Animal 18 (2024) 101091

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Loss of a grooming enrichment impacts physical, behavioural, and physiological measures of welfare in grazing beef cattle

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ARTICLE INFO

Article history:
Received 5 October 2023
Revised 17 January 2024
Accepted 22 January 2024
Available online 1 February 2024

Keywords:
Angus
Brush
Extensive management
Positive Welfare
Steers

ABSTRACT

Pasture-based beef cattle are raised in a range of production environments. Some paddocks may contain trees and other objects that allow for grooming, hence being naturally enriching, whilst others may be barren without these opportunities. Additionally, it is not uncommon for cattle to move between these enriched and barren environments as part of routine management. While the benefits of enrichment are well studied, how this 'enrichment loss' impacts cattle welfare as access to stimuli is removed is unknown. This trial assessed the impacts of the loss of an enriching object (grooming brush) on grazing beef cattle welfare and production characteristics. When grooming brush access was blocked, cattle became dirtier, showed reduced average daily gain, and had elevated faecal cortisol metabolites, although this varied according to the degree of initial individual brush use. Additionally, allogrooming and grooming on other objects were reduced when access to the brush was returned, potentially indicating a rebound effect. These results demonstrate that the loss of adequate grooming objects can impair the overall welfare of grazing cattle; however, further work is needed to determine exactly which natural or artificial objects provide adequate grooming opportunities.

Implications

Grazing cattle may experience a decline in welfare when access to a grooming brush is blocked, which can reduce both cattle comfort and productivity. In the current study, this was seen through dirtier coats, reduced weight gain, and elevated faecal cortisol levels for 'medium' and 'high' brush users when access was blocked. Continuous brush access is likely beneficial for cattle welfare; however, it is not yet known if naturally enriching objects in paddocks such as trees and stumps provide an equal grooming opportunity, and if cattle also experience a 'loss' if access to these natural objects is prevented.

Introduction

The environments grazing cattle are housed in can vary between countries, regions, and farms. For example, over 3.2 million km^2 of Australia's 7.7 million km^2 land area is predominately used for grazing approximately 21 million beef cattle (Australian Bureau of Statistics, 2021). The industry can be broadly classified into the Northern and Southern production systems. The former is characterised by large paddocks of native grasslands, while the latter is often more intensive with improved pastures employing rotational and strip grazing management (PricewaterhouseCoopers, 2011). Use of largely cleared improved pasture areas may reduce the likelihood of trees and other objects being present. Therefore, access to these natural enrichments could be constantly changing when cattle are regularly moved between environments, or alternatively if they are housed in large paddocks and voluntarily move to more barren areas to obtain water or adequate nutrition. Routine movement between areas containing natural enrichments such as trees and stumps that allow for grooming, through to other areas that may be 'barren' without these opportunities, may impact the welfare of these cattle.

Animals in extensive production systems typically show less restriction of their normal behaviours such as grazing and lying for extended periods of time when compared with intensive production systems such as feedlots (Petherick, 2005). However, variation in topography and resources of extensive environments may impact the expression of other natural behaviours. Cattle are motivated to groom, which includes self-grooming, allogrooming, and

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https://doi.org/10.1016/j.animal.2024.101091
1751-7311/

grooming on objects (Kilgour et al., 2012). The presence of trees and stumps in the environment presents natural opportunities for object grooming. When beef cattle at pasture were given a choice of enrichment objects, those that allowed grooming (tree stump and brush) were used more frequently than a chewing rope and woodchip pile (Dickson et al., 2022). Cattle at pasture have been shown to increase total grooming time when provided with trees (Kohari et al., 2007). Dairy cows are also highly motivated to access a grooming brush and will work as hard to access one as fresh feed (McConnachie et al., 2018). However, it is unclear if grooming on objects has a different underlying motivation than the similar behaviours of self-grooming and allogrooming expressed by cattle, and thus whether the absence and/or change in the level of availability of grooming objects may lead to a negative impact on animal welfare and potentially production. Access to a brush or other grooming device has been shown to increase the amount of self-grooming (Horvath and Miller-Cushon, 2019; Ninomiya, 2019), reduce allogrooming (Park et al., 2020), but also have no effect on these behaviours (Kohari et al., 2007). While grooming is an important and motivated behaviour of cattle, it is not yet clear if grooming on objects is a behavioural need, or if it can be replaced with the similar behaviours of self- and allogrooming.

The availability of suitable objects for grooming can impact cattle welfare. For example, brush provision has been shown to impact cattle behaviour, by reducing agonistic behaviours (Matković et al., 2020), allogrooming, bar licking (Park et al., 2020), and increasing eating time (Velasquez-Munoz et al., 2019). Additionally, it has been anecdotally observed that cattle with brush access may appear cleaner (DeVries et al., 2007), however, there have been no specific studies addressing this to the best of the authors' knowledge. In contrast, brush access has shown no impact on physiological measures such as weight gain (Ninomiya, 2019; Ninomiya and Sato, 2009; Park et al., 2020) or hair cortisol concentrations (Park et al., 2020) of beef cattle when housed in a feedlot environment. Brush access may also change other aspects of cattle behaviour, for example, enrichment reduces time spent inactive in calves (Bulens et al., 2014; Velasquez-Munoz et al., 2019). Although cattle are motivated to access a brush (McConnachie et al., 2018), it is not known how far cattle may travel in order to access one, or if this influences their daily distance travelled. Despite the research into impacts of brush use on dairy and feedlot cattle, no current research exists into the benefits of grooming brush provision to grazing beef cattle, or the impacts of the loss of brushes or alternate grooming objects on cattle welfare.

The loss of valued resources can impact both the behaviour and welfare of animals. Animals moved from enriched to barren or standard housing display a more pessimistic judgement bias than those initially housed in barren pens (Douglas et al., 2012) or those moving from standard to enriched housing (Bateson and Matheson, 2007). Stereotypic behaviours and/or aggression towards conspecifics are often increased following enrichment removal in pigs (Day et al., 2002; Munsterhjelm et al., 2009) and mice (Latham and Mason, 2010), along with decreased self-grooming, a behaviour associated with chronically stressed mice (Nader et al., 2012). Additionally, mice moved from enriched to standard housing will push a heavier weighted gate to re-access enriched housing than mice housed in standard cages from birth. There is also a positive correlation between cortisol levels following enrichment removal and this motivation to access enrichment (Latham and Mason, 2010). However, it is unknown if cattle experience these negative impacts of resource loss when unable to access appropriate objects for grooming, and how this might impact animal welfare and production.

The aim of the current study was to determine the impacts of loss of a valued grooming object (a brush) on pasture-based cattle welfare. Coat cleanliness, BW, cortisol concentration, grooming behaviours, and lying and stepping behaviours were monitored prior to and during enrichment loss. It was hypothesised that total grooming duration would decrease when brush access was blocked, and a rebound effect would be seen in the 'brush return' phase, in which brush use would increase. Self- and allogrooming behaviours were expected to increase during the 'loss' phase to compensate for lack of adequate object grooming opportunities. Finally, it was predicted that access to a brush would improve coat cleanliness, but its loss would have little to no impact on BW, cortisol levels, and lying and stepping behaviours.

