Effect of stress via ACTH administration and cortisol release on expression of key genes related to milk synthesis and apoptosis during mammary involution of Saanen goats.

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SUPPLEMENTARY FILE

Supplementary Figure

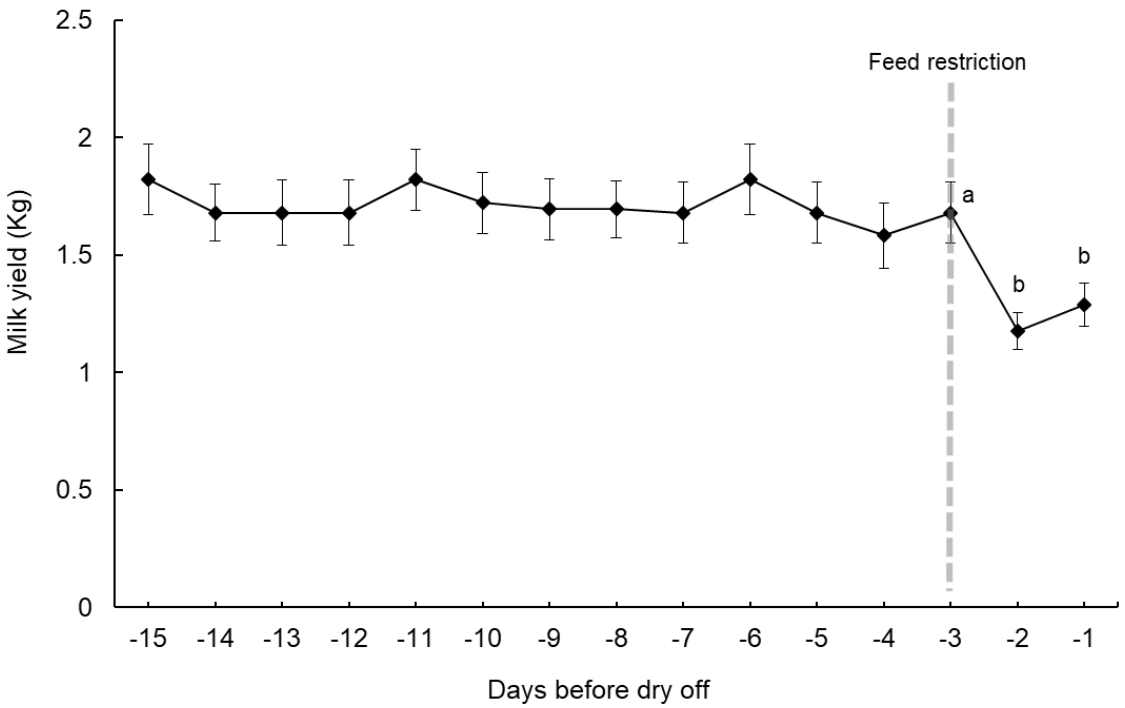

Figure S1. Milk yield (kg/day) of experimental goats (n = 30) during the last 15 days of lactation (from day -15 to 0, dry off was performed at day 0). All goats were subjected to feed restriction during the last 3 days before the start of dry. Data are presented as mean ± standard error of the mean. Means with different letters show significant differences ($p \leq$ https://pt.wiktionary.org/wiki/%E2%89%A4 0.05).

