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Behavioural effects of feed dilution and daily roughage provision in male Hubbard M77 broiler breeders during rearing

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ABSTRACT

A growing public interest in broiler chicken welfare is leading to an increase in the number of private companies committing to switch to slower growing hybrids, particularly those approved by schemes such as the Better Chicken Commitment. These slow growing chickens are often, however, a result of cross breeding a slow growing hybrid with a conventional hybrid. The Hubbard JA787 for example, is fathered by the M77 which has a conventional growth rate. As such, these broiler breeder males still experience feed restriction as is routine with other conventional hybrids. This study, therefore, aimed at investigating the effects of a combination of qualitative feeding restriction strategies (i.e. feed dilution and daily roughage) on several behavioural indicators of welfare in Hubbard M77 broiler breeder cockerels during the rearing period. Two hundred M77 broiler breeder cockerels were housed from 5 to 10 weeks of age in 12 pens (6 pens/treatment). The treatments were conventional feed restriction (Control) and feed with 20 % dilution with oat hulls and a daily provision per pen of 150 g of alfalfa roughage (D+R). In the home pen, novel object (NO) tests and frustration assessments during thwarted feeding were conducted, with responses captured on video. Additionally, four birds from each pen underwent a tonic immobility (TI) test. These behavioural tests were carried out weekly at 6, 8, and 10 weeks of age. After euthanasia, two feathers from each bird were plucked and examined macroscopically for fault bars. The results from the NO test introduced doubt as to whether the D+R diet reduced hunger in the short term (4 hours) or increased it in the longer term (24 hours). In the frustration test, control birds performed more behavioural transitions and spent more time pacing and pecking the feed box than D+R birds ($P < 0.05$), but these results were not consistent across weeks of age. Finally, no diet treatment differences were observed in the tonic immobility test or in the assessment of feather fault bars. We conclude that a combination of feed dilution with 20 % oat hulls and daily provision of roughage did not significantly improve M77 broiler breeder cockerel welfare during rearing according to the indicators assessed. Furthermore, the present study contributes to the growing literature suggesting that qualitative feed restriction, while promising in theory, in practice fails to deliver strong and consistent improvements to animal welfare.

1. Introduction

For the past several years, there has been a growing interest and concern for animal welfare, particularly the welfare of farmed animals. This concern comes not just from the public and consumers, but also from governments and private companies. As a result of this, we have seen a steadily rising number of companies committing to abide by higher animal welfare standards such as the European/Better Chicken Commitment (BCC, 2024). Currently, 384 European companies have pledged to follow BCC standards for 100 % of their supply by 2026 (Chicken Watch, 2018). The same pledge has been made by 238 companies in North America (Chicken Watch, 2018). The BCC policy restricts the hybrids that can be used for chicken meat production to the breeds that have a slower growth rate than the more conventionally used Ross 308 or Cobb 500. For example, the Ross 308 has a typical growth rate to 42 days of age of approx. 70 g/day, while the JA787, one of the breeds accredited by the BCC, has a growth rate of 45–50 g/day (Aviagen, 2022; Hubbard breeders, 2024). However, while the broiler chickens of slower growing breeds have slower growth rates, their parent breeders are often of conventional lines and therefore do not have a slower growth rate. The JA787, for example, is the offspring of the "dwarf" JA87 females with the conventional M77 males. The JA87

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females have a growth rate to 15 weeks of age of approx. 90.7 g/week compared to the equivalent of 115.7 g/week for the Ross 308 broiler breeder females (Aviagen, 2021; Hubbard, 2023). In comparison, the M77 males have a growth rate of 157.5 g/week to 15 weeks of age while the Ross 308 broiler breeder males grow 154 g/week to the same age (Aviagen, 2021; Hubbard, 2024). Therefore, these male broiler breeders still face a lot of the same welfare challenges seen in the Ross 308 broiler breeders, including feed restriction.