Material and methods

Animals and housing

The experiment was undertaken at CSIRO, FD McMaster Laboratory, Chiswick, Armidale, NSW, 2350, Australia from January to April 2023. Daily weather data (rainfall, temperature, humidity, and wind speed) were collected from an onsite weather station (Green Brain, 41 Vine Street, Magill, SA, 5072, Australia). Data were recorded every 5minutes, and returned a measure of daily total rainfall, and average daily temperature, humidity, and wind speed. The average 24-h daily temperature was 17.9 °C (SE ± 0.3 °C), the average daily rainfall was 3.8 mL (± 1.1 mL), the average humidity was 78.0% (± 1.0%), and the average wind speed was 4.4 km/h (± 0.2 km/h).

Four neighbouring paddocks were used, each approximately 2.25 hectares in size (Fig. 1) and surrounded by electric fencing that cattle were familiar with. All paddocks contained a similar mix of native and introduced pasture, along with a 400 kg rectangular bale of lucerne hay as the quantity of pasture was expected to decline over the course of the experiment. Each paddock also contained a water trough and a cattle brush (Redpath, 16 Bounty Place, Kelvin Gove, Palmerston North 4414, New Zealand), mounted 120 cm from ground to bottom of bristles.

The cattle brush was chosen as a form of grooming enrichment for multiple reasons. Firstly, diversity present in natural objects, such as trees and stumps, makes it more challenging to obtain four that are identical compared to achieving uniformity with artificial brushes. Secondly, brush use was higher than the use of a natural stump in a preliminary trial, and there was also less variation in how the brush was used compared to the stump (96% of use on brush was grooming, compared to 68% of usage on the stump being grooming; Dickson et al., 2022). Finally, brushes can be readily installed in a paddock while a tree takes a long time to grow, so it is an immediate solution for lack of grooming opportunity compared to natural objects for managers to action when enrichment levels are low.

Forty-eight Angus steers approximately 15 months of age were housed as eight groups (n = 6/group), across two cohorts. All cattle were sourced from a commercial producer, previously housed in a range of standard commercial paddocks and had no prior experience with cattle brushes.

Cattle were weighed using walk-over weigh scales in a crush (Tru-Test XR3000, Tru-Test, Banyo, QLD, Australia) approximately one week before the commencement of the study, and groups within cohorts were balanced for weight (average weight ± SE: Cohort 1 = 421 ± 20 kg; Cohort 2 = 426 ± 16 kg).

Study design and experimental protocol

Within cohorts, placement of groups into the experimental paddocks was staggered in groups of two by 48 h to better facilitate live behavioural observations, with Day 1 considered the day each

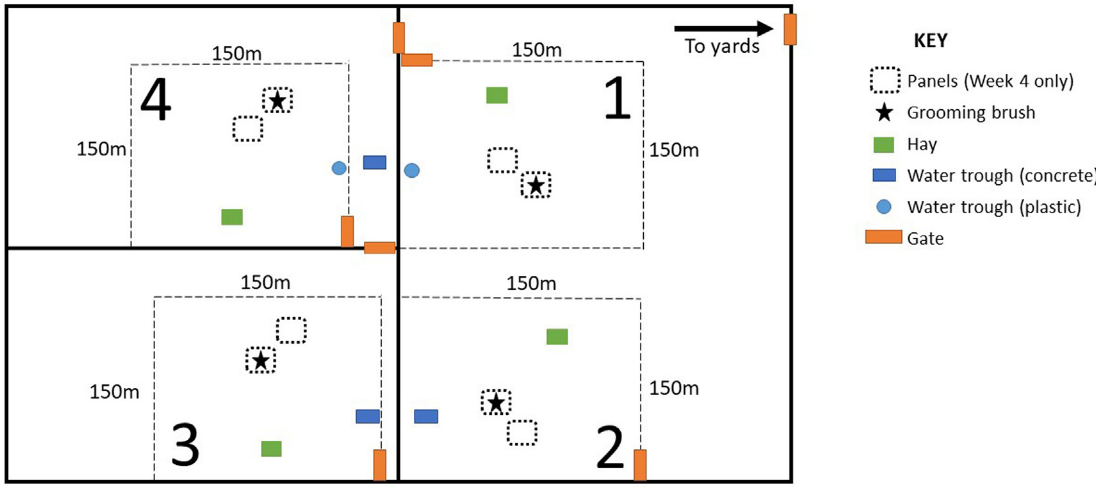

Fig. 1. Map of the 4 experimental paddocks (1–4), along with locations of water, hay, grooming brushes, and panels (during Week 4 only). Six Angus steers were housed in each paddock, and experienced 3 weeks of brush access 'Prior' to it being blocked, 1 week 'During' brush access being blocked, and 1 week 'After' brush access was returned. Schematic is not precisely to scale.

group was introduced to their paddock. Cattle were allotted two weeks to first acclimate to their paddocks, brushes, and groups before any behavioural observations occurred, to minimise brush novelty effects on grooming (Dickson et al., 2022). On Day 8, individuals were marked with the numbers 1–6 on their side and rump using livestock paint (Leader Products Pty Ltd, Craigieburn, VIC, Australia). Each animal was also fitted with an IceQube® logger (IceRobotics Ltd., Edinburgh, Scotland, UK) on their front left leg while restrained in a crush, to allow the assessment of lying and stepping behaviours. Cohort 2 was introduced into their experimental paddocks 5 days after Cohort 1 had finished testing and was removed.

All groups were allowed access to the brush for a two-week acclimation period, immediately followed by one week of observations and testing, totalling 3 weeks of brush access (Fig. 2). Brush access was then denied for a period of one week, by blocking access using panels arranged in a rectangle (3 × 2 × 1 m). A second identical set of panels was also installed in each paddock at this time, allowing a comparison of the levels of motivation to try and access the brush versus general curiosity towards the panels as a new object. This occurred between 1300 and 1400 h on the relevant day (Fig. 2). After one week, all panels were removed between 1100 and 1200 h on the relevant day to allow brush access for a final week. Cattle were also yarded at least once per week for a health check, BW measurement, and to reapply livestock paint if required (Days 8, 17, 24, 29, and 36).

Behavioural observations

Following the 2-weeks of acclimation, behavioural observations occurred over 4 consecutive 'Sessions', consisting of 2 morning and 2 afternoon periods across 2–3 days, in the week prior to brush removal ('Prior'), following brush removal ('During'), and when brush access was allowed again ('After') (Fig. 2). Two groups of cattle were continuously observed for 4 h during each period, as entry into the trial was staggered in groups of two by 48 h to enable this. For Cohort 1, this occurred from 0630 h to 1030 h during the morning period and 1545–1945 h in the afternoon period. For Cohort 2, these hours were changed to 0700–1100 h, and 1500–1900 h, to account for differing sunrise and sunset times. These early morning and late afternoon times were chosen as enrichment use increases during these periods (Dickson et al., 2022).

