Cabergoline decreases milk production in dairy ewes. By Caja et al.

Cabergoline, an inhibitor of prolactin secretion, was investigated in dairy ewes in late-lactation. Treatments were a single intramuscular injection at 3 doses of cabergoline: control (1.0 mL saline), 0.56 and 1.12 mg/ewe. Cabergoline supressed prolactin, decreased milk secretion and udder size but, all variables measured except udder volume, returned to control values after 5 d. No side or dose effects were detected. In conclusion, cabergoline may have interest to facilitate the decrease of milk production in dairy ewes as in the case of dry-off.

Suppression of prolactin and reduction of milk secretion by effect of cabergoline in lactating dairy ewes

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ABSTRACT

The effects of cabergoline, an ergot derivative and dopamine receptors agonist, were investigated in 30 ewes of 2 dairy breeds (Manchega, MN, n = 15; Lacaune, LC, n = 15). Ewes were in similar late-lactation stage but differed in milk yield according to breed (MN vs. LC, 1.02 ± 0.03 vs. 2.27 ± 0.05 kg/d). Treatments consisted of a single i.m. injection of cabergoline at different doses per ewe. Doses were: low (L, 0.56 mg), high (H, 1.12 mg) and control (CO, 1 mL saline). Milk yield was recorded daily (d −14 to 25), milk and blood sampled, and udder traits measured from d −2 to 14 after injection. No local reaction in the injection site, as well as on behavior and metabolic indicators of the ewes were detected after the cabergoline injection, but milk yield fell rapidly in both breeds (MN vs. LC, –54% vs. –27%), when compared to CO ewes. Cabergoline effects progressively disappeared after d 5 and no milk yield differences between treatments were detected from d 8 to 25 after injection. Milk fat and protein contents increased similarly (22% and 23%; respectively) in both breeds and at both cabergoline doses until d 5 and the effects disappeared thereafter. Plasma prolactin (PRL) decreased dramatically in the L and H treated ewes the day after injection, when compared to the CO ewes, and reached

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values below the detection limit of the assay between d 1 and 5, increasing similarly thereafter. On d 14, PRL values were 58% greater in the L and H treated than in the CO ewes, showing that PRL concentrations rebounded when the cabergoline effects ceased. Total udder volume correlated with milk accumulated in the udder (r = 0.77) of all groups of ewes throughout the experiment, suggesting its use as a non-invasive method for the estimation of milk stored in the udder. Udder volume was similar for the L and H ewes, but both values were lower than those of the CO ewes from d 1 to 14 after injection. No other effects on udder size were detected. In conclusion, cabergoline dramatically inhibited PRL secretion and decreased milk yield and udder volume of lactating dairy ewes. The L (0.56 mg/ewe) dose of cabergoline was as effective as the H (1.12 mg/ewe) in the 2 breeds of dairy ewes. These results suggest the interest of cabergoline to facilitate the decrease of milk production in dairy ewes (e.g., dry-off, illness care), although further research in pregnant dairy ewes and during the following lactation is still needed.

Key words: ergotic, dairy sheep, milk secretion, prolactin, udder.

INTRODUCTION

Late pregnancy and drying-off are challenging periods for dairy ruminants. Pregnant ewes are susceptible to ketone bodies toxemia and new intramammary infections (IMI) because of the increase of glucose demand and the decrease of immunocompetence (Shwimmer et al., 2008; Fthenakis et al., 2012; Zhao et al., 2019). The risks are greater in high-yielding and twin-bearing ewes (Oddy and Holst, 1991; Silva-del-Río et al., 2010), especially when using low energy diets or feed restriction during drying-off (Caldeira et al., 2007). Additionally, nutrient restriction at dry-off decreases 30% basal blood prolactin (PRL), as reported by Ollier et al. (2013) in dairy cows fed hay on the days preceding the dry-off.

58 Nutrient restriction at dry-off decrease the proliferation of mononuclear cells and
59 compromises immunocompetence (Ollier et al., 2014, 2015; Zhao et al., 2019). Cessation of
60 milking results in udder engorgement which leads mammary gland epithelium to apoptosis and,
61 if excessive, induces mammary inflammation and cell necrosis (Zobel et al., 2015). In dairy
62 sheep, where abrupt drying-off is commonly done, selective (i.e., IMI positive) or generalized
63 antibiotic therapy is recommended at drying-off to improve udder health and milk yield in the
64 following lactation (Gonzalo et al., 2004; Linage and Gonzalo, 2008). Consequently, reduction
65 of udder insults during dry-off may be a strategy to decrease the use of antibiotic therapies at
66 drying-off.

67 Under a physiological approach, an interesting method to facilitate the dry-off could be
68 inducing the cessation of milk production by interfering with the transmission of hormonal
69 signals from the pituitary gland (i.e., PRL inhibition). This approach has been proposed as a
70 management tool in dairy husbandry (Lacasse et al., 2019) to avoid inappropriate lactation or
71 to alleviate the nutritional stress in sick or injured lactating animals unable to support their level
72 of production.

73 Dopamine has a direct effect on the lactotrophs of the anterior pituitary by binding to their
74 D_2 receptors and reducing PRL exocytosis and gene expression (Fitzgerald and Dinan, 2008).
75 Ergotic (e.g., bromocriptine, cabergoline and metergoline) and non-ergotic (e.g., quinagolide)
76 derivatives also bind to D_2 receptors of the lactotrophs, and have shown to decrease PRL
77 secretion and milk production, although with differences in affinity, half-life and side-effects
78 (Bole-Feysot et al., 1998; Barlier and Jaquet 2006; Kvernmo et al., 2006). The use of PRL
79 inhibitors in lactating ruminants have been deeply reviewed by Lacasse et al. (2012, 2016,
80 2019) and Zhao et al. (2019).

81 Quinagolide injection proved to reduce plasmatic PRL and to be effective for milk
82 reduction, both in early- (Lacasse et al., 2011; Boutinaud et al., 2012) and late-lactating (Ollier

et al., 2013, 2014, 2015) dairy cows. Moreover, proliferation and survival of mammary epithelial cells after quinagolide treatment were fully restored by PRL injection (Lollivier et al., 2015; Lacasse et al., 2016), supporting the galactopoietic role of PRL in ruminants.

Cabergoline is a highly specific agonist of D_2 receptors with a long elimination half-life (Kvernmo et al., 2006; Odaka et al., 2014). The use of cabergoline, initially authorized by the European Medicines Agency (EMA, 2015) for facilitating the dry-off of cattle, decreased plasma PRL and accelerated udder involution reducing the secretory activity of mammary epithelial cells, udder engorgement and incidence of milk leakage in dairy cows (Bach et al., 2015; Boutinaud et al., 2016). A large-scale clinical study with 900 dairy cows in 63 farms (Hop et al., 2019), reported that cabergoline decreases under practical conditions the risks of milk leakage and of new IMI during the drying-off and post-calving periods. Nevertheless, despite the positive effects of cabergoline and the no food safety risks for consumers, when withdrawal period is respected (i.e., 32 d during dry-off or 8 milkings during lactation; EMA, 2015), its use in high-yielding dairy cows at late pregnancy has been associated to occasional adverse events, usually in the 24-h post-injection. These were recumbency and mortality, which were related to metabolic disorders (i.e., hypocalcemia, hypothermia, ataxia, adipsia, circulatory disorder and diarrhea). Therefore, the marketing authorization of cabergoline as Velactis (Ceva Animal Health, Libourne, FR) was first suspended (EMA, 2016) and finally its use banned in Europe (EMA, 2019), considering that the overall benefit-risk balance in dairy cows was negative.

