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ORIGINAL ARTICLE

Protected fatty acid supplementation during estrus synchronization treatment on reproductive parameters of dairy goats

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ABSTRACT

This study evaluated the effect of the protected fatty acid inclusion during estrus synchronization on reproductive parameters. Goats ($n = 32$) received progestagen sponges for 6 days and 200 IU equine chorionic gonadotropin and 30 μg d-cloprostenol were given on Day 5. No difference was found among control (C), 1% protected fatty acid inclusion (C + 1%) or 4% protected fatty acid inclusion (C + 4%) groups, respectively, in estrus (100.0, 100.0 or 90.9%), estrus duration (31.6 ± 12.3; 43.2 ± 12.9 or 40.8 ± 14.1 h), animals ovulating (100.0, 90.0 or 100.0%) or ovulation rate (1.3 ± 0.5; 1.1 ± 0.3 or 1.2 ± 0.4). The interval from sponge removal to ovulation and from estrus to ovulation, respectively, were shorter for C + 4% (45.2 ± 8.0 h; 18.3 ± 11.0 h) compared with C (56.3 ± 12.6 h; 30.6 ± 10.5 h) or C + 1% (57.7 ± 8.7 h; 30.3 ± 11.1 h). The average ovulatory follicle diameter was smaller for C + 4% (6.2 ± 0.7 mm) than C (7.5 ± 0.8 mm), but similar to C + 1% (7.0 ± 1.5 mm). Insulin, insulin-like growth factor 1, glucose and progesterone concentrations were similar among groups. The inclusion of protected fatty acid during synchronization treatment promoted no benefits on ovulation rate, but 4% anticipated the ovulation time.

Key words: *caprine, energy, ovulation rate, progesterone, PUFA.*

INTRODUCTION

Increasing the caloric density of rations by fat feeding has generally improved measures of cow reproduction, but positive effects on reproduction were not always observed (Santos *et al.* 2008). Supplemental polyunsaturated fatty acids (PUFA) have been shown to increase progesterone concentrations by enhancing development of luteal cells (Lucy *et al.* 1991), reducing uterine synthesis of prostaglandin F2α (PGF2α), delaying luteolysis (Williams 1989), and directly alleviating hepatic steroid metabolism (Reis *et al.* 2012). Additionally, supplemental PUFA may also increase circulating insulin concentrations, which in turn has also been shown to reduce hepatic expression of progesterone catabolic enzymes (Lemley *et al.* 2008). Cows fed fats enriched with essential fatty acids are likely to have more progesterone being synthesized due to a formation of a larger ovarian corpus luteum that is derived from a larger ovulatory follicle (Thatcher *et al.* 2008). No previous attempt has been made to analyze the effect of PUFA on reproduction of dairy goats. Our hypothesis was that this supplementation would promote benefits to their reproduction, as already shown in other species. Therefore, the aim of this study was to evaluate the supplementation of protected fatty acid during short-term protocol of estrus synchronization on reproductive end points of dairy goats.

MATERIALS AND METHODS

Location and experimental conditions

The study was conducted during February and March (beginning of breeding season) in the rural area of Piau, MG, Brazil (latitude 21°35′S and longitude 43°15′W). The average altitude was 435 m with Cwa climate (humid temperate with dry winters), according to Köppen classification. The goats were kept in an intensive system, within pens 15 m in length and 2 m wide

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which housed 10 goats each, providing 3 m^2 per animal. This research was reviewed and approved by the Animal Care Committee of Fluminense Federal University (UFF/109/09).