Two personnel recorded behaviours live using binoculars when necessary, using an annotation application (CSIRO AnnoLOG v 1.0.23, Little, B.A., 2018) installed on a Samsung Galaxy Tab A 7.0″ (Samsung, Seoul, Korea). This application allowed the ID of the animal performing a behaviour to be recorded, along with the time it occurred and the duration. All personnel were trained by a single individual (**ED**) who devised the behavioural ethogram (Table 1), and who was also present for each observation period. All personnel had experience using both the annotation application and a similar behavioural ethogram prior to the beginning of the trial. Cattle were well-habituated to humans, vehicles, and the equipment required for these live observations prior to behavioural observations beginning.

Due to large amounts of rainfall, Groups 1 and 2 were not observed in the afternoon of the 15th day and morning of the 16th day during the week 'Prior' to brush removal, and were instead observed in the afternoon and morning of the 20th day, and morning of the 21st day (Fig. 2).

Coat cleanliness

Coat cleanliness of individual animals was scored on a four-point scale, adapted from Reneau et al. (2005), by a single person (ED). The level of coat contamination of specific body areas – head,

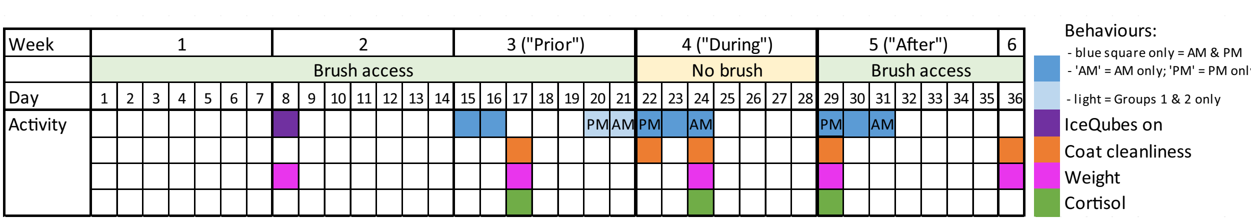

Fig. 2. Experimental timeline of events, depicting weeks of brush access, date IceQubes® were fitted, BW measures, faecal cortisol metabolite sampling, coat cleanliness scores, and behavioural observations across 8 groups of 6 Angus steers in paddocks.

Table 1
Ethogram of behaviours observed in Angus cattle housed at pasture when provided with, or denied, access to a grooming brush. The Animal ID, time a behaviour began, and its duration were recorded.

Behaviour	Description
Self-groom	An animal licks or scratches with its hoof any part of its own body.
Allogroom – Give	An animal licks or rubs its head on any part of another animal's body.
Allogroom – Receive	An animal is licked or rubbed on by another (can be 'receiving' from two animals at once).
Brush/Panels Groom	An animal was in physical contact (head, neck, back, or rump) with the brush or panels. The animal could be either moving its body against the object, or be still and leaning against it.
Brush/Panels Other	Any other interaction with the brush or panels (e.g. licking, sniffing).
Other Groom	Any other form of grooming (e.g. rubbing on hay bale or water trough).

neck, side, belly, rump, thigh, tail, and an average of the legs (Supplementary Figure S1) – was scored from 1 (very clean) to 4 (very dirty) for each animal (Supplementary Table S1).

On Day 22, cattle were scored in the paddock during addition of the panels, with the aid of binoculars as needed. On all other days of scoring, cattle were restrained in the crush following weighing to be scored.

Faecal cortisol metabolite concentrations

Faecal samples of individual animals were collected opportunistically between 1100 and 1230 h as groups were being herded to the yards, or manually while in the race as necessary. Samples were immediately placed into a mobile freezer upon collection and stored frozen for a maximum of 3 days. They were then placed in a drying oven at 60 °C until dry before being ground using a Retsch ZM 200 Ultra Centrifugal Mill with a 1 mm sieve. Samples were then stored in jars at approximately 4 °C until steroid extraction took place. For details on steroid extraction, see Supplementary Material S1.

Data and statistical analysis

All data analyses were performed in "R" (R Core Team, 2022). Main effects were considered significant at an alpha level of 0.05, and trends at an alpha level of > 0.05 < 0.10.

Data preparation

Data from the AnnoLOG application were directly imported into Excel and combined with daily weather data (24-h values for rainfall, temperature, humidity, wind gust speed). The time spent grooming by individuals (n = 48) was then summarised by day number, Session (n = 2), Week (n = 3), and Grooming Type (n = 4), resulting in a sample size of 2 304 events.

A principal component analysis (**PCA**) was performed using the "psych" package (Revelle, 2022) on the daily weather data (total rainfall, average temperature, humidity, and wind speed) from 16/1/23 to 5/4/23 inclusive. Principal components with eigenvalues greater than 1 were retained for use in models.

Individual animals were classed as 'high', 'medium', or 'low' brush users, based on their time spent grooming on the brush throughout observations in the 'Prior' week. These 'high' and 'low' groups were assigned based on +/- ½ SD from the global mean brush grooming time during the 'Prior' week. 'Low' brush users groomed the brush for less than an average of 44 seconds during a 4-h observation period (n = 20), 'medium' users between 44 and 156 seconds (n = 17), and 'high' users for greater than 156 seconds in a 4-h period (n = 11).

Grooming behaviours

All models were fit using the "lme4" package (Bates et al., 2015), and a description is provided in Table 2. All two-way interactions were initially fit and tested, but only those that reached significance are presented. The random nested effect of individual within group was also included. Model fit was checked by diagnostic plots and, if deemed to be poor, a transformation was applied as required. The significance of fixed effects was tested using the 'drop1' function, with a likelihood ratio test.

For analysis of the type of grooming, a two-stage modelling approach was taken, as many individuals did not perform all grooming types within a defined observation period. Firstly, a generalised linear mixed-effects model was fit for the *P* that a specific grooming event was performed by an individual, then a conditional linear mixed-effects model was fit for the duration of these events within a session (Table 2).

While the interaction between 'Week' and weather was significant for the total grooming duration model, there was a strong overlap between SEs (Supplementary Figure S2). The effect on outcomes showed no biologically relevant patterns (Supplementary Figure S3), and is therefore not presented for brevity.

IceQube® data

The IceQube® accelerometers provided data on standing and lying time (minutes), number of lying bouts, and number of steps

Table 2
All models used to analyse the grooming behaviour, coat cleanliness, faecal cortisol metabolites, average daily gain, and lying and stepping data of Angus steers housed at pasture experiencing brush loss. Weather is a numeric value derived from a PCA including average temperature, humidity, rainfall, and wind speed. Week refers to the week 'Prior' to brush access being blocked, 'During' the period of enrichment loss, and 'After' brush access was returned. Session refers to the time of the observation period (morning or afternoon). Type refers to grooming type (allogrooming, self-grooming, grooming on brush/panels, and any 'other' grooming). Brush Use classifies individuals as 'high', 'medium', or 'low' brush users according to their brush use in the week 'Prior' to brush access being blocked. Day Number is relative to the first day animals were placed into their experimental paddocks, and Interval refers to the time between being weighed to calculate ADG.