Few and controversial data are available on the use of dopamine agonists in small ruminants. Arlt et al. (2011) reported the inefficacy of cabergoline to cease inappropriate lactations in hobby goats. On the other hand, Lacasse et al. (2016) cited no effects on milk production of repeated injections of quinagolide (1 mg/d for 4 wk; B. Ponchon, V. Lollivier and M. Boutinaud, unpublished results), whereas a single cabergoline injection (1 mg; V. Lollivier

and M. Boutinaud, unpublished results) decreased milk yield (–28%) in dairy goats. The effects of dopamine agonists and the adequate dose for dairy ewes are unknown.

The objective of this work is to study the effects of 2 doses of cabergoline on PRL suppression and milk secretion to determine the suitable dose and the time-lasting effects in 2 breeds of dairy ewes in late-lactation.

MATERIALS AND METHODS

The study was conducted in the experimental farm of the SGCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona (UAB), in Bellaterra (Barcelona, Spain) during 2016. Animal-care conditions and management practices agreed with the Spanish Royal Decree 53/2013 on the protection of animals used for experimental purposes, the codes of recommendations for the welfare of dairy sheep of the Ministry of Agriculture, Alimentation and Environment of Spain (MAPA, 2007) and the procedures stated by the Ethical Committee of Animal and Human Experimentation of the UAB (Procedure #4992).

Ewe Management and Feeding

A total of 30 ewes of 2 dairy breeds (MN, Manchega, n = 15; LC, Lacaune, n = 15) in late-lactation (185 ± 11 and 186 ± 11 DIM, respectively; values are means ± SE) and kept as a single group on wood-chip bedded pens, were used. Their main characteristics (MN and LC, respectively) were: age (3.8 ± 0.5 and 2.7 ± 0.4 yr), BW (73.6 ± 2.5 and 67.6 ± 1.9 kg), BCS (3.08 ± 0.07 and 2.85 ± 0.07), and milk yield (1.02 ± 0.03 and 2.27 ± 0.05 kg/d). All ewes wore ruminal mini-boluses for electronic identification (20 g; Datamars, Bedano, SW) that were used for automatic milk recording (Ait-Said et al., 2014). Machine milking was conducted twice daily (0700 and 1700) in a 2×12 parallel stall milking parlor with automatic milk-recording

devices (MM25SG; DeLaval, Tumba, SE) with similar procedures to those described by Elhadi et al. (2019).

Diet consisted of a TMR ad libitum (forage:concentrate, 55:45%; DM basis) with alfalfa hay as forage and a farm-produced concentrate [ingredients: soybean hulls, 50.0%; barley grain, 10.0%; oats grain, 10.0%; gluten feed meal, 10.0%; rapeseed 00 meal, 5.0%; soybean oil, 5.0%; corn grain, 4.0%; bi-calcium phosphate, 2.5%; cane molasses, 2.0%; VitafacOvino-0.3 premix (DSM Nutritional Products, Madrid, ES), 1.0%; salt, 0.5%; as fed]. Additionally, all ewes received 100 g of corn whole grain in the individual feeders of the milking parlor at each milking to encourage their coming in. Nutrient requirements were calculated by INRAtion v.4.07 (Educagri éditions, Dijon, FR). Ewes had free access to water and commercial mineral blocks (Multi-Block; Agrària Comarcal del Vallès, Llerona, ES) in the pens.

Experimental Treatments

Ewes were blocked in 3 balanced groups of 10 animals (5 of each breed) according to age, BW, BCS, and milk yield, and submitted to a short-term lactation experiment divided in 2 periods: pre- (d –14 to –1) and post-injection (d 0 to 25). The experimental design consisted of a 2×3 factorial (breed×treatment) to which the ewe groups were randomly allocated. Treatments consisted of the dose of cabergoline (Velactis 1.12 mg/mL of cabergoline; Ceva Animal Health, Libourne, FR), intramuscularly injected into the middle of the left side of the neck after the p.m. milking, and were (cabergoline/ewe): low (L, 0.56 mg), high (H, 1.12 mg), and control (CO, 1 mL saline). The cabergoline doses used were achieved by the injection of 0.5 or 1.0 mL of Velactis per ewe, close to the EMA (2015) recommended treatment dose (RTD = 5.6 mg/cow or 7 to 10 µg/kg BW), equivalent to 0.49 to 0.70 mg of cabergoline for a standard ewe of 70 kg BW, and far from the 3×RTD (1.47 to 2.10 mg/ewe) and 5×RTD (2.45 to 3.50 mg/ewe) overdoses injected for the target animal safety studies (EMA, 2015). Although the cabergoline

157 diluent used in the Velactis product was a lipidic solvent mixture (i.e., dimethyl sulfoxide and
158 medium-chain triglycerides), we used sterile saline solution (0.9% NaCl; Laboratorios Grifols,
159 Parets del Valles, ES) in our control ewes to evaluate the whole local reaction to the commercial
160 product injection. Collected milk was discarded during the following week according to the
161 withdrawal recommendations.
162
163 ***Measurements, Sampling, and Analyses***
164 ***Reactions to the Injection.*** Local tolerance to cabergoline was evaluated on d 0, 1 and 7
165 post-injection using a severity score (0 to 3 points), according to the diameter of the adverse
166 reaction produced by the injection (0, none; 1, <2 cm; 2, between 2 and 8 cm; 3, >8 cm;
167 accuracy, 0.5 points). Special surveillance of the treated ewes was done at 8-h interval by a
168 technician supervised by the veterinarian responsible of the SGCE of the UAB during the 48-h
169 post-injection. General appearance, eating and drinking behavior and mobility of the ewes when
170 attending the twice-daily milking were monitored throughout the experiment.
171 ***Milk Yield.*** Milk yield of individual ewes was recorded by weight at each milking during
172 the whole experimental period (d –14 to 25), using the milk-flow and milk-recording automatic
173 units of the milking parlor (MM25SG, DeLaval). Data were uploaded using the AlPro software
174 7.2 (DeLaval) and daily reviewed to avoid incorrect values.
175 ***Milk Composition.*** Representative milk samples of each ewe were taken pre- (d −2 and −1)
176 and post- (d 1, 2, 5, 7 and 14) cabergoline injection for compositional analysis using
177 proportional milk samplers (DeLaval). Milk samples (50 mL) were composited according to
178 the daily milking intervals (a.m.:p.m., 60:40), preserved with an antimicrobial tablet (Bronopol;
179 Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) and stored at 4°C until
180 analysis. Milk samples were analyzed in the Dairy Herd Improvement Laboratory of Catalonia

(ALLIC, Cabrils, ES) for fat, total protein, lactose and urea (Milkoscan FT2; Foss, Hillerød, DK) and SCC (Fossomatic 5000, Foss).

Udder Size. Udder measurements were recorded before the p.m. milking on d −2 and −1, pre-treatment, and d 2, 5, 7 and 14 post-treatment, according to the agreed-upon FAO-M4 study protocol for dairy sheep (Labussière, 1983) with the ewes restrained by head-lockers in the milking stalls. Udder volume was estimated by water displacement using a 5-L bucket of warm water. Udder width was measured as the maximum udder width value by using a surgical thickness compass (Hauptner, Solingen, DE) and udder base-floor distance was taken by a measuring flexible tape, both from the back of the ewes.

Blood Measurements. Blood samples were taken from the jugular vein using 4-mL Vacutainer tubes with EDTA K2E 7.2 mg (BD; Belliver Industrial Estate, Plymouth, UK) 1-h after the a.m. milking (without corn supplement) and before feeding at d −1 (pre-treatment), and d 1, 2, 5, 7 and 14 (post-treatment). EDTA blood collecting tubes were used to avoid possible interactions in the hormonal enzymatic competitive analyses (Kohek et al., 2002). Plasma was obtained by blood centrifugation for 15 min at 2,000 × *g* and 4°C, transferred to 0.5 mL Eppendorf tubes and stored at −20°C for analysis.

