release) removal.

Table 1. Diet fed to ewes during estrous synchronization and early pregnancy.

Ingredient (g/Kg)	T0	T1
Oat hay	250	0
Coffee pulp	0	250
Corn	580	600
Soybean meal	110	90
Molasses	50	50
Mineral salt*	10	10
<i>Chemical composition</i>		
DM	89.2	89.0
Metabolizable energy Mcal/Kg	2.8	2.8
Crude Protein	13.05	13.0
Antioxidant capacity μmol (Trolox/g DM)	54.36	92.3

*Phosphorus (17.5%), Sodium (12.9%), Calcium (5.6%), Magnesium (3.4%).

T0: Control diet; T1: concentrate with 25% coffee pulp; DM: dry matter.

Estrous synchronization

At the beginning of the experiment, all ewes were pre-synchronized with two doses of 125 μg prostaglandin (Cloprostenol), 8 days apart. All ewes exhibited estrus after the second administration of prostaglandin, and therefore, it was assumed that they had *corpus lutea*, and only those that were cycling were used. Six days after the second prostaglandin administration, an intravaginal device impregnated with 0.3 g progesterone (CIDR) was inserted and left for 11 days. Eighteen hours after removing the CIDR, estrous detection initiated with the aid of rams equipped with a protective apron (to prevent mating), and continued at 8-hour intervals (Figure 1). Mating was performed at 8 and 16 h after estrous detection using 8 rams of the same breed with proven fertility. Estrous onset was recorded when the ewes allowed the rams to mount.

Pregnancy diagnosis

Gestation was diagnosed with the aid of real time - Sonovet 600 ultrasound equipment, and using a 7.5 Mhz transducer (Medison, Cypress, California, USA) on days 30 and 60 after removal of CIDR. To calculate the pregnancy rate, the number of ewes diagnosed as pregnant was divided by the number of ewes bred. Prolificacy or fecundity was

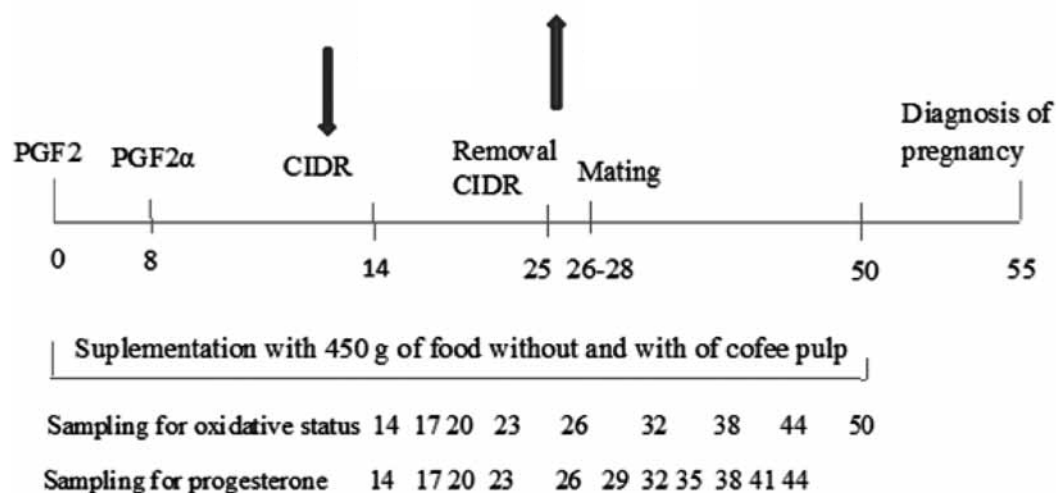


Figure 1. Synchronization and sampling protocols for measuring the oxidative state and circulating progesterone levels in control ewes and those receiving 25% coffee pulp in the feed concentrate.

expressed by dividing the number of lambs born by the number of ewes that lambled. Lamb weight at birth was also recorded during the first 12 hour after parturition.

Assessment of oxidative status

Plasma was obtained to measure oxidative status. Blood samples were collected from the jugular vein in 5 mL tubes with EDTA (ethylenediaminetetraacetic acid) at the beginning of the experiment (before supplementation), before CIDR insertion, and every three days up to day 26 (the day after removing the CIDR devices). After this, samples were collected every 6 days, up to day 50 of the experiment, when concentrate supplementation ended (Figure 1). Tubes were centrifuged at 2500 gravities for 10 min at 4 °C, and plasma was aspirated and stored at -40 °C until analysis.