Feed restriction is a strategy commonly used in the management of broiler breeders in order to slow their growth rate (Savory and Lariviere, 2000; Riber, 2020). If allowed to eat ad libitum, their fast growth rate brings about health issues and a decrease in reproductive health (De Jong et al., 2002). Nevertheless, this quantitative restriction, which is most severe during the rearing period, results in many other detriments to welfare, including hunger, feeding frustration, stereotypic behaviours, and severe feather pecking (Arrazola, 2018; Arrazola et al., 2020; D'Eath et al., 2009; Girard et al., 2017; Sandilands et al., 2005). Qualitative feeding restriction has been proposed as an alternative to the conventional quantitative restriction (Riber, 2020). The qualitative approach involves using fibers with little or no nutritional value to dilute the concentrated feed. Consequently, birds can be given larger portions without a large increase to their energy intake (Sandilands et al., 2006). This larger feed portion is anticipated to alleviate hunger by extending feeding time, increasing intestinal content, and prolonging feed passage time, thereby enhancing satiety, compared to the quantitative feeding restriction strategy (Hocking et al., 2004; Steenfeldt and Nielsen, 2012). In addition, giving larger amounts of feed increases the time spent feeding (Zuidhof et al., 1995). Therefore, qualitative restriction is expected to better meet the behavioural need for performing feeding behaviour.

The results of studies investigating the effectiveness of qualitative feeding are, however, often conflicting. For example, while a dilution using 20 % oat hulls had no effect on foraging behaviour in the home pen such as time spent feeding, it did reduce feeding intake motivation and frustration assessed with a feed intake motivation test and a frustration during thwarted feeding test (Riber et al., 2021; Riber and Tahamtani, 2020). Providing roughage as extra fibres, instead of using fibres to dilute the pelleted feed, improved plumage condition, likely due to a reduction in feather pecking behaviour, and reduced the number of feather fault bars but did not affect the motivation to explore or to forage (Riber and Tahamtani, 2020; Tahamtani et al., 2020). Furthermore, the vast majority of the previous research on this topic has focused on the effects of qualitative feeding restriction on the females, the pullets, as they are the ones responsible for egg laying. The only previous studies on broiler breeder males, using Ross 308 birds, tested the combination of feed dilution and daily roughage (Kittelsen et al., 2023; Tahamtani et al., 2024). The authors reported no adverse or beneficial health effects (Kittelsen et al., 2023) and some behavioural signs of reduced hunger such as reduced frustration, fear and motivation to explore, as assessed using thwarted feeding, tonic immobility and novel object tests, while also reporting an increase in feather fault bars, a result of feather follicle contraction in response to acute stress (Tahamtani et al., 2024). There is, therefore, still a large knowledge gap in regard to the effects of qualitative feeding restriction on broiler breeder males, especially those of hybrids other than Ross 308.

The objective of this study was to examine the impact of combining feed dilution and the daily provision of roughage (Dilution + Roughage) on various behavioural welfare indicators in Hubbard M77 broiler breeder cockerels throughout the rearing period (5–10 weeks of age) exactly as performed in Tahamtani et al. (2024). The birds in the control group (Control) received a standard feed for broiler breeders following their breed specifications (Hubbard, 2024). Both groups were evaluated for fear levels and exploratory motivation using Tonic Immobility (TI) and Novel Object (NO) tests. A relationship between hunger and the chickens' motivation to avoid fear-inducing stimuli and explore novel resources has been presented in previous studies. For example, Lindholm et al. (2018) showed that broiler breeders on skip-a-day schedules show more interest in an NO than birds fed daily, indicating increased risk-taking behaviour while fasting. Additionally, frustration was assessed during a thwarted feeding test, and the presence and severity of feather fault bars were examined. The hypothesis was that feed dilution combined with daily roughage would lead to reduced TI duration, longer distances to the NO, decreased frustration, and fewer and less severe fault bars. This research is part of a broader study investigating the effects of qualitative feeding restriction and daily roughage on various parameters, including home pen behaviour (Vasdal et al., in preparation) and health (Kittelsen et al., in preparation) in M77 broiler breeder cockerels.