Concentration of PRL in plasma was measured by ELISA sandwich type analysis (human PRL-ELISA KAPD1291, DIAsource Immunoassays, Leuven, BE) and the stopped ELISA plates were read in an automatic reader (iEMS Reader MF V.2.9–0; Labsystems España, Barcelona, ES) at 450 nm. Detection limit, intra and inter assay CV were 0.35 ng/mL, 5.5 and 6.5%, respectively.

Impact of cabergoline treatments on the metabolic status of the ewes was assessed by the plasmatic concentrations of glucose, lactate, γ-glutamyl transferase (GGT), phosphorus and creatinine, using an Olympus AU480 analyzer (Olympus Europa, Hamburg, DE) with the specific Reagent System of Olympus (OSR, Beckman Coulter, Krefeld, IE); the respective

206 analytical methods and reagents used were: hexokinase method (OSR6121), lactate oxidase
207 method (OSR6193), γ-glutamyl-3-carboxy-4-nitroanilide method (OSR6119, with a
208 concentration greater than 4 mmol/L), phospho molybdate method (OSR6122) and the Jaffé
209 method (OSR6178). Changes in absorbance were read at 340 nm, except for creatinine that
210 were read at 520 nm. Non-esterified fatty acids (NEFA) were determined by enzymatic
211 colorimetry (ACS-ACOD-MEHA; acyl-CoA synthetase, acyl-CoA oxidase, 3-methyl-N-ethyl-
212 N(β-hydroxy-ethyl) aniline) in the same analyzer using NEFA HR reagents (Fujifilm Wako
213 Chemicals, Neuss, DE) and read at 410 nm. Additionally, concentration of lactose in plasma
214 was used as indicator of the leakiness of the lactocyte tight junctions and was analyzed by
215 difference based on two enzymatic reactions using galactose dehydrogenase and β-
216 galactosidase, one measuring galactose and the other lactose and galactose (Boehringer
217 Mannheim/R-Biopharm, Darmstad, DE) in the Olympus AU480 analyzer and reading at 340
218 nm. The use of EDTA collecting tubes did not allow the analyses of Ca, K and Na in plasma.
219
220 ***Statistical Analyses***
221 Data were analyzed by the MIXED procedure for repeated measurements of SAS v.9.4 (SAS
222 Institute Inc., Cary, NC). The statistical mixed model to evaluate the similarity of the ewe's
223 groups during the pre-treatment period and the response to the treatments contained the fixed
224 effects of breed (MN and LC), cabergoline dose (L, H and CO), sampling time and
225 breed×treatment interaction, as well as the random effects of the experimental unit (the animal),
226 and the residual error. Values of the variables were computed for their respective treatment and
227 sampling dates, the means expressed as least squares means (LSM) and separated by pairwise
228 comparison using the PDIFF ADJUST=SCHEFFE test of SAS. Pearson correlation coefficients
229 (r) were calculated using the CORR procedure of SAS. Significance was declared at $P < 0.05$
230 and a tendency was considered when $P < 0.10$.

RESULTS AND DISCUSSION

Cabergoline Dosage. According to the mean BW of the dairy ewes enrolled in the experiment (MN and LC, 73.6 and 67.6 kg BW, on average, respectively), the chosen cabergoline doses (L and H, respectively) were slightly lower in the MN (7.6 and 15.2 µg/kg BW) than in the LC (8.3 and 16.6 µg/kg BW) ewes. The L dose fell in the range of the EMA (2015) recommended treatment dose of cabergoline (RTD = 7 to 10 µg/kg BW) and agreed with the dosage previously used for the dry-off of dairy cows (Boutinaud et al., 2016; 8.7 µg/kg BW). On the other hand, the H dose doubled the RTD but was far from the 3×RTD (21 to 30 µg/kg BW) and 5×RTD (35 to 50 µg/kg BW) overdoses injected for target animal safety studies (EMA, 2015). As previously indicated by Lacasse et al. (2016, 2019), and despite the inconsistency of some studies done in goats, a dose of 1.0 mg cabergoline (approximately 16.6 µg/kg BW for a 60 kg BW goat) was also used in high-yielding dairy goats (i.e., 3.5 kg/d milk) in early-lactation.

Reaction to Cabergoline Injection. Intramuscular injection of cabergoline at both L and H doses did not produce local or adverse reactions in the right site of the ewes' neck; only 3 MN ewes, 1 from each treatment (i.e., CO, L and H), showed a slight swelling reaction (score 1) to the injection. No swelling reactions were observed in the LC ewes characterized by having open fleeces and wool-uncovered necks, in comparison to MN, which may have allowed a more precise and clean i.m. injection. As a consequence, the values of swelling scores were very low and similar between treatments (0.10 ± 0.11, on average).

Regardless of the cabergoline dose used, no general reactions or apparent changes in the behavior of the L and H treated ewes (i.e., abatement, recumbency, lack of interest to the feed bunk or drinkers after feed offering), were detected during the 48-h post-injection and throughout the experiment, when compared to the CO ewes. Moreover, no changes in the

motion of the ewes, when being moved for the twice-daily milking or in their eating behavior in the milking parlor (i.e., refusal of corn whole grain) were recorded. Unfortunately, no data on local or adverse side-effects of the high dose of cabergoline used in the goat experiments were available for comparison (Lacasse et al., 2016, 2019).

Prolactin in Plasma. As shown in Figure 1, plasma concentrations of PRL in the CO ewes ranged between 12 and 24 ng/mL (17.8 ± 1.5 ng/mL, on average) from d −1 to 14; no differences were detected in both breeds ($P = 0.99$). On the contrary, concentrations of PRL dramatically fell in the cabergoline treated ewes (Figure 1) which values were under the detection limit (i.e., 0.35 ng/mL) during d 1 and 2 post-injection, slightly increased on d 5, and raised rapidly thereafter. The low PRL values persisted for both L and H doses from d 1 to 5 when compared to CO ewes (–86%, on average; $P < 0.001$; Table 1), with no differences between breeds ($P = 0.89$; Table 1) and raised after d 7 (Figure 1). Values of plasmatic PRL of our CO ewes 1-h after milking agreed with the basal values reported by Boutinaud et al. (2016) in Holstein dairy cows before milking (approximately, 16 ng/mL). Nevertheless, the effect of cabergoline injection on the PRL concentration of our lactating ewes was greater than the decrease reported by Boutinaud et al. (2016; –39%) in Holstein dairy cows at dry-off, when compared to conventionally dried cows and both fed a dry hay diet. This lower PRL difference may have been a consequence of the negative effect of feed restriction in the control cows, as previously reported by Ollier et al. (2013). Additionally, the difference between control and cabergoline injected cows in the Boutinaud et al. (2016) study, persisted for 8-d and disappeared on d 14, likewise as it was observed in our ewes (Figure 1). Moreover, –20% plasma PRL after cabergoline injection was reported in the Bach et al. (2015) study, but the authors did not mention the cow's BW and the blood sampling time with regard to milking, precluding the comparison with other data.

Lacasse et al. (2011) and Boutinaud et al. (2012) also reported decreases in the concentrations of PRL released at milking (–12 to –32%) in dairy cows treated with repeated injections of quinagolide during lactation (8-wk).

Interestingly, greater PRL values in plasma were detected on d 14 post-injection in the L and H ewes (58%, on average; $P < 0.001$), when compared to the CO ewes, indicating a PRL rebound effect after ceasing the cabergoline treatment (Figure 1). The PRL rebound was the response (paradoxical reaction) of the ewe's metabolism to return to its basal state (homeostasis) after having been modified by the injection of cabergoline. Evidence of PRL rebound can also be observed in the results of Ollier et al. (2013) in dairy cows injected with quinagolide for 4-d before dry-off (approximately 50% increase basal value at d 14 post-injection), but it was not visible in the Bach et al. (2015) and Boutinaud et al. (2016) dairy cows injected with cabergoline at dry-off.