Response	Predictors	Model	Transformation
Total duration grooming	Week (n = 3), Session (n = 2), Weather	LMM	Square-root of response
Type of grooming (*P*)	Week (n = 3), Session (n = 2), Grooming Type (n = 4), Weather	Binomial GLMM	None
Type of grooming (conditional)	Week (n = 3), Session (n = 2), Grooming Type (n = 4), Weather	LMM	Square-root of response
IceQube® data (steps, proportion time lying, lying bouts)	Week (n = 3), Brush Use (n = 3), Weather	LMM	None
Coat cleanliness	Day Number (n = 4), Brush Use (n = 3), Previous Score, Weather	LMM	None
Faecal cortisol metabolites	Day Number (n = 3), Brush Use (n = 3), Weather	LMM	Log of response
Average daily gain	Interval (n = 4), Brush Use (n = 3), Weather	LMM	None

Abbreviations: PCA = principal component analysis; ADG = average daily gain; LMM = linear mixed-effects model; GLMM = generalised linear mixed-effects model.

within 15-minute time periods. From this, the daily number of steps, number of lying bouts, and proportion of time spent lying was calculated. Days 15 to 34 inclusive were used for data analysis, excluding days when cattle were removed from their paddocks for weighing or panels were installed/removed (Days 17, 22, 24, and 29).

Models were again fit using the "lme4" package, with model fit and significance of fixed effects testing conducted as previously described. Predictors for each model are presented in Table 2.

The interactions between 'Week' and weather were significant for these models, but there was a strong overlap between SEs (Supplementary Figure S2). The effect on outcomes showed no biologically relevant patterns (Supplementary Figure S3), and is therefore not presented for brevity.

Use of brush vs control panels 'During' enrichment loss

The use of panels surrounding the brush compared to the control panels was analysed by the number of bouts at each, which consisted of any type of interaction (e,g, groom, lick, sniff). A new bout began when an animal either moved more than one body length away from the panels, or stood without interacting with the panels for at least 10 seconds. Each individual's total number of bouts at each set of panels over the 16 h of observation in the 'During' week was summed together for analysis.

A two-way ANOVA was performed testing the interaction between 'Panel Type' (Control, Brush) and 'Brush Use' ('high', 'medium', 'low'), with 'Group' also included as a fixed effect. After checking model residuals, a square-root transformation was undertaken on number of bouts so that normality assumptions could be met. Posthoc comparisons were conducted using Tukey's HSD test.

Other measures

Prior to analysis, the average coat score of each individual was also calculated for each day measured, as a model using individual body parts failed to converge. Average daily gain (**ADG**) was also calculated for individuals between each day they were weighed. Weather data from PCA were averaged on days between the collection of coat cleanliness and BW data, and faecal sampling days to be included in models.

Models were again fit using the "lme4" package, with model fit and significance of fixed effects testing conducted as previously described. Predictors for each model are presented in Table 2.

Similar to grooming behaviours, the interactions between'Day' and weather were significant for the faecal cortisol metabolites model, but there was a strong overlap between SEs (Supplementary Figure S2). The effect on outcomes showed no biologically relevant patterns (Supplementary Figure S3), and is therefore not presented for brevity. The effect of cumulative rainfall on coat cleanliness and ADG between measurement days was also investigated, but returned comparable results to principal component 1 (**PC1**) so was excluded for simplicity. The term 'Interval' and not 'Week' is used for the analysis of ADG data, as cattle were not weighed on the day brush access was first denied.

Results

Weather – principal component analysis

PC1 was the only component to have an eigenvalue larger than one (1.74), explaining 43% of variation, and was therefore included in later models. Higher values represented cold, wet, and low wind conditions (Supplementary Table S2).

Behavioural analysis

Type of grooming – P

For the *P* that a grooming event would occur by an individual within a session, the interaction between week and grooming type was significant (χ^2_8 = 15.63, P = 0.048). From Fig. 3A, cattle had a much higher *P* of self-grooming than any other grooming behaviour over each week. Whilst *P* of self- and allogrooming was observed at a relatively constant rate over the study, *P* of grooming on the brush and other objects declined in the week 'After' the brush was returned. Neither weather (χ^2_1 = 0.97, P = 0.325) nor Session (χ^2_1 = 0.81, P = 0.369) were significant.

Type of grooming – conditional

Considering only individuals that had performed at least one specific grooming event during a session, the interaction between week and grooming type was significant (χ^2_8 = 1 251.84, P < 0.001). From Fig. 3B, the average duration given an event occurred was highest for grooming on brush/panels, which reduced in the week 'During' brush removal and increased in the week 'After' the brush was returned. Additionally, the duration of allogrooming reduced in the week 'After' the brush was returned. Session was significant (χ^2_1 = 23.97, P < 0.001), with longer amounts of grooming performed in the morning period. Weather did not have a significant impact (χ^2_1 = 0.04, P = 0.834).

Type of grooming – summary

From Fig. 3, the *P* of grooming at least once on the brush or panels was constant throughout the weeks 'Prior' and 'During' brush access being blocked, but 'After' its return this likelihood reduced. However, when only panels were present ('During') duration of grooming on these decreased, before increasing 'After' the return of the brush, indicating that bout length likely increased as well. Although the *P* of allogrooming was constant, when brush access was returned, the duration of this behaviour was reduced. Similarly, time spent 'other' grooming also reduced in this period following brush access being returned, which was driven be a lowered *P* of this behaviour occurring. Finally, the performance of self-grooming was constant regardless of brush access.

Total grooming duration

Both Week (χ^2_2 = 9.78, P = 0.007) and Session (χ^2_1 = 10.57, P = 0.001) had a significant impact on total grooming performed, with more grooming occurring in the morning session than the afternoon, and in the week 'Prior' to brush access being blocked (Fig. 4A). Weather did not significantly impact total grooming duration (χ^2_1 = 0.27, P = 0.602).

Stepping behaviours

Brush access impacted number of steps (χ^2_2 = 31.71, P < 0.001), with more occurring during the week brush access was returned (Fig. 4B). Although 'low' brush users numerically took more steps, 'Brush Use' did not have an impact on the number of steps (χ^2_2 = 1.42, P = 0.493), but the number of steps increased under cool and wet conditions (estimate ± SE = 25.93 ± 10.93, χ^2_1 = 5.54, P = 0.019).

Lying behaviours

Brush access impacted the proportion of lying time (χ^2_2 = 7.43, P = 0.024) with more lying occurring during the weeks 'Prior' to brush removal (Fig. 4C). The proportion of time spent lying was not affected by the amount of 'Brush Use' (χ^2_2 = 3.30, P = 0.192), or weather (χ^2_1 = 1.40, P = 0.237).

Number of lying bouts was not influenced by brush access (χ^2_4 = 11.68, P = 0.020), 'Brush Use' (χ^2_1 = 0.26, P = 0.879), or weather (χ^2_1 = 1.90, P = 0.169).