Total antioxidant capacity was measured with the ferric reducing antioxidant power (FRAP) technique by Benzie and Strain (1996). The 6-hydroxy-2-5-7-8-tetramethyl-chroman-2-carboxylic acid (trolox) was used as a standard at different concentrations (0.2-1.6 nmol/μL) to measure the results.

Lipid oxidation was measured by the thiobarbituric acid reactive substances (TBARS) test, according to the technique by Ohkawa *et al.* (1979). The results were calculated using different concentrations (1-8 nmol) of malonaldehyde (MDA), recording acid hydrolysis from 1,1,3,3-tetraethoxypropane.

Progesterone measurement

Blood samples were collected from the jugular vein with vacutainer vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, New Jersey, USA) to measure plasma progesterone (P4). Samples were taken before inserting the CIDR's and every other day until day 44 of the experiment -which corresponded to one estrous cycle- after removing the CIDR (Figure 1). Plasma P4 was analyzed using the immunoenzyme assay (Immunometrics UK Ltd, 280 London, United Kingdom). The analytical sensitivity was 0.13 ng mL⁻¹, with 9.59 and 13.7% intra and inter-assay coefficient of variation, respectively.

Statistical analysis

An analysis of variance was conducted for antioxidant capacity and lipid oxidation, with treatment and sampling time as fixed effects. The statistical model used was:

$$Y_{ijk} = \mu + T_i + M_j + T_i M_j + A_{k(i)} + E_{ijk}$$

Where:

Y_{ijk} = response to the i^{th} treatment in the j^{th} sampling of the k^{th} replication.

μ = general mean.

T_i = effect of the i^{th} parturition.

M_j = effect of the j^{th} sampling.

T_{iM_j} = effect of the i^{th} treatment in the j^{th} sampling.

$A_{k(i)}$ = effect of the i^{th} treatment nested to the k^{th} animal.

E_{ijk} = experimental error.

A chi-squared test was used for fertility analysis and onset of estrus. The GENMOD of SAS was used for prolificacy. Statistical analyses were done using the SAS (2002) Version 9 (SAS Institute Inc, Cary, NC, USA).

Results

Reproductive variables

All ewes (100%) of both treatment groups exhibited estrus after removal of the intravaginal progestogen. Inclusion of 25% coffee pulp in the concentrate did not modify the onset of estrus after progestogen removal (31.61 ± 1.60 h for the control and 34.94 ± 2.61 h for the coffee pulp treatment). The pregnancy rate decreased ($p < 0.05$) by 21.96% at 30 days as an effect of including coffee pulp in the concentrate. However, no pregnancy loss was recorded after this time (the ewes diagnosed pregnant at 30 days were the same animals diagnosed pregnant at 60 days; Table 2).

Table 2. Reproductive variables of ewes supplemented with 250 g coffee pulp/Kg concentrate.

	T0	T1
Estrus (%)	100	100
Estrous onset (hours)	31.61 ± 1.60	34.94 ± 2.61
Gestation at 30 days (%)	(21/21) 100 ^a	(15/19) 78.94 ^b
Gestation at 60 days (%)	(21/21) 100 ^a	(15/19) 78.94 ^b
Parturition (%)	(21/21) 100 ^a	(15/19) 78.94 ^b
Prolificacy	1.52	1.50
Lamb weight at birth (Kg)	6.85 ± 0.42	7.09 ± 0.52

T0: ewes fed 450 g concentrate, (control diet); T1: ewes fed 450 g concentrate with 25% coffee pulp. Different superscript letters (^{a,b}) within the same row indicate statistically significant differences ($p < 0.05$).

Oxidative status

An interaction effect was observed between blood sampling time and treatment on antioxidant capacity. Antioxidant capacity was higher in ewes supplemented with 250 g coffee pulp/Kg than in control ewes at the sampling prior to progestogen insertion. No differences were found between treatments ($p < 0.05$) on the other sampling days. The antioxidant capacity started to decrease from progestogen removal until 22 days into pregnancy in both treatments.