Given that cabergoline half-life in cows is 19-h (EMA, 2015) and its high affinity for dopamine D_2-like receptors (Kvernmo et al., 2006), the occurrence of the PRL rebound on d 14 after injection may be related to a mid-term feed-back of pituitary's lactotrophs, decreasing the release of natural dopamine, which will result in a rise of PRL. Mechanism of PRL rebound in rats after dopamine withdrawal was explained by Chen et al. (1993) and Chang and Shin (1999) who demonstrated that dopamine acts on D_2 receptors both to inhibit and to stimulate PRL release. We hypothesize that this may be related to the decrease of the activity of tyrosine hydroxylase (TH), the rate-limiting key enzyme in the biosynthesis and availability of catecholamines (i.e., dopamine, noradrenaline and adrenaline) during the cabergoline treatment. Gordon et al. (2008) showed that TH binds to dopamine in high- and low-affinity binding sites, and dissociation of TH from dopamine markedly increases TH activity that will lead to greater PRL concentration in blood.

304 ***Milk Yield.*** All of the ewes were healthy at the start of the experiment and showed high milk
305 yields for late-lactation before applying the treatments (Figure 2). Lactation persistency,
306 estimated as the linear slope of the milk yield curve according to the stage of lactation was –18
307 g/d for the CO ewes (y = –0.0178x + 1.44; r = 0.92, $P < 0.001$) during the whole experiment (d
308 –14 to 25), with differences in both breeds. Persistency was inverse to the level of production
309 of each breed (MN, –6 g/d; LC, –28 g/d; $P = 0.004$), the lower the yield, the higher the
310 persistency.
311 Milk yield fell rapidly during the first 5 d after treatment (Figure 2 and Table 1) for both
312 cabergoline doses (L vs. H, –40% vs. –22%; $P < 0.001$) and ewe breeds (MN and LC, –54%
313 and –27%; $P < 0.001$), when compared to CO ewes. No differences were detected between the
314 L and H doses in both breeds (Table 1), the cabergoline×breed interaction being not significant
315 ($P = 0.99$). On average, the injection of cabergoline produced a sudden and marked decrease of
316 milk yield (–31%; $P < 0.001$) immediately after the treatment and the slope (d 1 to 5) of the
317 milk regression was –64 g/d of milk, on average. Milk yield progressively increased after d 5,
318 and no differences were detected between the CO and the cabergoline treated ewes on d 8 after
319 injection ($P = 0.23$) and thereafter (d 9 to 25; $P = 0.49$). Nevertheless, all ewes showed an
320 unexpected increase in milk yield between d 8 to 10 (26%, on average), followed by a milk
321 drop on d 11 and 12 (–20%, on average; Figure 2), without differences between the CO and the
322 cabergoline treated ewes ($P = 0.90$). As a result, the persistency of the cabergoline treated ewes
323 throughout the whole experiment (d –14 to 25) was on average –23 g/d (y = –0.0233x + 1.35;
324 r = 0.85, $P < 0.001$), similar to the CO ewes ($P = 0.35$) and without differences between the H
325 and L doses ($P = 0.85$). Again, persistency was inverse to the yield of each breed (MN, –16 g/d;
326 LC, –30 g/d; $P = 0.026$).
327 Milk yield also declines faster in quinagolide treated cows (daily injections for 8-wk) during
328 lactation than in control cows (Lacasse et al., 2011; approximately –15%), but no effects of a

329 single injection of cabergoline have been tested during lactation. Milk decrease after the
330 cabergoline injection in our ewes duplicated the above indicated value in dairy cows.
331 The increase in milk yield after d 11 was unlikely produced by the PRL rebound because the
332 parallel raise in milk yield of the CO ewes and the numerically greater concentration of PRL in
333 the plasma of the L treated ewes (Figure 1).
334 ***Milk Composition.*** On average, milk fat (Figure 3a) and milk protein (Figure 3b) contents
335 of our ewes increased rapidly from d 1 to 5 (22 and 23%, respectively; $P < 0.001$) after the
336 cabergoline treatment. Despite these increases in milk component concentrations, daily yields
337 of milk fat and milk protein tended to decrease ($P = 0.07$ and $P = 0.10$, respectively; Table 1)
338 as a result of the decrease in milk yield produced by the cabergoline injection. The effects on
339 milk fat and protein contents disappeared after d 7 post-injection, accompanying the recovery
340 of milk yield previously discussed after d 5. On the contrary, no differences in milk lactose
341 content were detected between treatments on average ($P = 0.11$), although the content of lactose
342 in the milk of the cabergoline treated ewes was lower than in the CO ewes on d 2 and 5 (Figure
343 3c; $P = 0.042$). Milk lactose yield decreased by effect of cabergoline (Table 1; $P = 0.003$),
344 although no differences were detected between the L and H treated ewes. The differences in
345 milk composition among treatments decreased after d 5 and no differences in the concentration
346 of milk components were detected at d 7 and 14 post-injection.
347 Despite the reported drop in milk yield of quinagolide treated cows during lactation (Lacasse
348 et al., 2011), only numerically greater milk fat and milk protein contents are seen in milk;
349 nevertheless, daily yields of both milk fat and milk protein decrease, agreeing the results
350 obtained in our ewes. Moreover, marked decreases of milk lactose content during the last 4-wk
351 of treatment and of lactose yield were reported by Lacasse et al. (2011) in the quinagolide
352 treated cows, agreeing with our results in cabergoline treated ewes during lactation. Conversely,
353 Boutinaud et al. (2016) did not find effects on the composition of mammary secretions (i.e., fat,

354 protein and lactose) of Holstein cows treated with cabergoline at dry-off, although the
355 composition of mammary secretions after ceasing milking is not directly comparable to that of
356 milk obtained at milking during lactation.

357 No effects of cabergoline treatment were detected on the SCC of our ewes ($P = 0.50$; Table
358 1) that, despite being in late-lactation, showed a low SCC (5.40 ± 0.26, on average, equivalent
359 to 250,000 cells/mL).

360 All effects of cabergoline on milk composition of our ewes disappeared after the first week
361 post-treatment and no differences with the CO ewes were detectable at d 14.

362 ***Metabolic Indicators.*** Impact of cabergoline treatments on the metabolic status of the ewes
363 was assessed by the plasmatic concentrations of several metabolic indicators during the critical
364 period post-treatment (d 1 to 5), as well as for glucose and creatinine during the whole
365 experiment (data not shown). No effects of cabergoline treatment were detected on glucose,
366 NEFA, lactate and the GGT liver enzyme (related to glutathione metabolism, amino acid
367 absorption and protection against oxidation) as shown in Table 1. Moreover, glucose and
368 creatinine in plasma were steady throughout the whole experimental period ($P = 0.97$).
369 Plasmatic values of NEFA tended to be greater in the LC ewes, according to their greater milk
370 production ($P = 0.08$; Table 1) but did not differ by cabergoline treatment ($P = 0.89$) indicating
371 that cabergoline did not produce metabolic stress in our ewes. Similar results were obtained by
372 Ollier et al. (2014) in quinagolide treated dairy cows during drying-off.

373 Although it was not possible to analyze Ca and Mg values because of the EDTA collecting
374 blood tubes, no differences in plasmatic P values were detected (4.50 ± 0.31 mg P/dL, on
375 average) between treatments ($P = 0.89$) or breeds ($P = 0.49$). According to Venjakob et al.
376 (2017), there are positive associations between serum Ca and P concentrations in dairy cows,
377 suggesting that no differences in blood Ca should be expected as a result of the cabergoline
378 injection in our ewes. Lacasse et al. (2019) concluded that lowering the PRL concentration is

unlikely to be responsible of a reduction in blood Ca and to cause hypocalcemia, as it was suspected in the cabergoline banning decision of EMA (2016, 2019). Nevertheless, it should be stressed that the use of cabergoline is still suspended for cattle in the EU.