2. Materials and methods

2.1. Ethical statement

This study was approved by the Ethical Board of the Norwegian Food Safety Authority, license number 30003. The study was conducted at the Norwegian University of Life Sciences' research facility.

2.2. Animals and housing

This study followed the methods outlined in Tahamtani et al. (2024). We used 200 Hubbard M77 broiler breeder cockerels aged 5–10 weeks. Incubation, hatching, sex-sorting and toe clipping were all performed in a commercial hatchery. The birds were not beak trimmed. They were then transported to the rearing farm, where males (N = 600) and females (N = 8000) were housed in separate compartments within the same room with wood shavings used as litter over the concrete floor. All rearing management regarding density, lighting, environmental enrichment, and feeding schedule adhered to the breeding company's recommendations and Norwegian regulations (Landbruks- og matdepartementet, 2006).

At 5 weeks old, 200 cockerels were selected based on their live weight, aiming to match the Hubbard breeding manual's performance objectives for that age (approx. 960 g; Hubbard S.A.S, 2024). Experienced poultry handlers caught and crated the cockerels, transporting them for 6.5 hours in a vehicle equipped with climate control to the Centre for Husdyrforsøk at the Norwegian University of Life Sciences.

Upon arrival at the experimental facilities, the birds were randomly distributed into 12 pens located in the same room, each measuring 296 × 60 × 71 cm (length × height × depth), with 16 or 17 birds per pen, resulting in a density of 8–8.5 birds/m^2, consistent with common practices in Norway. Norwegian law permits a density of up to 15 birds/m^2 (Landbruks- og matdepartementet, 2006). Wood shavings were used as litter material and environmental enrichment was provided in every pen as a 70 cm jute rope (ø: 20 mm) hanging inside the pen. The birds had ad libitum access to water via nipple drinker lines (4 nipples per pen). The physical environment inside the room, such as temperature, and lighting, were maintained according to the Hubbard M77 males parent stock management handbook and were uniform across pens. The light period started at 13 hours at 5 weeks of age (from 07:30–20:30), gradually decreasing each week until it reached 10 hours (from 07:30–17:30) at 10 weeks of age. Light intensity was maintained at 10 lux.

The birds were given 6 days to habituate before the study commenced. During this habituation period, all cockerels received the same feed as at the rearing farm. The treatment diets were introduced on the sixth day following their arrival at the experimental facility.

2.3. Experimental treatments

Six pens received one of the two diets, i.e. Control or Dilution + Roughage (D+R). The placement of the pens was balanced in the room to account for minor variations in physical conditions such as temperature, humidity, and activity near the door versus the back of the room. Control and treatment pens were alternated throughout the room, with both treatments represented in each row.

Both diet concentrates were presented as pellets (ø: 2.5 mm). Daily feed amounts were based on recommendations from Hubbard and typical growth curves for broiler breeder cockerels in Norway. The nutritional specifications of a commercial rearing diet were used to formulate the control concentrate. For the D+R concentrate, the control concentrate was diluted with 20 % oat hulls, reducing the metabolizable energy (ME) and digestible amino acid content approximately one-fifth, allowing for a 20 % higher feed portion per bird per day (Table 1). The pelleted feed for both treatments was distributed manually once daily at 09:00 by scattering it on the pen floor. Approximately 15 minutes later, 150 g of alfalfa roughage was distributed to each D+R pen via hay feeders (25 × 9 × 17 cm) attached to the side of each pen.

2.4. Data collection

Three behavioural tests were performed on the birds every 2 weeks at 6, 8 and 10 weeks of age. These were the Novel Object (NO) test, a frustration test, and a Tonic Immobility (TI) test.

