List of abbreviations

AW	abrupt weaning
D0	day of separation/weaning
D1	1 d after weaning
D-1	1 d before weaning
D2	2 d after weaning
D-2	2 d before weaning
D3	3 d after weaning
D-3	3 d before weaning
D-4	4 d before weaning
D7	7 d after weaning
D8	8 d after weaning
EIA	enzyme immunosorbent assay
FGM	fecal glucocorticoid metabolites
FL	fenceline weaning
IQR	interquartile range
IRR	incidence rate ratio
NF	nose-flap weaning

Appendix 1.

Salivary cortisol extraction and assay

Salivary cortisol was quantified using a commercial Enzyme Immunosorbent Assay (EIA) kit (The SALIMETRICS LLC, State College, Pennsylvania, USA) following the manufacturer's protocol. Briefly, samples, controls, and standards were plated in duplicates, followed by addition of conjugate, shaken for 5 min at 500 RPM, and incubated for 1 h at room temperature. Plates were washed 4 times, tetramethylbenzidine was added, mixed for 5 min at 500 RPM, and incubated for 25 min at room temperature. Reaction was stopped with 2M sulfuric acid, and absorbance was read at 450 nm. Cortisol concentrations were determined using a 4-parameter logistic curve and expressed as μg/dL of saliva.

Appendix 2.

Fecal glucocorticoid metabolites extraction, assay and validation

At the Toronto Zoo, FGM were extracted from 0.5 ± 0.02 g of wet feces by adding 5 mL of 80% methanol-distilled water (1) overnight at room temperature with continuous mixing on a plate shaker. Fecal samples were centrifuged at 2400 x *g* for 10 min, and the supernatant was decanted and stored in tightly capped glass vial at -20ºC until analysis.

The FGM were analyzed using a double-antibody EIA 11-oxoetiocholanolone 72T mini kit (Arbor Assays, Ann Arbor, Michigan, USA) following the manufacturer's protocol, with slight modifications (2). Briefly, fecal extracts diluted in assay buffer, along with controls and standard, were prepared in duplicates and added to glucocorticoid antibody reagent pre-coated plates. Horseradish peroxidase conjugate and 11-oxoetiocholanolone antiserum were added, mixed, and incubated for 2 h at room temperature. Plates were washed using a 405VR microplate washer (BioTek Instruments, Winooski, Vermont, USA), followed by addition of tetramethylbenzidine and incubation for 30 min at room temperature. Finally, sulfuric acid (1.8M) was added, and absorbance was measured using a spectrophotometer at 450 nm. The FGM concentrations were expressed as ng/g of feces.

Parallel displacement between standard curve and serial dilutions of pooled fecal extract indicated specificity ($t = 0.39$, $P = 0.69$, $df = 8$). Samples were assayed at dilutions corresponding to 50% binding. Recovery of known amounts of 11-oxoetiocholanolone was calculated by increasing concentrations of the hormone standard added to an equal volume of pooled fecal extracts of known concentration. Recovery test had an average of 114.9 ± 10.2%, indicating no interference from fecal components. Intra-assay CV was 12.9%, inter-assay CVs were 7.5% (28% binding), 12.3% (51% binding), and 11.1% (64% binding), all < 15%.

References (Appendix 2).

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Table S1. List of cow behaviours observed and their respective definitions.

Behaviour	Description
EATING	Consuming vegetation from the pasture, with head close to the ground, while standing still or moving slowly (S2)
STANDING	Maintaining an upright position on extended legs (S34)
LYING	Resting in any positions with all legs folded under body (S35)
WALKING	All 4 legs are moving with head raised at least 2 steps (S34)
PACING	Walking faster than in normal walking speed (S2) or walking back and forth
VOCALIZING	Any visually observed behaviour that suggests the cow is calling (i.e., mouth open and diaphragm contracting) including making sounds through mouth (S2)