No differences in plasma lactose were detected by effect of the cabergoline treatment in our ewes ($P = 0.84$; Table 1), although marked differences were observed according to breed ($P = 0.048$), the LC having greater values than MN ewes. This result agree with previous data in the same breeds and with the fact that LC ewes have greater milk yield and are more tolerant to milk accumulation between milkings than the MN are (Castillo et al., 2008). Consequently, no tight junction disruption (leakiness) was produced in our ewes despite their level of production, as a result of the injection of cabergoline. Milk lactose yield reduction in our cabergoline treated ewes (Table 1) could be explained by the reduction of lactose synthesis in the mammary epithelial cells and not by leaking through cellular tight junctions.

Udder Traits. Involution of the udder induced by the cabergoline treatment was assessed by the changes in its anatomical measurements. The greater the reduction of the udder size, the better the effectivity of the dry-off facilitation treatment. Udder size will be an objective non-invasive criterion to monitor the involution of the udder during the dry-off. Data of the udder volume (range, 1.00 to 3.42 L) and the corresponding milk yield obtained at the p.m. milking (range, 0.07 to 1.41 kg) of our ewes throughout the experiment (d –2 to 14) correlated ($y = 0.4034 x – 0.226$; $r = 0.77$, $P < 0.01$; Figure 4) and explained more than one-half (59%) of the variation of milk accumulated in the udder. Udder volume differed between breeds in our ewes (MN, 1.57 ± 0.07; LC, 2.23 ± 0.07; $P < 0.001$), agreeing with their differences in milk yield. Additionally, the volume of the udder tissue (non-milk volume of the udder) estimated by difference also correlated with the total volume of the udder ($r = 0.82$, $P < 0.001$), the udder tissue of the LC ewes being 26% greater than in the MN ewes ($P = 0.002$), as previously reported by Rovai et al. (2008). Labussière (1983), in a prospective study on the milkability of

404 different dairy breeds in the Mediterranean, reported positive correlations between udder
405 volume and daily milk yield (r = 0.40 to 0.71), although the reported correlations vary with the
406 age of the ewes and the milk fraction considered, as also indicated by Fernández et al. (1983)
407 in Manchega dairy ewes (r = 0.17 to 0.85). Similarly, udder width (MN, 12.6 ± 0.3; LC, 14.8 ±
408 0.3; $P < 0.001$) and base-floor distance (MN, 34.1 ± 1.1; LC, 28.3 ± 1.1; $P < 0.001$) also were,
409 on average, different between breeds in our ewes, the greater the yield the greater the size of
410 the udder.

411 With regard to the cabergoline treatments, volume and width of the udder (Figure 5) were
412 similar for the ewes treated with the L and H doses of cabergoline ($P = 0.36$ and $P = 0.56$,
413 respectively) but, on average, udder volume was lower (–18%; $P < 0.005$) and udder width
414 tended to be lower (–6%; $P = 0.09$) than those of the CO ewes from d 2 to 5 (Table 1).
415 Nevertheless, no differences in udder volume between treatments were detected in the LC ewes
416 ($P = 0.57$). Interestingly, despite not having differences in milk yield between CO and both L
417 and H treatments at d 14 post-injection, udder volume of the cabergoline treated ewes remained
418 smaller than in the CO ewes (Figure 5a). This result may be consequence of a reduction of the
419 secretory tissue of the udder (i.e., mammary gland involution), which was only observed in the
420 case of the treated MN ewes ($P = 0.045$), but that did not affect milk yield in the late stage of
421 lactation of our ewes. There is also the possibility of a side-effect of the PRL rebound on
422 oxytocin secreted at milking, agreeing the oxytocin-PRL positive feedback suggested by
423 Kennett and McKee (2012) in rats, which may have reduced the amount of residual milk in the
424 cabergoline treated ewes. This last hypothesis needs further research.

425 No effects of treatments were detected on the base-floor distance of the udder of either breed
426 of ewes ($P = 0.53$; Table 1) indicating that pre-milking udder volume and, to a less extent the
427 pre-milking udder width, were the only useful morphological traits to assess udder involution.
428

CONCLUSIONS

Cabergoline temporarily inhibited PRL and markedly decreased milk secretion and udder volume, but increased most milk components in lactating dairy ewes. The effect disappeared after 5 d and milk yield and milk composition did not differ from control values when milking was maintained for more than 20 d after injection. The effect on udder volume lasted longer than the effect on milk yield, which may have relation with the PRL rebound after cessation of cabergoline treatment and needs further research. The L dose (0.56 mg/ewe) was as effective as the H dose of cabergoline (1.12 mg/ewe) for the reduction of lactation, without differences between them with regard to udder traits. Overall, the use of 0.56 mg/ewe of cabergoline as a dry-off facilitator may be a strategy of interest in high-yielding dairy ewes in order to reduce the feed restriction stress (i.e., ketosis risk) and antibiotic therapy at dry-off.

No apparent adverse reactions were detected and our data do not support the suspicion that use of cabergoline, at the recommended treatment dose, may be related to hypocalcemia or mammary epithelial cell tight junctions disruption in lactating dairy ewes. Additionally, the results of this study may be useful to understand the use of PRL inhibitors as a management tool in dairy small ruminants. Further research on the use of cabergoline on pregnant dairy ewes at the dry-off and during the following lactation is needed. Finally, it should be stressed that the use of cabergoline is currently suspended in the EU for cattle and that its use in dairy sheep will require a specific approval by EMA.

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458

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617 **Table 1.** Lactational effects of a single injection of cabergoline at different doses[1] during d 1 to 5 post-injection in two breeds of dairy ewes in late-lactation

618 (data are LS means ± SEM)

	Manchega			Lacaune					Effect (*P*-value)		
Item	CO	L	H	CO	L	H	Mean	±SEM	Cabergoline	Breed	Interaction[2]
Milk yields											
Milk, kg/d	0.87[a]	0.40[b]	0.62[ab]	1.84[a]	1.34[b]	1.56[ab]	1.11	0.12	0.001	0.001	0.99
ECM[3], kg/d	1.03[a]	0.59[b]	0.88[ab]	1.88	1.55	1.82	1.29	0.13	0.050	0.001	0.95
Fat, g/d	80[x]	48[y]	72[x]	138[x]	115[y]	137[x]	98	11	0.07	0.001	0.93
Protein, g/d	63[x]	37[y]	54[x]	116[x]	101[y]	116[x]	81	8	0.10	0.001	0.97
Lactose, g/d	36[a]	16[b]	22[ab]	80[a]	52[b]	63[ab]	45	6	0.003	0.001	0.96
Milk contents											
Fat, %	9.15[b]	11.74[a]	11.64[a]	7.49[b]	8.60[a]	8.82[a]	9.57	0.54	0.001	0.001	0.36
Protein, %	7.28[b]	9.26[a]	8.63[a]	6.31[b]	7.51[a]	7.44[a]	7.74	0.41	0.001	0.001	0.63
Lactose, %	4.13	3.87	3.58	4.33	3.90	4.03	3.97	0.16	0.11	0.20	0.60
SCC, log_{10}/mL	5.24	5.36	5.36	5.28	5.76	5.42	5.40	0.25	0.50	0.43	0.74
Plasma											
Prolactin, ng/mL	19.34[a]	0.65[b]	0.56[b]	19.21[a]	1.22[b]	0.91[b]	6.98	2.37	0.001	0.89	0.99
Glucose, mg/dL	62	60	60	60	64	59	61	2	0.61	0.81	0.42
NEFA, mmol/dL	0.075	0.083	0.070	0.101	0.088	0.114	0.087	0.011	0.89	0.08	0.16
Lactate, mmol/L	0.93	1.32	1.21	1.02	1.12	0.92	1.04	0.21	0.49	0.43	0.88
GGT[4], IU/L	58	60	47	77	88	67	66	12	0.53	0.12	0.80
P, mg/dL	4.23	4.57	4.28	4.73	4.44	5.03	4.50	0.31	0.89	0.49	0.34
Creatinine, mg/dL	0.68	0.71	0.71	0.64	0.67	0.65	0.68	0.03	0.69	0.08	0.98
Lactose, µmol/L	10.8	6.8	8.0	35.8	31.9	39.8	21.2	12.3	0.84	0.048	0.97
Udder traits[5]											
Volume, L	1.96[a]	1.41[b]	1.35[b]	2.36	2.13	2.19	1.90	0.13	0.005	0.001	0.20
Udder tissue, L	1.28[a]	1.24[a]	1.09[b]	1.52	1.43	1.57	1.35	0.11	0.74	0.002	0.36
Width, cm	13.2	12.3	12.3	15.3[x]	14.2[y]	14.8[xy]	13.7	0.5	0.09	0.001	0.83
Base-floor, cm	31.9	34.0	36.4	28.4	31.8	28.5	31.2	1.9	0.53	0.001	0.47