Dietary treatment, estrus synchronization treatment and mating

Toggenburg nulliparous ($n = 32$) goats 8 months of age were selected and allocated according to category, body weight (BW) and body condition score (BCS, range 1–5), respectively, into three treatments with different amounts of protected fatty acid inclusion: Control group (C), with no inclusion ($n = 11$; 32.9 ± 7.0 kg, BCS 2.8 ± 0.3); Group C + 1%, with 1% ($n = 10$; 32.5 ± 4.3 kg, BCS 2.8 ± 0.3); and Group C + 4%, with 4% of protected fatty acid on the diet ($n = 11$; 32.3 ± 3.8 kg, BCS 2.8 ± 0.2). After the adjustment period (22 days), when all animals were receiving the maintenance diet (Group C diet), experimental diets were offered according to groups, for 6 days (period of estrus synchronization). Diets were calculated according to the National Research Council (2007) and were equally balanced for all other components than protected fatty acid (Megalac-E®, QGN, Camaçari, Brazil). It is important to note that also energetic levels were balanced by the use of other ingredients such as corn meal, soy and sorghum bran to compensate the diet of animals receiving protected fatty acid. Animals were fed with grass (*Pennisetum purpureum* – 2.0 kg/animal/day) and concentrate (0.5 kg/animal/day), achieving 1.0 kg of dry matter intake per animal per day. Mineralized salt and drinking water were available ad libitum.

For estrus synchronization, intravaginal sponges impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Tecnopec, São Paulo, Brazil) were used for 6 days. All goats received 200 IU equine chorionic gonadotropin (eCG; Novormon 5000®, Tecnopec) i.m. (intramuscularly) and 30 µg d-cloprostenol (Veteglan®, Calier, Barcelona, Spain) i.m. 24 h before sponge removal. After sponge removal, estrus was monitored with the use of fertile bucks twice a day (07.00 and 19.00 hours).

Ultrasonography

Transrectal ovarian ultrasonography was performed in all goats daily (by the same operator) during progesterone treatment (Days 0–6) and every 12 h after device removal until detection of ovulation, or until 96 h after device removal (if ovulation was not detected). All examinations were conducted with a B-mode transrectal ultrasonographic scanner with 8.0 MHz transducer (Áquila Vet Pie Medical – Nutricell, Campinas, Brazil). Does were maintained in a standing position, fecal pellets were removed manually (with a finger), and 20 mL of carboxymethylcellulose gel was placed into the rectum. Ovaries were located and the number, diameter and position of ovarian follicles ≥ 3 mm were recorded. The day of ovulation was defined as the day when the largest follicle, previously identified, was no longer detected. The preovulatory follicle diameter was considered the last measurement obtained before ovulation.

Blood sample collection and assays

Blood samples were obtained 1 day before sponge insertion, on Days 2, 4, 6 and 10 days after sponge removal, always in the pre-prandial period. Blood samples were collected by jugular veinpuncture, into tubes (Vacutainer®, Becton Dickinson and Company, São Paulo, Brazil), either without ethylenediaminetetraacetic acid (EDTA) for insulin-like growth factor 1 (IGF1) and insulin serological analyses, with EDTA for progesterone plasmatic analysis or with sodium fluoride (Glistab®, Labtest, Lagoa Santa, Brazil) for glucose plasmatic analysis. Samples were centrifuged for 15 min and both serum and plasma were stored at -20°C for posterior analyses. Analyses were carried out with commercial radioimmunoassay kits for insulin (Coat-a-Count®, DPC; Diagnostic Products Corporation, Los Angeles, CA, USA), IGF1 (IGF1 with extraction; Gênese®, São Paulo, Brazil) and progesterone (Coat-a-Count®; Diagnostic Products Corporation), whereas for glucose, a colorimetric kit was used (Labtest®, Lagoa Santa, Brazil).

Variables and statistical analyses

The interval to estrus (from sponge removal to first acceptance of mounting); estrus duration (interval from the first to last acceptance of mounting); interval from onset of estrus to ovulation; interval from sponge removal to ovulation; animals ovulating rate (number of does with confirmed ovulation/number of does evaluated by ultrasonography × 100); ovulation rate (number of ovulations per doe); average ovulatory follicle diameter; insulin, IGF1, progesterone and glucose concentrations, were analyzed using Statistic Analytical System (SAS Institute Inc., Cary, NC, USA). Parametric variables were compared by analysis of variance (ANOVA) and the intergroup differences were determined by Student-Newman-Keuls (SNK) test. Data related to animals in estrus and animals ovulating were analyzed by Chi square test (χ^2) and $P < 0.05$ was considered to be statistically significant. The results are described as mean ± SD.