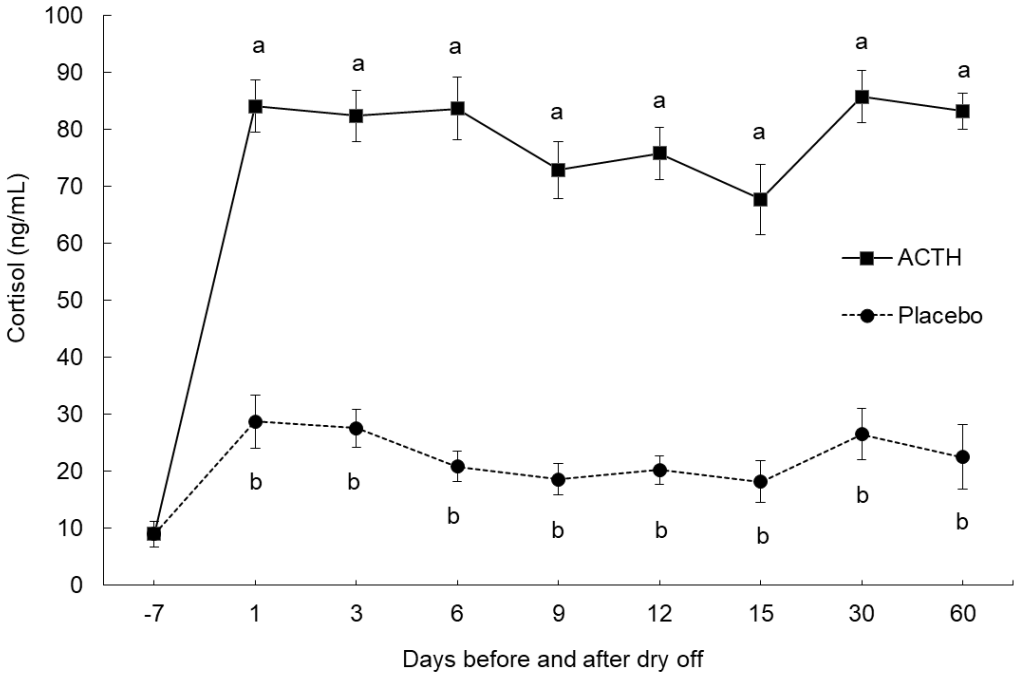

Figure S2. Plasma cortisol concentration (ng/mL) released by Saanen goats after ACTH administration or Placebo (n = 15) before (day -7) and during the dry off (days 1, 3, 6, 12, 15, 30, and 60). Data are presented as mean ± standard error of the mean. Means with different letters show significant differences ($p \leq$ https://pt.wiktionary.org/wiki/%E2%89%A4 0.05).

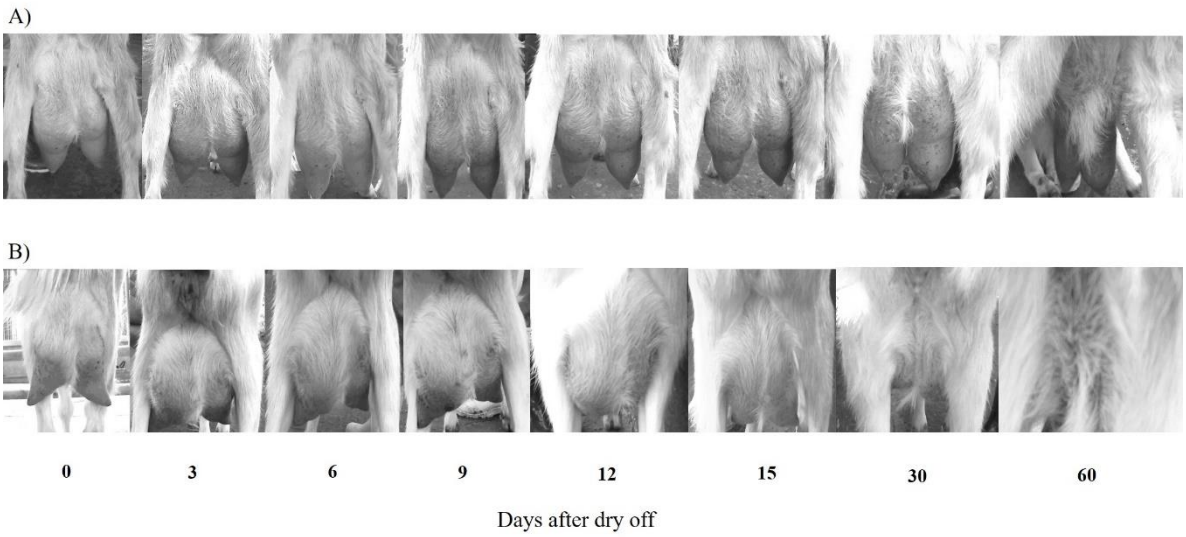

Figure S3. Evolution of mammary gland morphology of one representative goat showing persistent lactation (A) and one representative dry goat showing total mammary involution (B). The mammary involution was considered total when the udder had a morphology similar to those observed in non-lactating and non-pregnant goats, 100% of the goats of both treatments considered dry on days 30 and 60 of dry off showed total involution of the mammary gland. The goats were considered dry when the volume of mammary secretion collected was less than 50 mL and showed total mammary involution during the dry period.