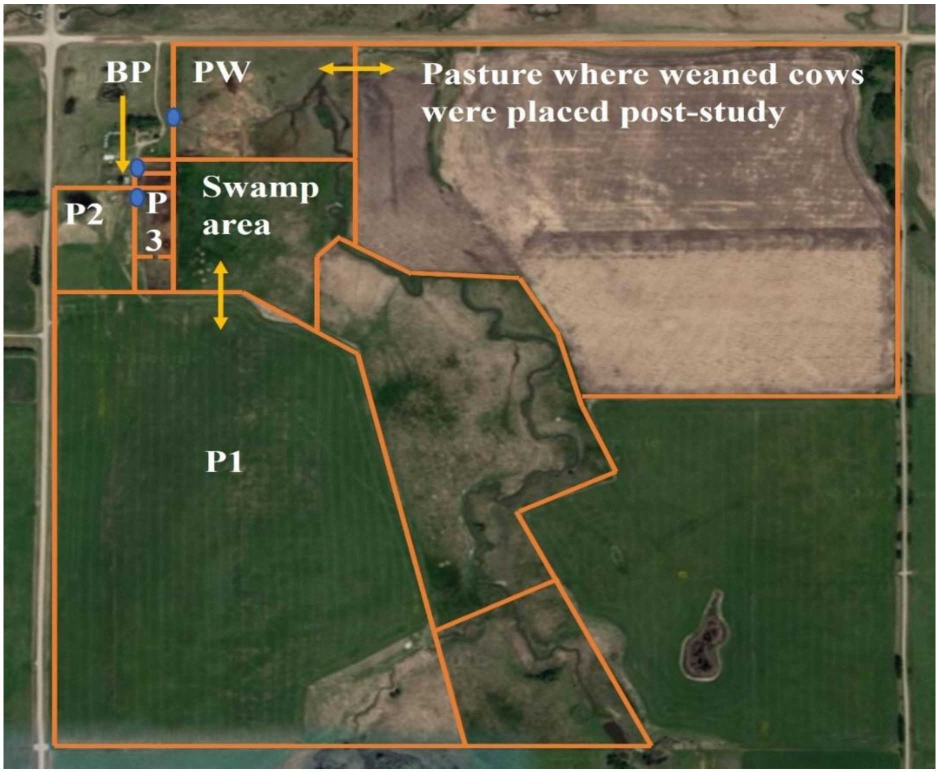

Fig. S1. Map of pasture area where the study was conducted. P1 – Pasture 1 where cow-calf pairs yet to be weaned were placed. Cow-calf pairs had access to hay and water trough through the swap area. P2 – Pasture 2 where cows were observed for behavioural recordings. P3 – Pasture 3 where calves were fenceline-weaned. BP – Processing barn. PW – Pasture with access to water for weaned cows or cow-calf pairs yet to be weaned.