RESULTS

Sexual behavior and ultrasonography end points

There was no difference ($P > 0.05$) among groups C, C + 1% and C + 4% for animals in estrus, interval to estrus, estrus duration, animals ovulating or ovulation rate. However, the interval from onset of estrus to ovulation was shorter ($P < 0.05$) for C + 4% (18.3 ± 11.0 h) when compared to other groups (C: 30.6 ± 10.5 h; C + 1%:

30.3 ± 11.1 h) whereas no differences ($P > 0.05$) were detected between C and C + 1%. Similarly, the interval from sponge removal to ovulation was shorter ($P < 0.05$) for C + 4% (45.2 ± 8.0 h) than C (56.3 ± 12.6 h) or C + 1% (57.7 ± 8.7 h). The average ovulatory follicle diameter was smaller ($P < 0.05$) on C + 4% (6.2 ± 0.7 mm) than C (7.5 ± 0.8 mm), but similar to C + 1% group (7.0 ± 1.0 mm) (Table 1).

Insulin, IGF1, glucose and progesterone concentrations

Plasma glucose concentrations did not differ among groups ($P > 0.05$). Interestingly, for all groups, on the day of sponge removal, higher concentrations were obtained in comparison with other times ($P < 0.05$; Table 2). Conversely, serum concentrations of insulin and IGF1 neither differed among groups nor at time of sample collection ($P > 0.05$; Table 2). Plasma progesterone concentrations did not differ among diets ($P > 0.05$); but, for all diets, at 10 days after sponge removal, higher concentrations were detected when compared to other times ($P < 0.05$; Table 3).

DISCUSSION

Approximately 97% of the goats were detected in estrus, with only one goat (C + 4%) not demonstrating estrus. Likewise, 97% of the goats ovulated in the present study with only one goat (C + 1%) failing to ovulate, similar to 96% earlier reported after the use of a short-term treatment using a different progesterone device for estrus induction (Souza *et al.* 2011). Estrus duration ranged from 31 to 43 h, which is within goat species range and the ovulation rate was similar among all groups. This result indicates that there was no effect of protected fatty acid supplementation on these parameters. The impact of short-term supplementation on ovulation rate may depend on the follicular status, circulating concentrations of glucose and metabolic hormones, hormone dynamics, and the pool of follicles available at the time the

Table 1 Reproductive end points from goats receiving no (Control; C), 1% or 4% of protected fatty acid inclusion in diets during short-term protocol of estrus synchronization (mean ± SD)

	C	C + 1%	C + 4%
Reproductive end points			
Animals in estrus (%)	100.0 [a]	100.0 [a]	90.9 [a]
Interval to estrus (h)	28.0 ± 12.0 [a]	32.8 ± 11.6 [a]	30.4 ± 9.5 [a]
Estrus duration (h)	31.6 ± 12.3 [a]	43.2 ± 12.9 [a]	40.8 ± 14.1 [a]
Animals ovulating (%)	100.0 [a]	90.0 [a]	100.0 [a]
Ovulation rate	1.3 ± 0.5 [a]	1.1 ± 0.3 [a]	1.2 ± 0.4 [a]
Interval from estrus to ovulation (h)	30.6 ± 10.5 [a]	30.3 ± 11.1 [a]	18.3 ± 11.0 [b]
Interval from IVS removal to ovulation (h)	56.3 ± 12.6 [a]	57.7 ± 8.7 [a]	45.2 ± 8.0 [b]
Average ovulatory follicle diameter (mm)	7.5 ± 0.8 [a]	7.0 ± 1.0 [a,b]	6.2 ± 0.7 [b]

[a,] [b]Different letters within rows differ ($P < 0.05$) IVS, intravaginal sponges

Table 2 Circulating concentrations of glucose (mg/dL), insulin (μIU/mL), IGF1 (ng/mL) at different times, from goats with no (Control; C), 1% or 4% protected fatty acid inclusion in diets during short-term protocol of estrus synchronization (mean ± SD)