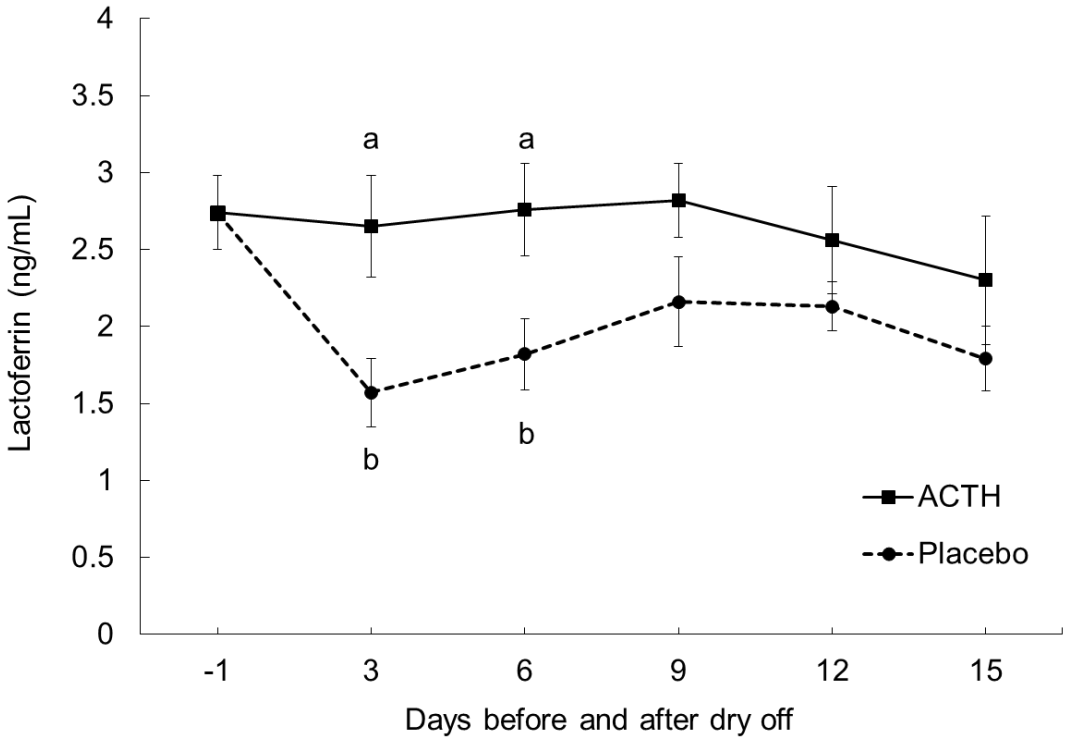

Figure S4. Lactoferrin concentration (ng/mL) in milk and mammary secrections of the Saanen goats after ACTH administration or Placebo (n = 15) before (day -1) and during the dry off (days 3, 6, 12 and 15). Data are presented as mean ± standard error of the mean. Means with different letters show significant differences ($p \leq$ https://pt.wiktionary.org/wiki/%E2%89%A4 0.05).