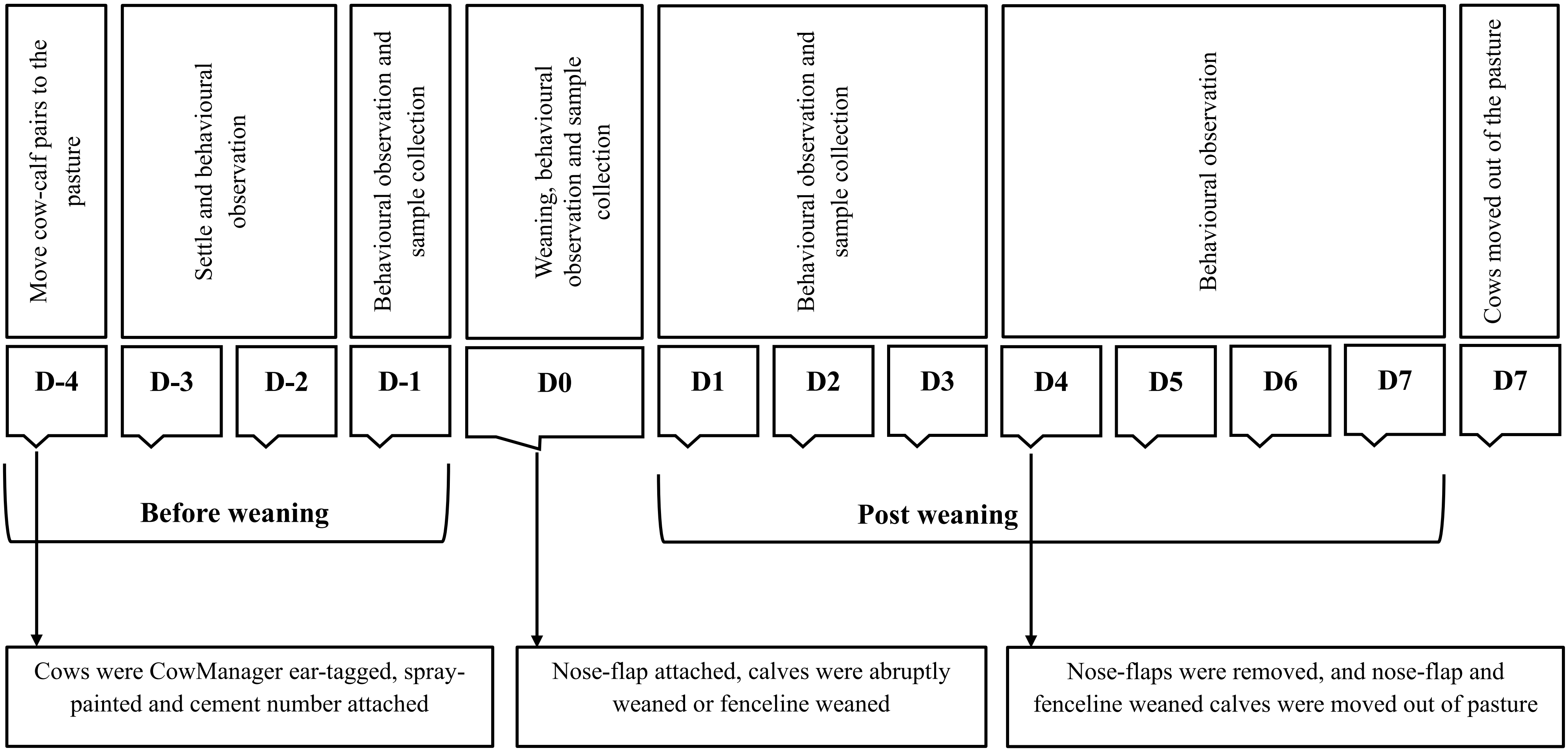

Fig. S2. The timeline of enrollment for the 30 cow-calf pairs in their respective weaning treatment groups over the 2-week period