	C ($n = 11$)	C + 1% ($n = 10$)	C + 4% ($n = 11$)
Glucose (mg/dL)			
One day before IVS insertion	58.0 ± 5.5 [b]	57.1 ± 6.3 [b]	53.6 ± 5.0 [b]
Two days after IVS insertion	56.5 ± 8.3 [b]	56.1 ± 5.6 [b]	57.7 ± 5.4 [b]
Two days before IVS removal	54.6 ± 5.0 [b]	54.3 ± 6.8 [b]	57.4 ± 5.4 [b]
On the day of IVS removal	65.3 ± 6.5 [a]	63.3 ± 6.9 [a]	69.1 ± 8.9 [a]
Ten days after IVS removal	54.2 ± 6.7 [b]	51.7 ± 3.5 [b]	53.8 ± 9.5 [b]
Insulin (μUI/mL)			
One day before IVS insertion	9.1 ± 4.5 [a]	8.6 ± 2.2 [a]	6.8 ± 3.0 [a]
Two days after IVS insertion	6.9 ± 1.8 [a]	9.0 ± 5.7 [a]	6.5 ± 3.2 [a]
Two days before IVS removal	7.3 ± 6.0 [a]	9.8 ± 3.4 [a]	9.8 ± 5.0 [a]
On the day of IVS removal	7.8 ± 3.0 [a]	9.0 ± 3.5 [a]	7.4 ± 6.4 [a]
Ten days after IVS removal	10.0 ± 5.6 [a]	7.1 ± 4.1 [a]	5.3 ± 2.7 [a]
IGF1 (ng/mL)			
One day before IVS insertion	334.9 ± 33.9 [a]	331.4 ± 31.7 [a]	372.3 ± 31.6 [a]
Two days after IVS insertion	342.6 ± 45.6 [a]	189.7 ± 79.7 [a]	239.7 ± 52.8 [a]
Two days before IVS removal	308.1 ± 47.5 [a]	305.1 ± 52.8 [a]	305.3 ± 43.4 [a]
On the day of IVS removal	295.3 ± 45.4 [a]	267.8 ± 27.0 [a]	314.5 ± 48.3 [a]
Ten days after IVS removal	272.6 ± 46.4 [a]	290.3 ± 26.9 [a]	344.8 ± 44.8 [a]

[a,] [b]Different letters within columns (each hormone) differ ($P < 0.05$) IGF1, insulin-like growth factor 1; IVS, intravaginal sponges

Table 3 Circulating concentrations of progesterone (ng/mL) at different times from goats with no (Control; C), 1% or 4% protected fatty acid inclusion in diets during short-term protocol of estrus synchronization (mean ± SD)

	C	C + 1%	C + 4%
Progesterone (ng/mL)			
One day before IVS insertion	1.7 ± 3.8 [b] (11)	2.0 ± 3.8 [b] (9)	0.1 ± 0.1 [b] (11)
Two days after IVS insertion	1.6 ± 3.3 [b] (11)	1.5 ± 2.7 [b] (10)	0.5 ± 0.9 [b] (11)
Two days before IVS removal	0.3 ± 0.3 [b] (11)	0.2 ± 0.2 [b] (9)	0.1 ± 0.1 [b] (10)
On the day of IVS removal	0.2 ± 0.2 [b] (11)	0.2 ± 0.1 [b] (8)	0.1 ± 0.1 [b] (8)
Ten days after IVS removal	7.6 ± 3.9 [a] (11)	7.0 ± 2.0 [a] (10)	7.0 ± 3.1 [a] (11)

[a, b]Different letters within columns differ ($P < 0.05$) Numbers in brackets represent numbers of animals IVS, intravaginal sponges

supplement is fed, making it difficult to obtain consistency in the results in different laboratories (Viñoles *et al*. 2005).

On average, does from all groups manifested estrus within 31 h after sponge removal, which is desirable since it was demonstrated that fertility decreased significantly for goats coming into estrus later than 30 h (late estrus) (Baril *et al*. 1993). We can speculate that the protected fatty supplementation was not efficient to induce a raise of estradiol production by follicles, which would possibly shorten this interval. Interestingly, Robinson *et al*. (2002) reported controversial data regarding estradiol concentrations in cattle. Probably, these differences may be due to the amount or moment of fat inclusion as well as the species.