2.4.1. Novel object test

A Novel Object (NO) test was used to assess the impact of the diet treatments on the exploratory behaviour of the birds in their home pens. On test days, the NO test was conducted once in the morning, one hour before feeding (8:00) and again in the afternoon, four hours after feeding (13:00). Each time, a novel object, varying in shape and colour, was placed in the pens at the back corner near the door, and the birds' responses were recorded for 10 minutes using video cameras (Sony Handycam HDR-CX405) mounted outside a neighbouring pen. The two objects presented on the same day were of approximately the same size. The NOs included: white plastic balls (ø: 75 mm), yellow plastic doorstoppers (height: 135 mm), green toy plastic rakes (height: 15 cm), Coca-Cola aluminium cans (volume: 330 ml), blue plastic frisbees (ø: 21 cm), and orange plastic cones (height: 30.5 cm) (Tahamtani et al., 2024). Three pens from each treatment group were exposed to one object in the morning test and a different object in the afternoon test, with the order reversed for the other three pens. During the video analysis, the latency for the first bird to touch the NO was recorded. Additionally, four zones were assigned in each pen: Zone 1 was recorded when birds were in physical contact with the NO. Zones 2–4 were equal thirds of the pen (99 cm×71 cm each) with zone 2 being the closest, and zone 4 being the furthest from the NO. The number of birds in each zone was noted every 30 seconds for the first 5 minutes and every minute for the last 5 minutes. These data were used to calculate the estimated odds of the birds to be in a zone close to the NO during the NO test.

2.4.2. Frustration test

The frustration test was used to assess the response of the birds during thwarted feeding. The test was performed at 11:00, between the two NO tests described above. Frustration was induced by the placement of a closed transparent box filled with feed inside the home pen. The box allowed the birds to see the feed, but not to access it. The feed used corresponded to each diet treatment. The birds were habituated to the empty plastic boxes for the three days prior to the test day. The birds' behavioural responses were recorded for 5 minutes using video cameras (Sony Handycam HDR-CX405). During the analysis of the videos, two focal animals were randomly selected, and continuous recoding of their behaviour was performed using the ethogram presented in Table 2 and the event-logging software BORIS (Friard and Gamba, 2016). The total duration of each behaviour and the number of transitions between different behaviours were calculated for each focal bird. An increased occurrence of behavioural transitions is considered an indicator of frustration (Roper, 1984; Tinbergen, 1951).

2.4.3. Tonic immobility

The birds' fear response relative to the diet treatment was assessed using the TI test. This test was performed on 4 birds/pen, giving a total

Table 2
Ethogram used for data collection during the frustration test (Adapted from Riber and Tahamtani, 2020).

Behaviour	Description
Pecking the box	The focal bird pecks, often in a stereotyped manner, the transparent box with feed. Includes pauses between pecks (= bouts*)
Standing	The focal bird stands on the ground with both feet.
Pacing	Horizontal or vertical movement of body, such as running, walking, jumping and hopping without performing any other type of behaviour.
Drinking	Having the beak in touch with the drinker. Includes the pauses between sips (= bouts*).
Foraging	Pecking and scratching the ground. Includes the pauses between each of the described elements (= bouts*).
Pecking object	Pecking, often in a stereotyped manner (i.e. several uniform pecks without moving its body) at fixtures in the pen (e.g. wall, drinking line (not nipples), etc.). Includes pauses between pecks (= bouts*). Does not include pecks to the transparent box with feed.
Feather pecking	Pecking the feathers, except the head, of the other bird. Includes the pauses between pecks (= bouts*), which often involves following the recipient bird.
Toe pecking	Pecking the toes or feet of the other bird. Includes the pauses between pecks (= bouts*).
Preening	Manipulating own plumage with the beak. Includes the pauses between each contact between beak and feathers (= bouts*).
Comfort behaviour	Wing flapping, stretching legs or wings and feather ruffling/shaking (outside the context of dustbathing). Includes the pauses between each of the described elements (= bouts*).
Aggressive behaviour	Aggressive pecking (forcefully pecking directed towards the head (generally) of the other bird - either the peck results in contact or causes an avoidance response/squat in the target chick). Hopping towards the other bird, frontal threatening (the two birds have an upright position towards each other). Leaping towards the other bird (= hopping on the spot), may involve kicking and wing-flapping. Includes the pauses between each of the described elements (= bouts*).