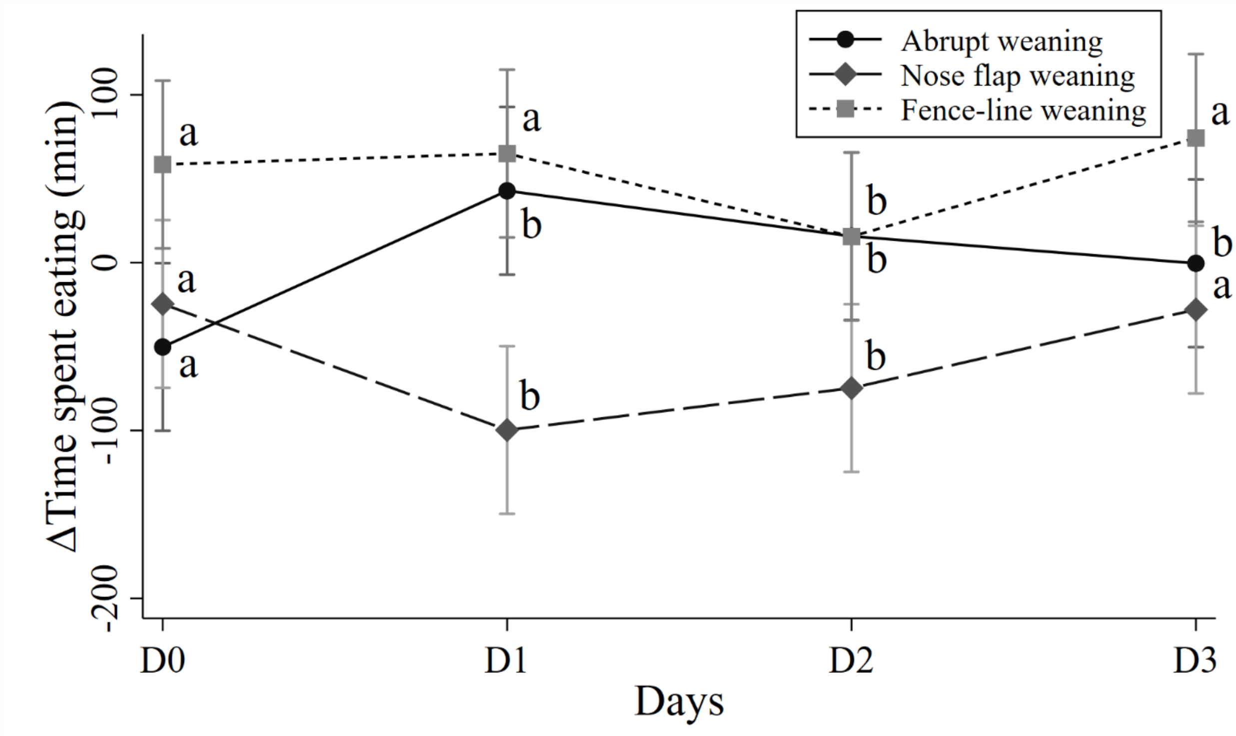

Fig. S3. Estimated marginal means for Δtime spent eating (in min) from a multi-level mixed linear regression model of the effects of treatment (weaning methods), and days (day of weaning and post-weaning days). The 95% predicted confidence intervals based on standard errors of model estimates are shown for each predicted value. Δ – The change in time spent eating by cows for each respective day compared to the day before weaning within each group. D0 – The day of weaning. D1 – 1 d after weaning. D2 – 2 d after weaning. D3 – 3 d after weaning. Within a weaning method, Δtime spent eating by cows between day of weaning and post-weaning days, as identified with different letters "a, b" in the figure.

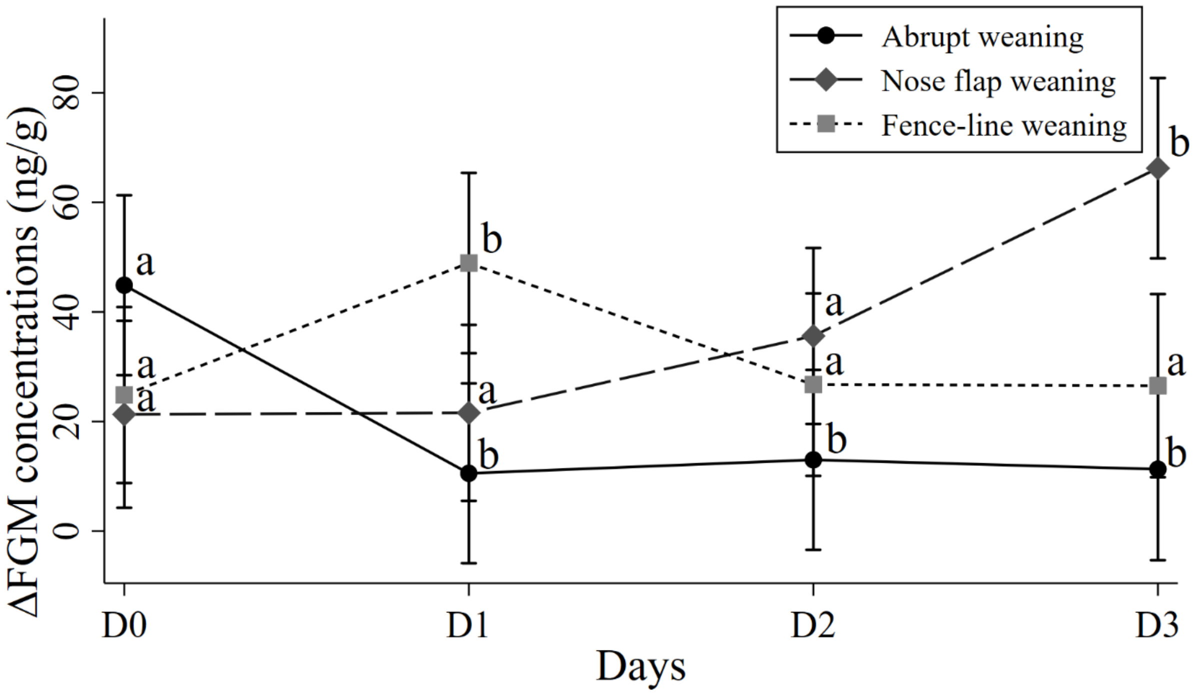

Fig. S4. Estimated marginal means for the change in fecal glucocorticoid metabolites concentrations on cows from multi-level mixed linear regression model of the effects of treatment (weaning methods) and days (day of weaning and post-weaning days). The 95% predicted confidence intervals based on standard errors of model estimates are shown for each predicted value. Δ – The change in fecal glucocorticoid metabolites concentrations in cows for the specified day of weaning compared to the day prior to weaning within each group. FGM – Fecal glucocorticoid metabolites concentrations. D0 – The day of weaning. D1 – 1 d after weaning. D2 – 2 d after weaning. D3 – 3 d after weaning. Within a weaning method, ΔFGM concentrations in cows between day of weaning and post-weaning days were significant, as identified with different letters "a, b" in the figure.

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