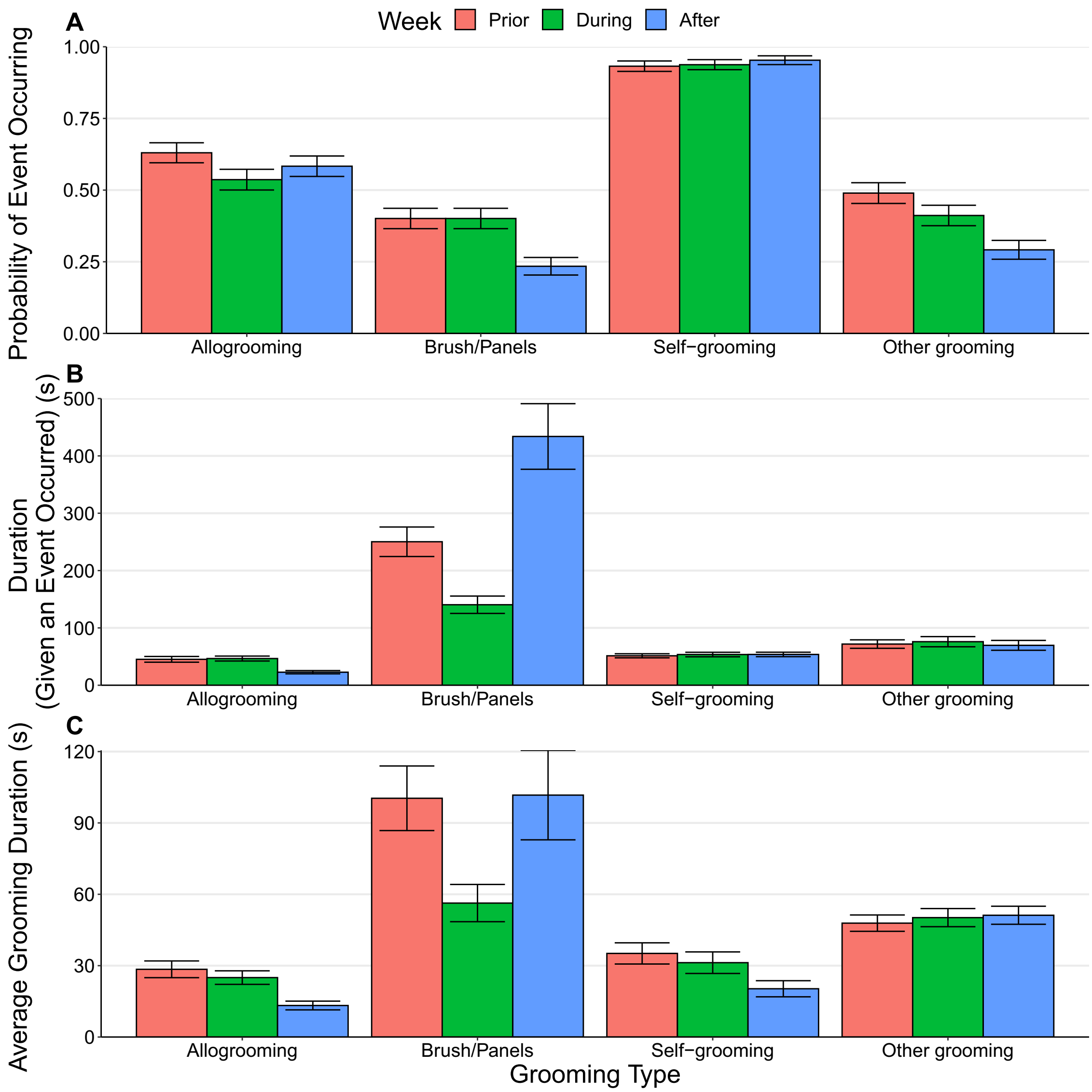

Fig. 3. Grooming behaviours observed in a session (4 h) of individual Angus steers housed in groups of 6 at pasture. The *P* (± SE) of a specific grooming event occurring **(A)**, duration (± SE) of grooming, given the individual performed the grooming type at least once within the observation period **(B)**, and raw average (± SE) duration individual cattle spent performing specific grooming behaviours **(C)** are presented. Allogrooming, self-grooming, grooming on a brush ('Prior' and 'After' weeks) or panels ('During' week), and other grooming were recorded.

Use of control vs brush panels 'During' enrichment loss

The interaction between 'Brush Use' and 'Panel Type' was significant ($F_{2,\ 83}$ = 6.50, P = 0.002), with 'low' brush users having fewer interactions at either set of panels, 'high' brush users having the most, and 'medium' brush users interacting with the brush panels much more than the control panels (Fig. 5). 'Brush Use' was significant as a main effect in isolation ($F_{2,\ 83}$ = 13.16, P < 0.001), but 'Panel Type' was not ($F_{1,\ 83}$ = 1.88, P = 0.174). 'Group' was also significant ($F_{7,\ 83}$ = 6.90, P < 0.001), with posthoc analysis revealing that Group 7 interacted with panels more than other groups.

Other measures

Coat cleanliness

Day number had a significant effect on coat cleanliness (χ^2_3 = 54.49, P < 0.001), whilst individual's previous cleanliness score tended to impact the current score (χ^2_1 = 3.09, P = 0.079), indicating that brush access impacts coat cleanliness. Cattle were cleanest on Day 22 when brush access was first blocked, dirtiest at the end of the 'no brush' period on Day 29, and became less dirty when brush access had been allowed for a week on Day 36 (Fig. 6). However, the amount of brush use in the first week did not have an impact on the cleanliness score (χ^2_2 = 1.07, P = 0.585), nor did weather (χ^2_1 = 0.07, P = 0.799). From Fig. 6, the body parts most impacted by brush access were the head, neck, rump, and thigh.

Faecal cortisol metabolite concentrations

The interaction between brush use in the first week of observations and sample number was significant (χ^2_4 = 12.61, P = 0.013), with individuals classed as 'low' brush users having higher cortisol concentrations than 'high' users prior to brush removal, with this trend reversing following brush access being prevented, particularly following one week of brush access being prevented (Fig. 7). Weather did not have a significant impact on faecal cortisol metabolite concentrations (χ^2_1 = 0.14, P = 0.711).