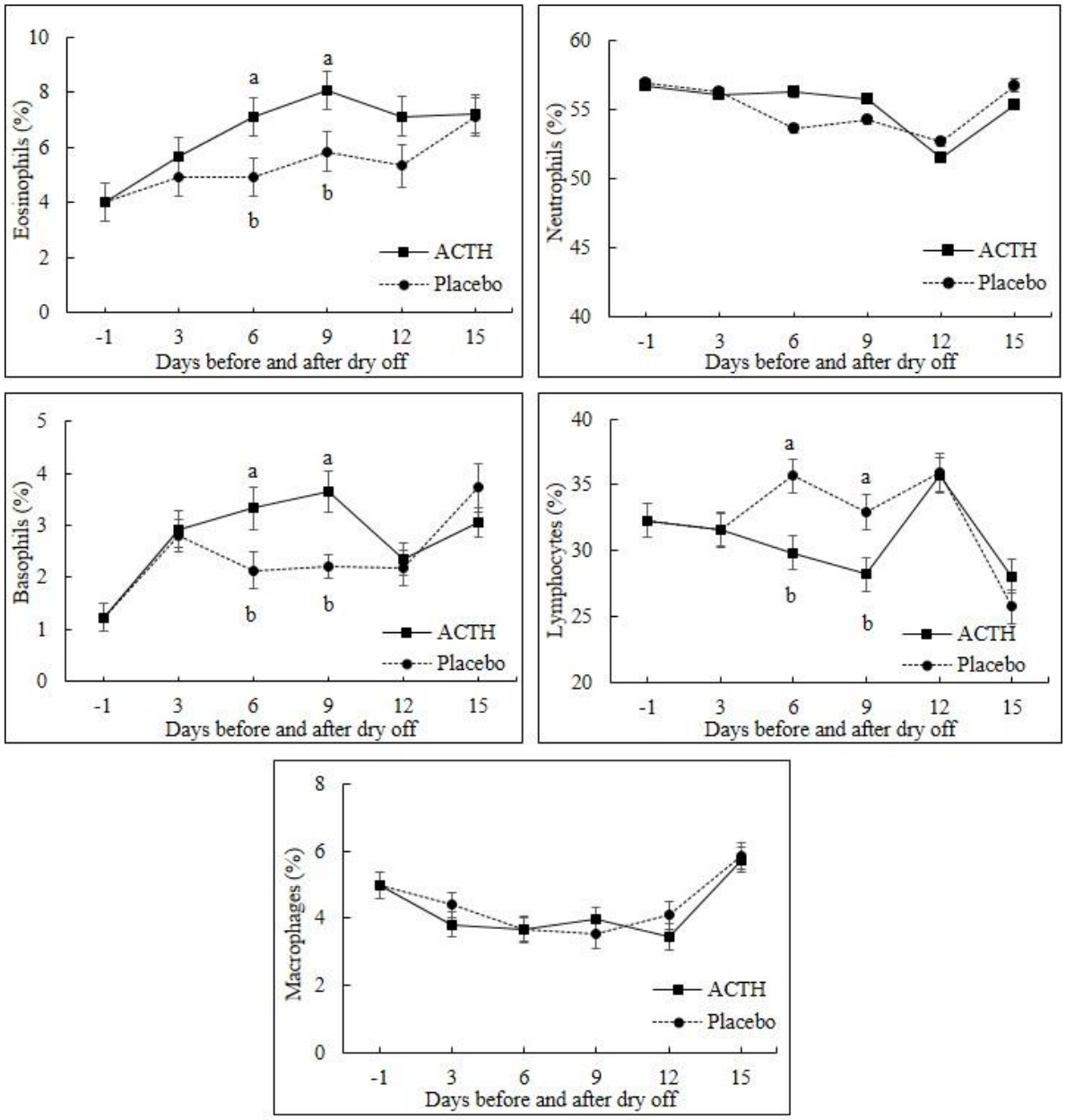

Figure S5. Leucocyte count percentage in milk and mammary secrections of the Saanen goats after ACTH administration or Placebo (n = 15) before (day -1) and during the dry off (days 3, 6, 12 and 15). Data are presented as mean ± standard error of the mean. Means with different letters show significant differences ($p \leq$ https://pt.wiktionary.org/wiki/%E2%89%A4 0.05).

Supplementary Table

Table S1. Sequence of primers used in the reverse transcription quantitative PCR.

Gene	Primer sequences	Accession number[1]
IGF1R	5’-TGGAGTGCTGTATGCCTCTGT-3’ 5’-GGTCTCGGGCTCATCCTT-3’	XM_005694951
PIK3CA	5′-TCAACCATGACTGTGTGCCA-3′ 5′-CCATCAGCATCAAATTGGGCA-3 ′	XM_018047551
AKT1	5′-CCTGCCCTTCTACAACCAGG-3′ 5′-GTCTTGGTCAGGTGGCGTAA-3′	NM_001285750
MTOR	5′-CGTCTCGCTTGTACTTTGGG-3′ 5′-GCTGCTTGGAGATTCGTCTG-3′	NM_001285748
CSN2	5'-ACAGCCTCCCACAAAACATC-3 ' 5’-AGGAAGGTGCAGCTTTTCAA-3’	EF558564
LALBA	5'-ACCAGTGGTTATGACACACAAGC-3' 5'-AGTGCTTTATGGGCCAACCAGT-3'	NM_001285635
LF	5’-GACCTCTGCCTTGGAATGTATC-3’ 5’-ATCTAGCCACAGCTCCCTGGAG-3’	DQ522303
GAPDH	5'-GGTGATGCTGGTGCTGAG-3' 5'-TGACAATCTTGAGGGTGTTG-3'	AJ431207

Insulin-like growth factor type 1 receptor (IGF1R); Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA); AKT serine/threonine kinase 1 (AKT1); mechanistic target of rapamycin kinase (MTOR); beta-casein (CSN2); lactalbumin alpha (LALBA); lactoferrin (LF); glyceraldehyd-3-phosphate dehydrogenase (GAPDH).

[1]https://www.ncbi.nlm.nih.gov/genbank/