Lipid peroxidation was not modified by treatment ($p > 0.05$), but there was a change resulting from the sampling time ($p < 0.05$). No changes were recorded before supplementation or during synchronization. However, one day after removing progestogen, when ewes began showing estrus, an increase in lipid oxidation was observed relative to that at the onset of the experiment (when ewes were not pregnant). Nevertheless, lipid oxidation started to decrease after the beginning of gestation, until it reached values below those at 16 and 22 days of pregnancy (Figure 2).

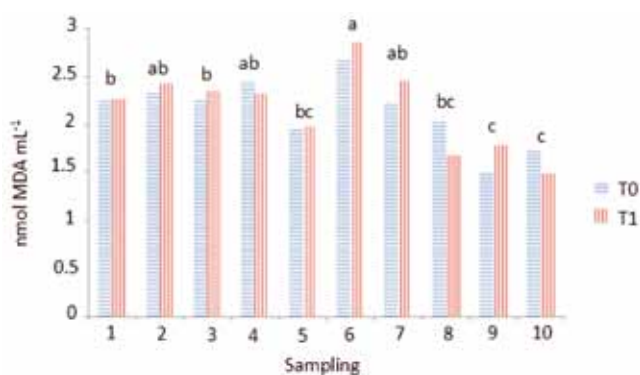
Progesterone

Supplementation with 25% coffee pulp in the concentrate did not modify ($p < 0.05$) plasma progesterone at any sampling time (Table 4). Plasma progesterone increased after progestogen insertion

Table 3. Mean (± SE) antioxidant capacity of ewes during estrous synchronization using progestogen and early gestation.

Sampling	FRAP (nmol trolox mL ⁻¹)	
	T0	T1
Before supplementation	221.31 ± 5.11	213.81 ± 5.39
Insertion of progestogen	179.04 ± 4.92 ^b	221.21 ± 5.18 ^a
3 days with progestogen treatment	230.15 ± 5.11	234.39 ± 5.39
6 days with progestogen treatment	210.17 ± 5.11	223.07 ± 5.39
9 days with progestogen treatment	207.03 ± 5.38	192.83 ± 5.39
1 day after progestogen removal	250.27 ± 5.11	247.38 ± 6.61
4 days of gestation	239.96 ± 5.11	217.39 ± 6.61
10 days of gestation	200.23 ± 5.11	195.39 ± 6.61
16 days of gestation	186.15 ± 5.69	181.31 ± 6.61
22 days of gestation	178.87 ± 5.38	170.71 ± 7.19

T0: ewes fed 450 g concentrate (control diet); T1: ewes fed 450 g concentrate with 25% coffee pulp. FRAP: ferric reducing antioxidant power.

**Figure 2.** Lipid peroxidation of ewes during estrous synchronization using progestogen during early gestation. Sampling: 1 (before supplementation); 2 (progestogen insertion); 3 (3 days with progestogen treatment); 4 (6 days with progestogen treatment); 5 (9 days with progestogen treatment); 6 (1 day after progestogen removal); 7 (4 days of gestation); 8 (10 days of gestation); 9 (16 days of gestation); 10 (22 days of gestation).

T0: ewes fed 450 g concentrate (control diet); T1: ewes fed 450 g concentrate with 25% coffee pulp.

in both treatments, but it decreased in the sampling conducted the day after CIDR removal. Later on, as gestation advanced, progesterone concentration increased again.

Table 4. Mean (\pm SE) progesterone concentration of ewes during estrous synchronization and early gestation.

Sampling day	Progesterone (ng/ml)	
	T0	T1
Insertion of progestogen	4.80 \pm 1.36	3.82 \pm 1.57
3 days with progestogen	8.06 \pm 0.46	8.21 \pm 0.50
6 days with progestogen	8.33 \pm 0.54	9.22 \pm 0.57
9 days with progestogen	9.36 \pm 0.60	8.46 \pm 0.63
1 day after progestogen removal	0.47 \pm 0.04	0.55 \pm 0.05
4 days of gestation	0.65 \pm 0.07	0.56 \pm 0.09
6 days of gestation	3.23 \pm 0.21	2.75 \pm 0.24
9 days of gestation	6.03 \pm 0.44	5.92 \pm 0.54
12 days of gestation	7.76 \pm 0.61	7.27 \pm 0.72
15 days of gestation	8.89 \pm 0.58	7.49 \pm 0.69
18 days of gestation	9.38 \pm 0.54	9.61 \pm 0.66

T0: ewes fed 450 g concentrate, (control diet); T1: ewes fed supplement of 450 g concentrate with 25% coffee pulp.