Average daily gain

'Interval' had a significant effect on average daily gain (χ^2_3 = 35.38, P < 0.001), with weight gain dropping during the period when brush access was denied (Fig. 8). Weather did impact ADG (es-

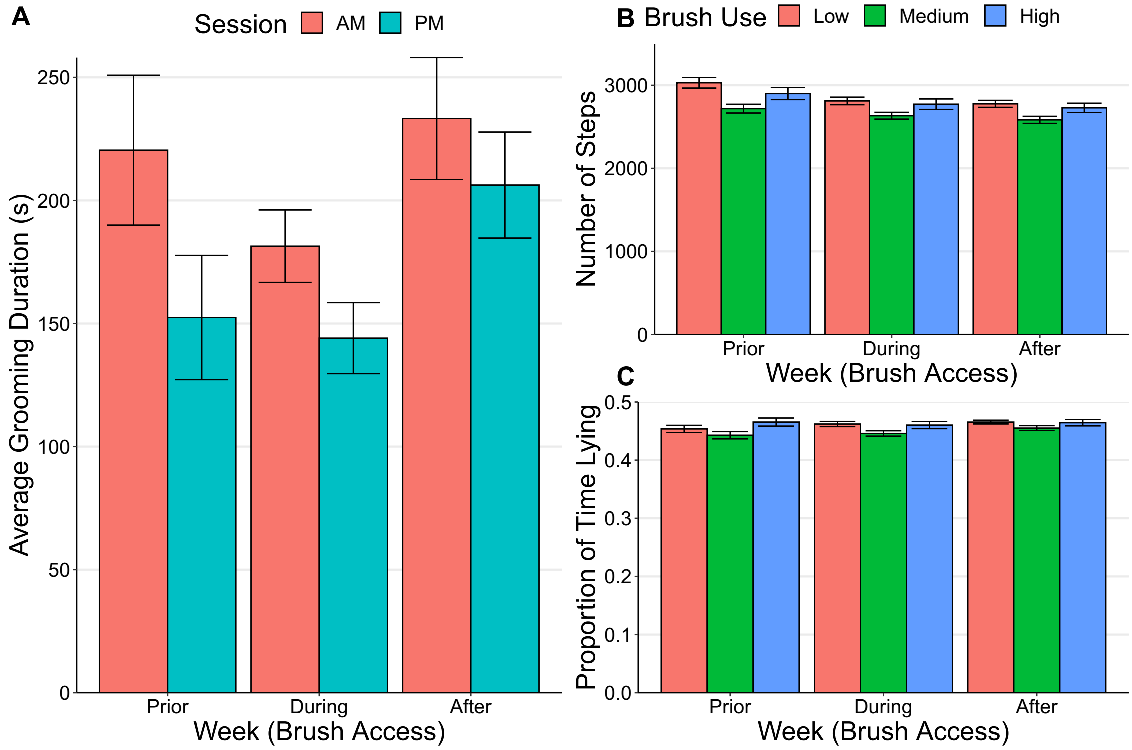

Fig. 4. Average total grooming (allogrooming, self-grooming, brush/panel grooming, and other grooming) duration (seconds) **(A)**, average daily number of steps **(B)**, and average daily proportion of time spent lying **(C)** (±SE) of individual Angus steers, housed in groups of 6, within a 4-h observation period in either the morning or afternoon session. Access to a grooming brush was allowed during the 'Prior' week, blocked for the 'During' week, and returned for the 'After' week. Individuals were classified as 'high', 'medium', or 'low' brush users according to their brush use in the week 'Prior' to brush access being blocked.

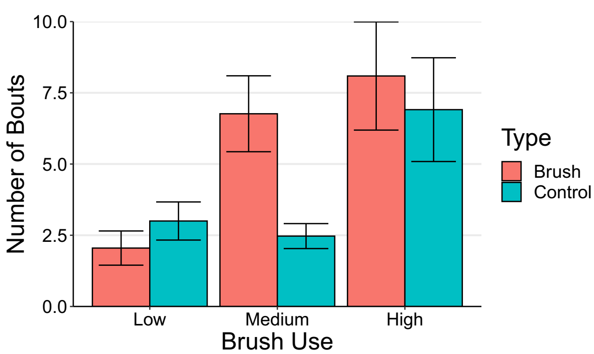

Fig. 5. Total mean number of interaction bouts (±SE) with panels surrounding a brush vs control panels performed by individual Angus steers, housed in groups of 6 at pasture. Bout number is the sum of 16 observation hours in the week 'During' brush access being blocked. Steers were classified into three phenotypes based on 'low', 'medium', or 'high' brush use prior to access being blocked via the panels.

timate ± SE = 0.39 ± 0.11, $\chi^2_1 = 11.79$, $P < 0.001$), with ADG increasing under wet, cold, and still conditions. However, the amount of brush use in the first week of observations did not significantly impact ADG ($\chi^2_2 = 0.30$, $P = 0.863$).

Discussion

The current study aimed to investigate the effects of the removal of adequate grooming objects on the behaviour and performance of grazing beef cattle that had previously been able to access these stimuli. Overall, grooming behaviours did not change when access to the brush was blocked, but when it was returned, both allo-grooming and grooming on other objects were reduced. Additionally, interaction with the brush and control panels differed based on an individual's brush use in the week 'Prior' to brush access being blocked, whereby 'medium' brush users interacted with brush panels more than control panels. During the brush loss phase, cattle gained less weight, had dirtier coats, and had increased faecal cortisol metabolites, indicating welfare may have been impaired.

The prediction that total grooming duration would be reduced in the period 'During' brush access being blocked was somewhat supported. Grooming in this period was lower than in the week 'Prior' to brush access being blocked, but not 'After' its' return, partly conflicting with previous studies stating that brush access increases total grooming (DeVries et al., 2007; Kohari et al., 2007). This may be because brush use was relatively low for the animals studied, with an average of 100 seconds use by individuals during a 4-h observation period, accounting for approximately 50% of total grooming duration. This is similar to the relative proportion of time grooming on trees to total grooming of grazing beef cattle (Kohari et al., 2007), but far less than the 90% of total grooming performed on brushes of indoor-housed dairy cows (DeVries et al., 2007). Other studies have reported brush use ranging anywhere from 2 to 31 minutes per day (DeVries et al., 2007; Foris et al., 2021; Mandel et al., 2017; Newby et al., 2013; Toaff-Rosenstein et al., 2016; Toaff-Rosenstein and Tucker, 2018). These differences in brush could be for a multitude of reasons such as sampling period (4 vs 12 h), seasonal differences, breed differences (dairy vs beef), health issues (mange: Moncada et al., 2020; metritis: Mandel et al., 2017), type of brush (stationary brush used less frequently than a mechanical brush; Strappini et al., 2021), environmental differences influencing time budgets (grazing vs indoor-housed and fed energy-dense diet), group size, and brush location (Foris et al., 2023).