[a,b]Within a row and breed, values with a different superscript differed ($P < 0.05$); [x, y]Within a row and breed, values with a different superscript tended to differ ($P < 0.10$).

[1]Treatments: CO, 0 mg; L, 0.56 mg; H, 1.12 mg cabergoline per ewe.

[2]Cabergoline × Breed.

[3]Energy corrected milk = Milk yield × [0.071× (Fat, %) + 0.043 × (Total protein, %) + 0.2224], according to Bocquier et al. (1993).

[4]γ-glutamyl transferase.

[5]Data from d 2 to 5 post-injection.

FIGURE CAPTIONS

Figure 1. Effects of a single injection of cabergoline at different doses on plasma PRL of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes were significant from d 1 to 7 (***, $P < 0.001$).

Figure 2. Effects of a single injection of cabergoline at different doses on milk yield of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes tended or were significant from d 1 to 5 (*, $P < 0.05$; **, $P < 0.01$).

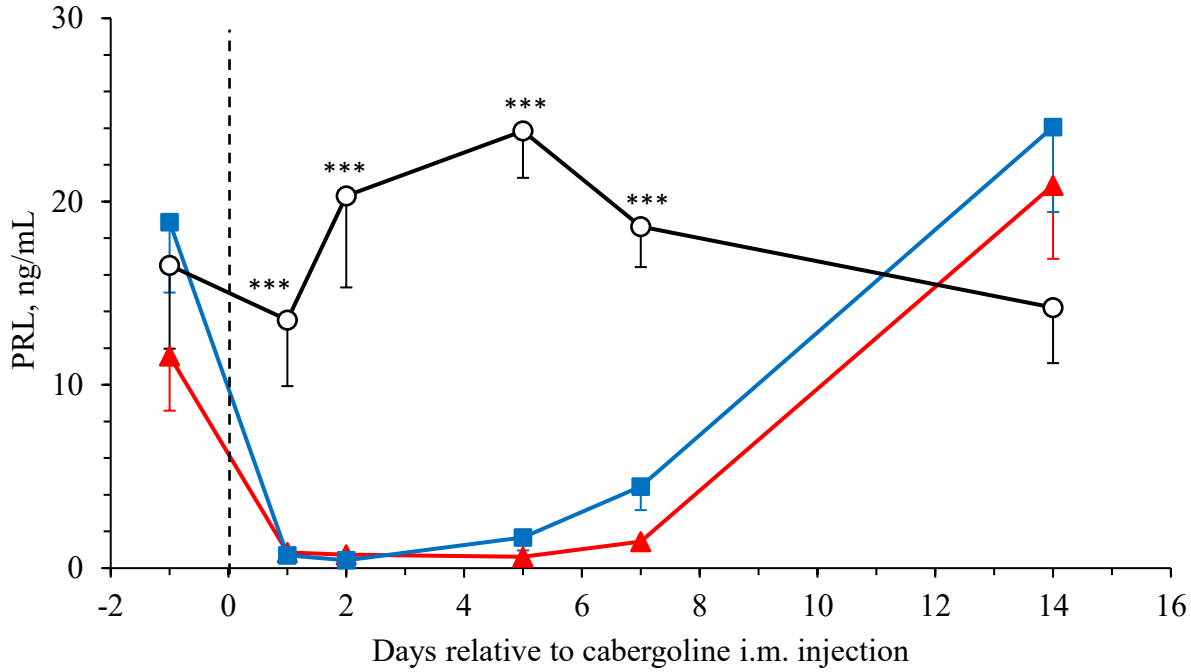
Figure 3. Effects of a single injection of cabergoline at different doses on milk composition (a, fat; b, protein; c, lactose) of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences in fat and protein milk contents between control and cabergoline i.m. injected ewes were significant from d 1 to 5 (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Differences in lactose milk content were significant at d 2 and 5 (*, $P < 0.05$).

Figure 4. Correlation (y = 0.4034 x – 0.226; r = 0.77, $P < 0.01$; n = 180) between udder volume and milk yield at p.m. milking of lactating Manchega and Lacaune dairy ewes after a single injection of cabergoline at different doses. Doses of cabergoline: ▲ (H, 1.12 mg; n = 60, r = 0.77; $P < 0.05$), ■ (L, 0.56 mg; n = 60, r = 0.83; $P < 0.05$), and ○ (CO, 0 mg; n = 60, r = 0.66; $P < 0.05$).

Figure 5. Effects of a single injection of cabergoline at different doses on the udder traits (a, volume; b, width) of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes were significant from d 2 to 14 (***, $P < 0.001$) in udder