CIDR: intravaginal device for releasing progesterone.

Discussion

Reproductive variables

Inclusion of 25% coffee pulp in the concentrate did not modify the onset of estrus, which occurred 31.61 h after removal of the CIDR in the control treatment and 34.94 h in the coffee pulp treatment. This is similar to the 36.2 h reported by Cleeff *et al.* (1998), but greater than the 26.5 h after CIDR removal reported by Godfrey *et al.* (1999).

The lower fertility in ewes fed 450 g concentrate with 25% coffee pulp may possibly be due to the 0.572% caffeine in coffee pulp used in this study. It is corresponding to 643.5 mg consumption of caffeine per day. Caffeine is a methylxanthine, which is absorbed rapidly in humans and crosses into the placenta. In women, it has been observed that when less than 100 mg caffeine is consumed in early pregnancy (6 to 12 weeks), there is a higher risk of a miscarriage (Cnattingius *et al.*, 2000) and losses of

fetuses after 20 weeks of pregnancy (Hammer *et al.*, 2005). After the first diagnosis of pregnancy on day 30 and suspension of coffee pulp in the diet, there were no more embryo or fetus losses. The same ewes pregnant at 30 days were diagnosed pregnant at 60 days and gave birth.

Feeding ewes with coffee pulp during the first 25 days of gestation did not modify birth weight. In this respect, Hammer *et al.* (2007) and Clausson *et al.* (2002) found no differences in birth weight or gestation time between women who consumed coffee with caffeine and those that consumed decaffeinated coffee. Bracken *et al.* (2003) observed that consumption of moderate amounts of caffeine does not reduce birth weight, but daily consumption of more than 600 mg of caffeine was detrimental. In this experiment, no differences in birth weight were found, possibly because ewes were supplemented with coffee pulp only during the first month of gestation, with the greatest fetal growth occurring during the last third of gestation.

Inclusion of 0, 5, 10, and 15% coffee hulls in the total diet of dairy cow has no adverse effects on milk production (Cipriano *et al.*, 2006). Moreover, coffee hulls have been administered as 25% of the concentrate fed to dairy cows in a proportion of 60:40 forage:concentrate. Also, ruminal fermentation (Soares *et al.*, 2007a), digestibility and production and composition of milk (Soares *et al.*, 2007b) have been assessed and it has been determined that their inclusion is possible in lactating dairy cows. This is the first study with animals in which it was determined that inclusion of coffee by-products, such as pulp, causes adverse effects in fertility. Usually, breeding occurs when dairy cows are still lactating. In other ruminants, such as sheep and goats, breeding happen immediately after weaning. The use of coffee pulp at 25 percent is not recommended, as it reduces the probability of pregnancy.

Oxidative status

Oxidative status has been assessed mainly during peri-partum, when susceptibility to lipid peroxidation increases (Bernabucci *et al.*, 2005). However, little is known about oxidative state during estrous synchronization and early gestation.

The inclusion of coffee pulp increased antioxidant capacity before progesterone insertion. Antioxidant capacity can be modified mainly for three reasons: first, a greater antioxidant intake; second, a greater use of antioxidants; and third, greater enzymatic synthesis of antioxidants. In the present study ewes were in the same physiological state so the difference in antioxidant capacity may have been due to a greater antioxidant intake from coffee pulp. In this regard, Mumford *et al.* (2015) found that antioxidant concentrations are associated with steroidogenesis, while Sönmez *et al.* (2009) reported that intravaginal progesterone sponges increased lipidic oxidation, thus the antioxidant effect of coffee pulp observed before progesterone insertion was not observed in ewes with progesterone or in pregnant ewes because there are other factors in these stages that may have masked the antioxidant effect of coffee pulp.

Regarding the effect of sampling times on antioxidant capacity, it is important to emphasize that in both treatments antioxidant capacity was at its maximum the day after progesterone removal, and it decreased progressively as gestation advanced. In a similar study, Mohebbi-Fani *et al.* (2012) found that vitamins with antioxidant capacity (such as vitamin A, E, and C) decrease from the beginning of gestation; so early gestation involves a reduction in antioxidant capacity. Although there is no record of antioxidant capacity during the first few days of gestation, other researchers have found that it decreases a few days before parturition (Turk *et al.*, 2013). Lipid oxidation generally follows the same trend as antioxidant capacity, tending to decrease as gestation advances, suggesting that decrease in antioxidant capacity is due to the fact that antioxidants were used to decrease lipid oxidation. More studies are needed to determine the importance of antioxidants at the onset of gestation and their probable benefits for reproductive traits.