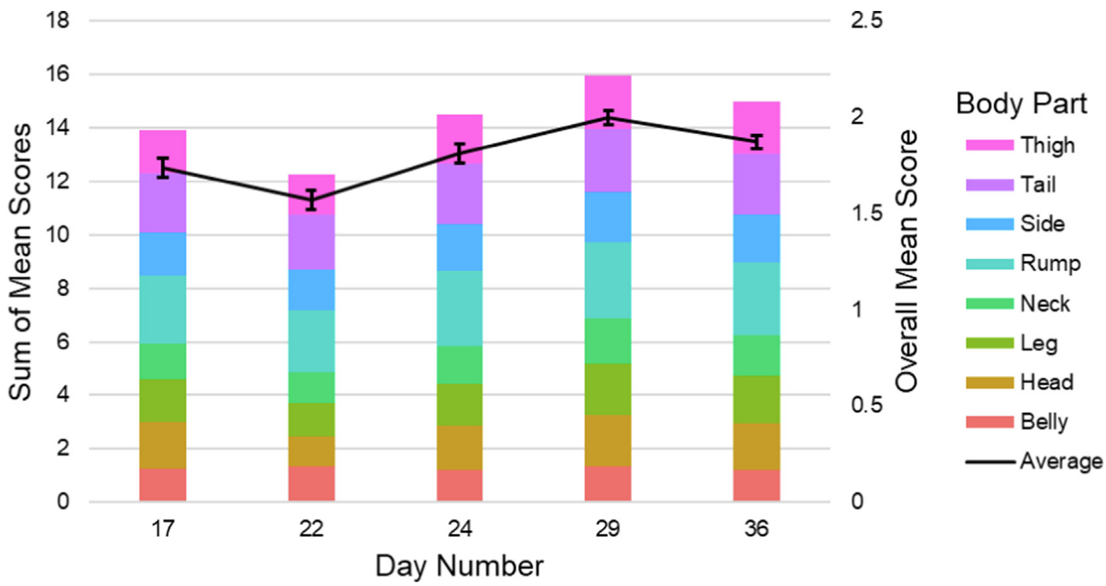

Fig. 6. Raw mean score data (± SE) for coat cleanliness by body type of individual Angus steers (bar graph), and overall mean score data (± SE) used for data analysis (line graph). Access to a grooming brush was blocked on Day 22, and returned on Day 29. Day 17 was not included in the linear mixed-effects model, as there was no 'previous score' for this day.

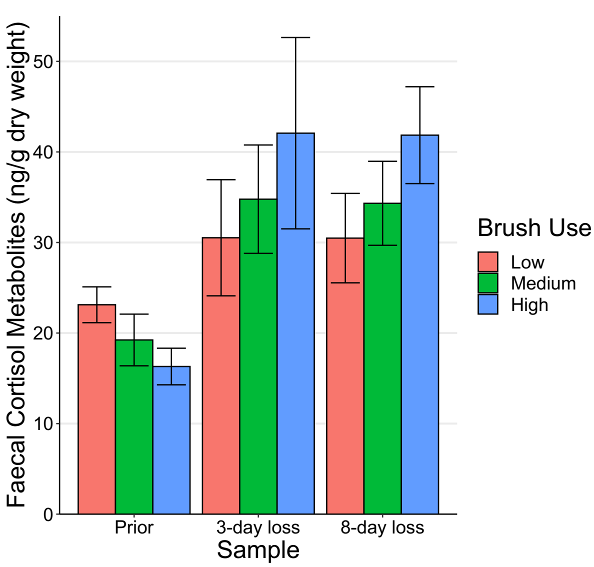

Fig. 7. Faecal cortisol metabolite concentrations (ng/g dry weight) (± SE) of individual Angus steers housed at pasture, in groups of 6. The first sample ('Prior') was taken when access to a brush was allowed, second sample after 3 days of no brush access, and third sample after 8 days of no brush access. Individuals were classified as 'high', 'medium', or 'low' brush users according to their brush use in the week 'Prior' to brush access being blocked.

An alternative explanation for total grooming duration not increasing from the 'During' to 'After' phase is due to the panelling that was installed during the loss period being associated with a relatively high level of grooming (approximately 1 minute per individual per 4 h). If cattle were observed later during this week, or the panels were present in some capacity from the beginning of the study, it is possible panel habituation would reduce any novelty effect on grooming (Dickson et al., 2022). Cattle that used the brush the most 'Prior' to its removal performed more bouts at the panels the week 'During' blocked brush access, whilst individuals that used the brush the least performed the least bouts at panels, likely due to different levels of grooming motivation. Interestingly, 'medium' brush users performed more bouts at the brush panels compared to the control panels, but this was not seen for either 'low' or 'high' brush users. As cattle have been shown to be motivated to access a grooming brush and will push a weighted gate to do so (McConnachie et al., 2018), this was unexpected. It is possible that the 'high' brush users had a higher motivation to groom on any object, whilst the 'medium' users, with lower grooming motivations, were more selective. It is presumed the brush may be a more valuable grooming resource as cattle could use it to access more body areas.

Cattle became dirtier when brush access was blocked, particularly the head, neck, rump, and thigh. Anecdotally, cattle were observed grooming these body parts on the brushes the most, although this was not formally recorded in the current study. This observation is reflected in the literature, which reports that cattle most commonly groom their head and neck on brushes (Horvath et al., 2020; Van Os et al., 2021; Zobel et al., 2017), and brush access also increases grooming of backs and tails (DeVries et al., 2007; Ninomiya, 2019). Although cattle were relatively clean, even during the period when brush access was denied, and a significant improvement in coat cleanliness was not seen after brush access was returned for a period of one week, it is possible that with continuous access cleanliness would further improve. After 6–7 weeks of brush access, dairy calves were cleaner during weaning than controls (Horvath and Miller-Cushon, 2019; Horvath et al., 2020), however, brush access did not impact coat contamination for beef calves (Bulens et al., 2014), or hair shedding scores for feedlot steers (Park et al., 2020). Poor coat cleanliness can reduce cattle welfare, by impacting thermoregulation (Aggarwal and Upadhyay, 2013), potentially irritating the skin (Nafstad, 1999, as cited in Hedman et al., 2021), pose a mastitis risk for cow/calf systems (Huxley and Whay, 2006), and in extreme cases can compromise hygiene at the abattoir (Torres, 2004). Therefore, access to appropriate grooming substrates, whether natural or artificial, may help to maintain coat cleanliness and prevent a range of health and welfare issues.

Brush loss did not impact the performance of self-grooming, but the amount of both allogrooming and 'other' grooming decreased when the brush was returned. This is somewhat in line with other reports of brush access having no impacts on self-grooming

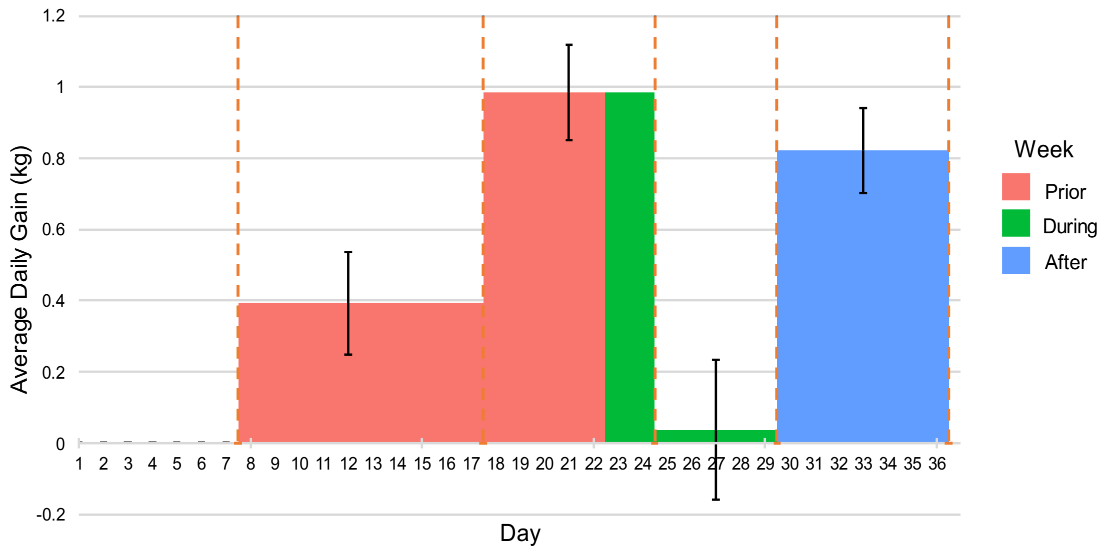

Fig. 8. Average daily gain (± SE) of individual Angus steers housed at pasture, in groups of 6. Dashed orange lines indicate days cattle were weighed. Access to a grooming brush was blocked on Day 22 using panels, and returned on Day 29.