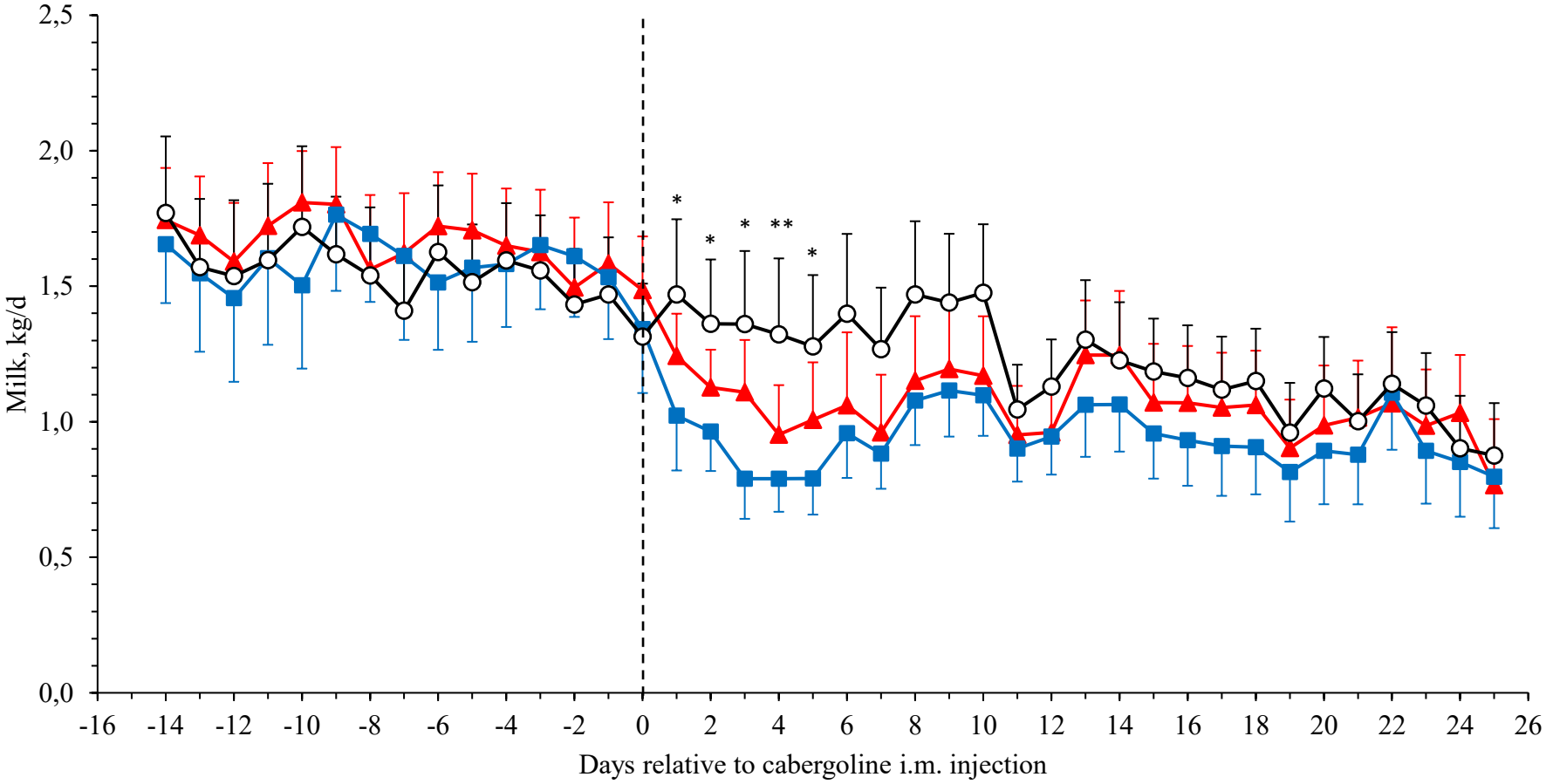
658 volume, and were significant at d 5 (*, $P < 0.05$) or tended to decrease (+, $P < 0.10$) at d 2 and
659 7 in udder width.
660
661

Figure 1.


30
20
10
0
PRL, ng/mL
***
***
***
***
-2
0
2
4
6
8
10
12
14
16
Days relative to cabergoline i.m. injection

667 **Figure 2.**

668

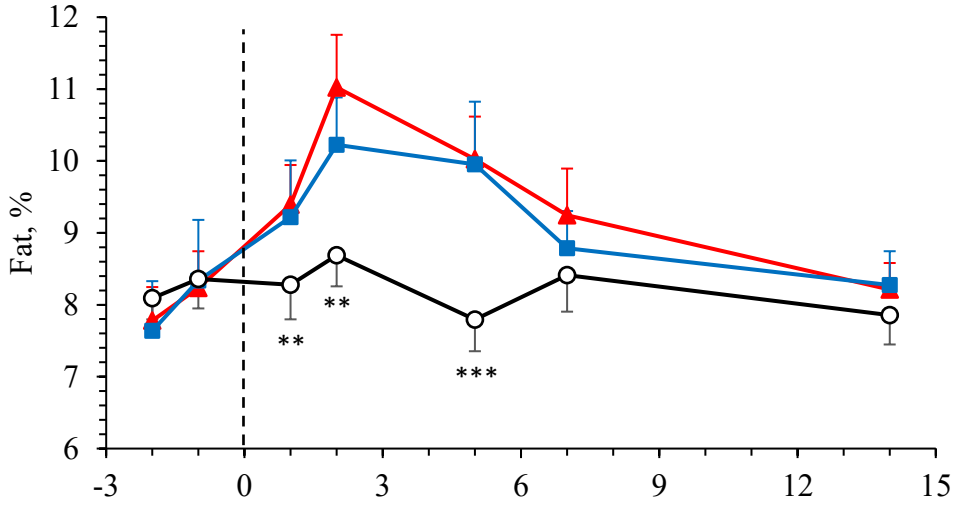


669

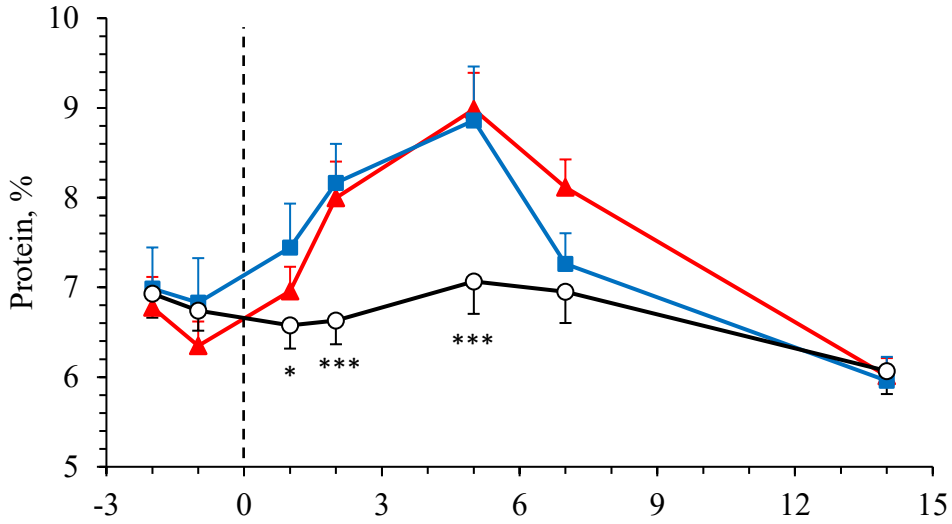
670

Figure 3.

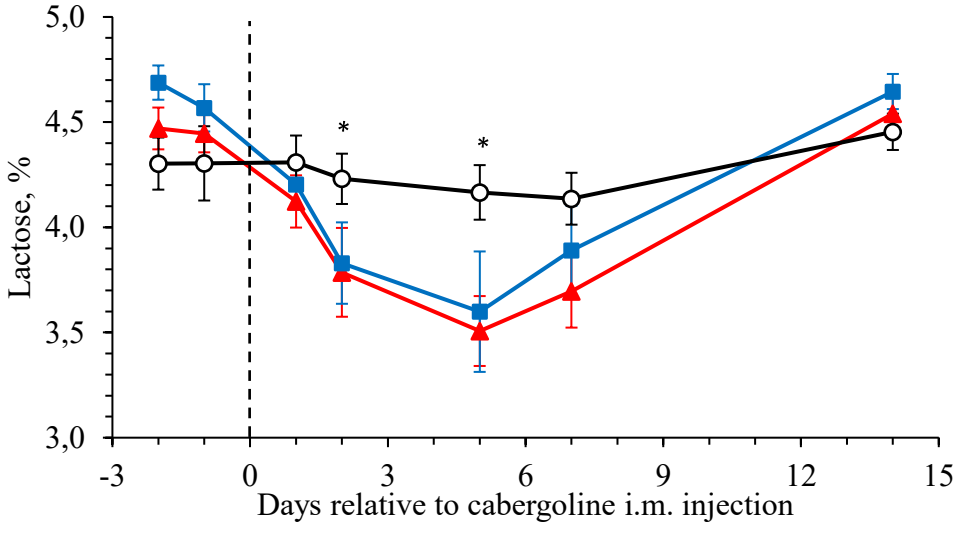
a)