The interval from onset of estrus to ovulation and the interval from sponge removal to ovulation were both shorter ($P < 0.05$) for C + 4% (18; 45 h, respectively) when comparing the other groups (~30; 57 h). It is noteworthy that to know the expected time of ovulation depending on the treatment used is essential in order to correctly apply, for example, a timed artificial insemination program. Progesterone concentrations were similar among groups at sponge removal and 10 days after. We hypothesize that if the blood sample collections would have been daily performed after sponge removal, an earlier increase in progesterone concentrations in the C + 4% group would be observed due to the anticipation of ovulation. Moreover, the average ovulatory follicle diameter was smaller ($P < 0.05$) in C + 4% (6.2 mm) than other groups (7-7.5 mm). It is reasonable to assume that since this group had a shorter interval from estrus onset to ovulation, the follicles had a lower growth rate and therefore ovulated follicles of smaller diameters. Interestingly, in sheep, the use of calcium salt of long-chain fatty acids from corn oil in diet improved the number and diameter of the ovulatory follicles as well as ovulation rate (Herrera-Camacho *et al*. 2008).

For cattle, considering the total availability of diet energy, it was suggested that when diet contains forage, the fat inclusion rate should be ~2-3% and not over 4%, otherwise the energetic density of diet will not be increased (Hess 2008). Perhaps, this pattern may be similar for goats and the level of protected fatty acid included in diet may have been low for the C + 1% group and high for the C + 4% group, justifying the absence of benefits on reproductive parameters. According to Titi and Awad (2007), high levels of fat may reduce the dry matter digestibility by the rumen, causing a lower availability of energy.

Plasma glucose concentrations did not differ among groups ($P > 0.05$). However, for all groups, on the day of sponge removal, higher concentrations (63-69 mg/dL) were obtained in comparison with other times ($P < 0.05$). Viñoles *et al*. (2005) demonstrated that concentrations of glucose reached the peak at the third day after the start of a short-term supplementation and at the sixth day it was still high, in both supplemented and control ewes. Coincidently, in the current study the highest concentrations were also obtained on day 6 after the start of supplementation. In fact, it has been demonstrated that the pattern of glucose and insulin concentrations occurs in a wave manner, increasing and decreasing (Zabuli *et al*. 2010). Our results are in accordance with Green *et al*. (2008) who reported no significant influence for ewes fed with rumen-protected PUFA on glucose concentrations.

Protected fatty acids cause a raise in hepatic gluconeogenesis which is associated with a raise in insulin and IGF1 plasmatic circulation, known to increase ovarian follicles population (Thomas & Williams 1996). However, in the current study, serum IGF1 and insulin concentrations were similar among groups or regarding the different times evaluated ($P > 0.05$). Likewise, Green *et al*. (2008) supplemented ewes with PUFA and neither insulin nor IGF1 concentrations differed in relation to diet. Circulating concentrations of insulin and IGF1 may not be a good indicator of their real bioavailability for follicles and therefore samples from follicular fluid should also be analyzed for further information.

At 10 days after sponge removal, higher concentrations of progesterone were detected in comparison with other times. The reason is easily explained by the fact that at this time, animals were pregnant and therefore secreting higher concentrations of progesterone due to active corpora lutea (CL). In the current study, plasma progesterone concentrations were similar among groups. Ghoreishi *et al*. (2007) showed that ewes fed with different amounts of protected fatty acid had an increase on plasma progesterone concentration. Feeding calcium salts of fish oil, beginning early post partum, had no positive or negative effect on progesterone

concentrations during a programmed estrous cycle (Bilby *et al*. 2006). Furthermore, Moussavi *et al*. (2007) failed to detect any effect of feeding either fishmeal or calcium salts of fish oil on progesterone concentrations during a programmed estrous cycle until day 15. Robinson *et al*. (2002) found no diet × sample day interaction or effect of diet on progesterone concentrations in beef heifers supplemented with fat. The authors emphasized that although treatment effects appeared to increase with time, there was no diet × estrous cycle interaction. Certain PUFAs may increase serum concentration of insulin and this could reduce hepatic expression of some enzymes of the cytochrome P450 complex that catabolize progesterone (Lemley *et al*. 2008). We had no difference in the pattern of insulin concentrations and therefore no influence in progesterone concentrations.

Conclusions

In conclusion, from the present results it appears that supplementation with 1% or 4% protected fatty acid in diet during short-term protocol of estrus synchronization was not efficient to improve reproductive end points such as follicular population or ovulation rate of nulliparous dairy goats. Further detailed studies are necessary to propose different percentages of inclusion for goat species in order to identify an optimal amount to positively affect reproductive end points.

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