(Horvath et al., 2020; Kohari et al., 2007; Newby et al., 2013), reducing allogrooming (Meneses et al., 2021; Park et al., 2020), and reduced grooming on other pen objects (DeVries et al., 2007). However, other studies have shown conflicting results, with brush access increasing self-grooming (Horvath and Miller-Cushon, 2019), and having no impact on allogrooming (Horvath and Miller-Cushon, 2019; Kohari et al., 2007). This reduction in allo- and 'other' grooming may be evidence of a rebound effect, in which some grooming behaviours were replaced by grooming on the brush. However, the performance of self-, allo- and 'other' grooming did not increase in the week 'During' brush access being blocked. Thus, it is suggested that these grooming behaviours are a separate behavioural need to grooming on objects. As the reduction in allogrooming coincides with dirtier coat scores, it is possible that cattle were not willing to groom themselves or each other if the recipient was dirtier. Further investigation is required into the mechanisms behind changes in allogrooming seen in the current study. The relationship between allogrooming, herd social interactions and cattle welfare warrants additional research.

In the current study, weather did not significantly impact faecal cortisol metabolite concentrations but did significantly impact ADG, with ADG reducing during dry, hot, and windy conditions. This study was conducted during late summer/early autumn, and weather was not extreme (average daily temperature of 17.9 °C and rainfall of 4.0 mL). Although cattle in a feedlot spend less time eating under conditions with low relative humidity, low wind speed, and when temperatures exceed 21.6 °C, this does not impact growth performance (Schwartzkopf-Genswein et al., 2002). It is important to note that the interpretation of these data may be limited by the short time frame over which ADG was estimated. Daily fluctuations in feed and water intake may have a potential impact on ADG, and therefore, longer measurement periods are recommended (Wang et al., 2006).

Both faecal cortisol metabolite concentrations and ADG were significantly impacted by brush access, suggesting that the loss of brush access was stressful, as ADG decreased and faecal cortisol metabolites increased during the period brush access was denied. In some species, grooming (or somatosensory stimulation) is thought to release oxytocin, which then decreases the activity of the HPA axis and sympathetic nervous system (reviewed in Uvnäs-Moberg et al., 2015), resulting in physical changes such as reduced heart rate and lowered cortisol concentrations. In cattle, allogrooming, brushing, and stroking by humans are associated with higher serum oxytocin concentrations (Chen et al., 2015), reduced plasma cortisol levels (Wredle et al., 2022), reduced heart rate (Laister et al., 2011; Sato and Tarumizu, 1993), and calmer behaviour (Proctor and Carder, 2014, 2015a,b). This is supported by the current study, as the interaction between the level of brush use and sample number in the current study was significant. 'Low' brush users had higher faecal cortisol metabolites than 'high' users in the week 'Prior' to brush access being blocked, with this trend reversing during the week 'During' access being blocked. Although previous studies on brush provision to cattle show no impact on cortisol concentrations (Park et al., 2020) or ADG (Horvath et al., 2020; Park et al., 2020; Velasquez-Munoz et al., 2019), the current study focuses on the loss of adequate grooming opportunities, and when other captive species are denied access to valued resources, they experience a spike in cortisol (Cronin et al., 2008; Mason et al., 2001). Therefore, this increase in stress in the period 'During' brush access being blocked could be linked to a negative affective state due to the experience of loss or frustration. Alternatively, as the total duration spent grooming did not differ between treatment weeks, it is possible that grooming on the brush provided a different type of tactile stimulation. For example, coarse bristles as opposed to smooth metal panelling, or on different body parts, may have stimulated oxytocin release. Finally, this increase in stress may have been due to muddier coats – resulting from blocked brush access – rather than changes in affective state, but this cannot currently be confirmed.

In general, a change in daily activity has not been seen in cattle when provided with a brush (Horvath et al., 2020; Meneses et al., 2021), however, some studies have noted an increase in play behaviour (Bulens et al., 2014) and increased activity (Velasquez-Munoz et al., 2019) for dairy calves. Additionally, for some species, activity increases when access to an important resource is blocked (Cooper and Appleby, 1995; Cronin et al., 2005; Mason et al., 2001; Yue and Duncan, 2003). This was not seen in the current study, in which the number of steps taken increased the week 'After, when brush access was returned, suggesting that cattle may walk further to access this resource. However, longer lying times were seen in the week 'Prior' to brush access being blocked, suggesting the cattle may have been experiencing frustration. The exact mechanisms behind this are unknown, but as lying time has the potential to influence welfare (reviewed in Tucker et al., 2021), this poses an interesting avenue for further study.

Conclusion

The current study provides evidence that pasture-based beef cattle did not change their object, self- and allo-grooming behaviours when access to a grooming brush was blocked, but upon its return, they decreased their time allogrooming and grooming on other objects. Additionally, coat cleanliness decreased during the period when brush access was prevented, along with a reduction in average daily gains and an increase in faecal cortisol concentrations for 'medium' and 'high' brush users, suggesting that the loss of adequate grooming substances can impair the overall welfare of cattle. The main limitations of the current study are that the effects of weather could not be controlled, and there were still objects present in the paddocks which allowed for grooming behaviours to be expressed, which may not occur in all production systems. Suggested future directions are to determine how long-term loss or continuous provision and removal of enrichment impacts cattle welfare, the development of a paddock-based test to accurately measure the affective state following enrichment provision and removal, and whether the potential impacts of enrichment loss are the same for other artificial and natural grooming objects such as trees.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101091.

Ethics approval

The procedure of this study was approved by the CSIRO Agriculture Animal Ethics Committee (Armidale, ARA 22-33), under the New South Wales Animal Research Act 1985.

Data and model availability statement

The data were not deposited in an official repository, but are available on request from the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

CRediT authorship contribution statement

Emily J. Dickson: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Jessica E. Monk:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Caroline Lee:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Paul G. McDonald:** Writing – review & editing, Supervision, Formal analysis. **Edward Narayan:** Writing – review & editing, Resources. **Dana L.M. Campbell:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Acknowledgements

Thank you to Troy Kalinowski, Jim Lea, Sue Belson, and Tim Dyall for their assistance with data collection, and Harsh Pahuja for his assistance with laboratory analysis.

Financial support statement

This research was funded by Meat and Livestock Australia Ltd., project number P.PSH.0807. E.J.D. was supported by an Australian Government Research Training Program (RTP) Scholarship through the University of New England, Armidale, NSW, Australia.

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