Fat, %
12
11
10
9
8
7
6
-3
0
3
6
9
12
15
**
**
***

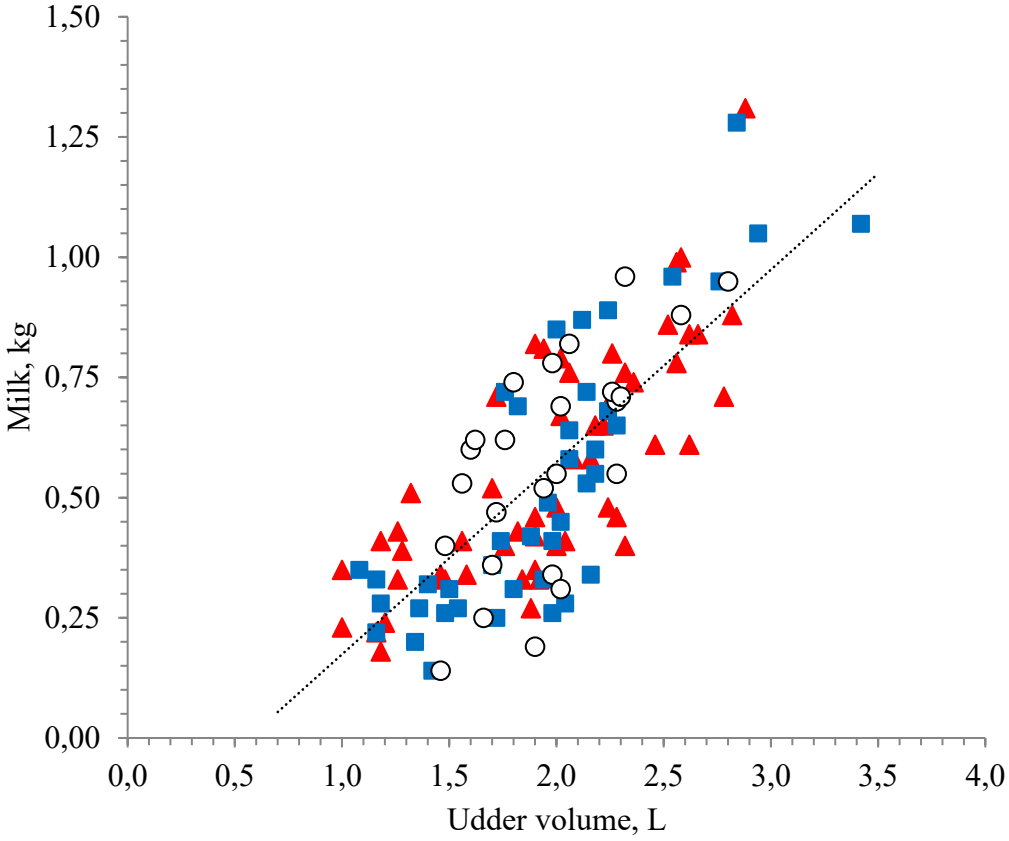
b)


Protein, %
10
9
8
7
6
5
-3
0
3
6
9
12
15
*
***
***

c)


Lactose, %
5,0
4,5
4,0
3,5
3,0
-3
0
3
6
9
12
15
*
*
Days relative to cabergoline i.m. injection

678 **Figure 4.**

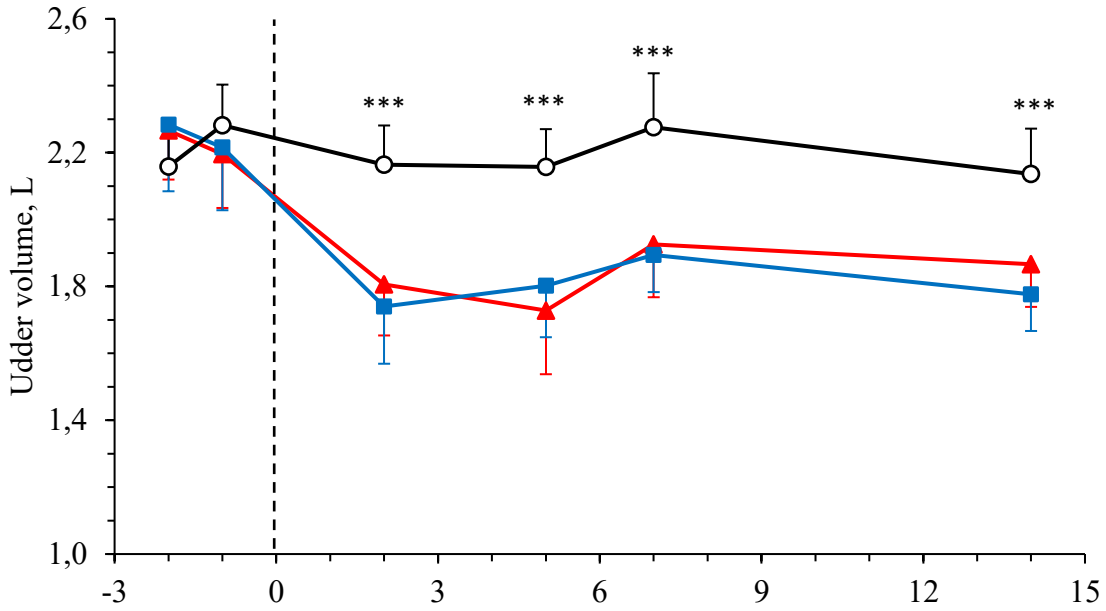


679

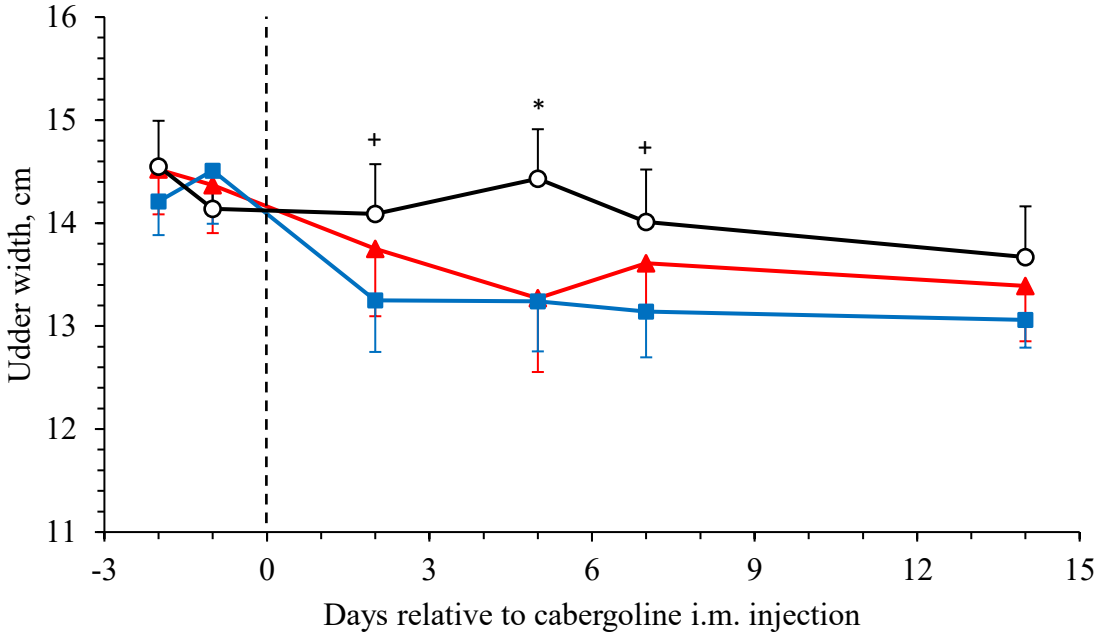
680

Figure 5.

a)


2,6
2,2
1,8
1,4
1,0
Udder volume, L
***
***
***
***
-3
0
3
6
9
12
15

b)


16
15
14
13
12
11
Udder width, cm
+
*
+
-3
0
3
6
9
12
15
Days relative to cabergoline i.m. injection