Progesterone

Inclusion of coffee pulp did not modify progesterone concentration. In this respect, it was observed that ascorbic acid (an antioxidant) increases progesterone concentration in women who exhibit a defective luteum phase (Henmi *et al.*, 2003). In ovariectomized sheep, administration of 20 mg caffeine did not affect the concentration of other reproductive hormones

such as FSH (Follicle-Stimulating Hormone) and LH (Luteinizing Hormone). However, prolactin concentration increased with caffeine administration (Scaramuzzi *et al.*, 1997). In the present experiment, neither antioxidants nor caffeine in coffee pulp modified progesterone concentration in ewes during synchronization and early gestation. For this reason, it is possible that the lower fertility observed in ewes supplemented with coffee pulp was due to other causes not related to a hormonal imbalance.

It is concluded that supplementation with 25% of coffee pulp did not affect the onset of estrous, estrous response, prolificacy, or plasma progesterone concentrations of ewes. However, it increased antioxidant capacity before insertion of progesterone. Even so, supplementation with 25% of coffee pulp during estrous synchronization or early gestation is not recommended, as it reduces fertility. Further research is required to determine whether lower levels of coffee pulp could cause an adverse effect on reproduction.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this study.

References

- Al-Gubory KH, Fowler PA, Garrel, C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol* 2010; 42:1634-1650.
- Arellano GM, Ramirez, CA, Mancera TT, Pérez MG, Saucedo CG. Antioxidant activity of fermented and non-fermented coffee (*Coffea arabica*) pulp extracts. *Food Technol Biotechnol* 2011; 49:374-378.
- Benzie IF, Strain, JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 1996; 239:70-76.
- Bernabucci U, Ronchi B, Lacetera N, Nardone A. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *J Dairy Sci* 2005; 88:2017-2026.
- Bracken MB, Triche EW, Belanger K, Hellenbrand K, Leaderer BP. Association of maternal caffeine consumption with decrements in fetal growth. *Am J Epidemiol* 2003; 157:456-466.
- Cipriano RF, Garcia R, Freitas AW, Lima de Souza A, Valadares FSC, Gomes PO, Sampaio RJP, Gonçalves TR, Cipriano R G. Intake and digestibility of lactating dairy cows fed diets containing coffee hulls. *R Bras Zootec* 2006; 35:2154-2162.

- Clausson B, Granath F, Ekblom A, Lundgren S, Nordmark A, Signorello LB, Cnattingius S. Effect of caffeine exposure during pregnancy on birth weight and gestational age. *Am J Epidemiol* 2002; 155:429-436.
- Cleeff JV, Karsch FJ, Padmanabhan V. Characterization of endocrine events during the peri-estrous period in sheep after estrous synchronization with controlled internal drug release (CIDR) device. *Domest Anim Endocrinol* 1998; 15:23-34
- Cnattingius S, Signorello LB, Annerén G, Clausson B, Ekblom A, Ljunger E, Blot AJ, McLaughlin JK, Petersson G, Rane A, Granath F. Caffeine intake and the risk of first-trimester spontaneous abortion. *N Engl J Med* 2000; 343:1839-1845.
- Ferreira BA, Aguiar AP, Pérez OJ, Santos VB, Cardoso RM. Antinutritional factors of the hull and dehydrated pulp of coffee (*Coffea arabica L.*) stored in different periods. *Rev Bras Zootec* 2001; 30:1325-1331.
- Fleisch A, Werne S, Heckendorn F, Hartnack S, Piechotta M, Bollwein H, Thun R, Jannet F. Comparison of 6-day progestagen treatment with Chronogest® CR and Eazi-breed™ CIDR® G intravaginal inserts for estrous synchronization in cyclic ewes. *Small Ruminant Research* 2006; 107:141-146.
- Fthenakis GC, Arsenos G, Brozos C, Fragkou IA, Giadinis ND, Giannenas I, Mavrogianni VS, Papadopoulos E, Valasi I. Health management of ewes during pregnancy. *Anim Reprod Sci* 2012; 130:198-212.
- García E. Modificaciones al sistema de clasificación de Köppen. 3a edición. Instituto de geografía. UNAM. México, DF; 1985.
- Godfrey RW, Collins JR, Hensley EL, Wheaton JE. Estrous synchronization and artificial insemination of hair sheep ewes in the tropics. *Theriogenology* 1999; 51:985-997.
- Hammer BB, Aagaard NE, Vaeth M, Henriksen TB, Olsen J. Coffee and fetal death: A cohort study with prospective data. *Am J Epidemiol* 2005; 162:983-990.
- Hammer BB, Obel C, Brink HT, Olsen J. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. *BMJ* 2007; 334:1-6.
- Hashemi M, Safadian M, Kafi M. Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. *Small Ruminant Research* 2006; 65:279-283.
- Henmi H, Endo T, Kitajima Y, Manase K, Hata H, Kudo R. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect. *Fertil Steril* 2003; 80:459-461.
- Mohebbi-Fani M, Mirzaci A, Nazifi S, Shabbooe Z. Changes of vitamins A, E, and C and lipid peroxidation status of breeding and pregnant sheep during dry seasons on medium-to-low quality forages. *Trop Anim Health Prod* 2012; 44:259-265.
- Mumford SL, Browne RW, Schliep KC, Schmelzer J, Plowdwn TC, Michels KA, Sjaardan LA, Zarek SM, Perkins NJ, Messer LC, Radin RG, Wactawski-Wende J, Schisterman EF. Serum antioxidants are associated with serum reproductive hormones and ovulation among healthy women. *J. Nutr* 2015; 146:98-106.
- NRC. 1985. Nutrient Requirements of Sheep, Sixth Revised. Washington, D.C.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95:351-358.
- SAS (Statistical Analysis System), 2002. SAS Proceeding Guide, Versión 9. SAS Institute, Cary, NC, USA.
- Scaramuzzi RJ, Downing JA, Williamson S, Pollard I. The circulating concentrations of FSH, LH and prolactin in the oestradiol-implanted ovariectomized ewe treated with caffeine. *Anim Reprod Sci* 1997; 45:273-282.
- Seekallu SV, Toosi BM, Zeigler A, Rawlings NC. Effects of estradiol and progesterone on circulating LH and FSH secretion, and ovarian antral follicle growth in anestrus ewes. *Small Ruminant Research* 2010; 91:178-85.
- See-Ling L, Sirajudeen KNS, Jan JMH. Increase in maternal adiposity and poor lipid profile is associated with oxidative stress markers during pregnancy. *Prev Med* 2013; 57:S41-S44.
- Soares OA, Souza CJM, Valadares FSC, Assis AJ, Monteiro ATR, Navajas RL, Dos Santos PD, Soares OG. Replacing corn with coffee hulls or soyhulls in diets of dairy cows: chewing activity, ruminal metabolism, nitrogen utilization and microbial protein synthesis. *R Bras Zootec* 2007a; 36:205-215.
- Soares OA, Souza CJM, Valadares FSC, Assis AJ, Araújo TRM, Diniz VRF, Dos Santos PD, Soares OG. Replacing corn with coffee hulls or soyhulls in dairy cows diets: intake, nutrient digestibility, and milk production and composition. *R Bras Zootec* 2007b; 36:1172-1182.
- Sönmez M, Bozkurt T, Türk G, Gür S, Kizil M, Yüce A. The effect of vitamin E treatment during preovulatory period on reproductive performance of goats following estrous synchronization using intravaginal sponges. *Anim Reprod Sci* 2009; 114:183-192.
- Stefanidou EM, Caramellino L, Patriarca A, Guido M. Maternal caffeine consumption and sine causa recurrent miscarriage. *Eur J Obstet Gynecol Reprod Biol* 2011; 158:220-224.
- Turk R, Podpečan O, Mrkun J, Kosec M, Flegar-Mestrić Z, Perkov S, Starič J, Robić M, Belić M, Zrimsek P. Lipid mobilization and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. *Anim Reprod Sci* 2013; 141:109-115.
- Vik T, Bakkeiteig LS, Ulla TK, Lund-Larsen K, Jacobsen G. High caffeine consumption in the third trimester of pregnancy: gender-specific effects on fetal growth. *Paediatr Perinat Epidemiol* 2003; 17, 324-331.