* If another behaviour was performed during the pauses, a new bout was set to have commenced when the behaviour was resumed.

Table 1
Diet composition information for the experimental diets provided.

	Age (wk)	ME (MJ/kg)	Protein (g/kg)	Crude fiber (%)	Soluble NSP[a] (%)	Nonsoluble NSP[a] (%)	Daily amount of feed g/bird/day[b]
Starter							
Control	5	11.8	165	5.18	2.92	14.4	49
Grower							
Control	6 – 10	11.2	135	6.2	2.98	18.09	50–60
Diluted	6–10	9.0	110	11.6	2.74	30.71	60–72
Alfafa	6–10	3.6	168	27.1	Total NSP 55 %		ca. 8–9

[a] Non-Starch Polysaccharide.
[b] The daily amount increased according to the weight of the birds. This shows the increase from the first week to the last week.

of 24 birds/treatment. The testing order was randomized each test day on each test week (i.e. one testing day per week in weeks 6, 8 and 10 of age). Two observers conducted the test between 10:00 and 14:00 so to not disrupt the feeding or the light schedules. The number of birds tested by each observer from each pen was balanced to eliminate observer bias. To perform the test, a bird was randomly selected and carried by the observer from the home pen to an adjacent room, where the observers conducted the test at opposite ends to avoid disturbing each other (approx. 10 m apart). The state of tonic immobility was achieved by placing the bird on its back in a V-shaped wooden cradle, following the methods described by Tahamtani et al. (2024). The bird was held in place with one hand on its chest and the other covering its head for 10 seconds. After the slow removal of the observer's hands from the bird, if the bird righted itself within 5 seconds, the observer attempted to induce TI again. Induction to TI was tried up to a maximum of 3 attempts. If TI could not be induced after 3 attempts, a 4 was recorded as the number of attempts necessary and the bird was returned to the home pen without collecting data on TI duration. Once TI was induced, the observer stood motionless nearby, within sight of the bird, but without making direct eye contact, in order to catch the bird when it righted itself and avoid injury to the bird. The latency to perform head movements and the total duration of TI, i.e., until the bird righted itself, were recorded. TI was terminated by the observer if it lasted longer than 10 minutes. After the test, the bird was returned to its home pen.

2.4.4. Feather fault bars

At the end of the experiment (i.e. 10 weeks of age), all birds were euthanized and underwent a postmortem examination, with results to be detailed in (Kittelsen et al., in preparation). The birds were stunned using blunt force trauma to the head, followed by euthanasia through cervical dislocation. During the postmortem assessment, two feathers were plucked from each bird: the left primary 8 (P8, the third outermost flight feather) and the left scapular 1 (Sc1, central scapular feather) (Arrazola and Torrey, 2019).

The plucked feathers were placed in individual freezer-grade plastic bags and labelled with the bird ID and pen number. These bags were stored in a freezer at -5°C for later analysis. After thawing, a single observer, blind to the experimental diets, examined all feathers macroscopically for the presence of translucent lines perpendicular to the rachis (i.e., fault bars) by holding them under light and using a magnifying glass (2.25x magnification). The faults were categorized by length and severity: minor (< 5 mm), moderate (≥ 5 mm), and severe (≥ 5 mm with broken barbules on the fault bar) (Arrazola and Torrey, 2019). Feathers that were broken or very dirty were excluded from the analysis.

2.5. Statistical analysis

Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC), following the methodology of Tahamtani et al. (2024). The mixed procedure was used to analyse the latency to approach the novel object in the NO test. The model included the fixed factors diet treatment, week of age, time of day, and their interactions, as well as pen as a random effect. The glimmix procedure was used to analyse the probability of the birds to be in a zone closer to the NO during the NO test. The model used an ordered multinomial distribution, and had diet treatment, time of day, and their interaction as fixed effects. Pen number was also included in the model, as a random factor. For this model, Bonferroni corrections were applied to adjust the critical alpha value for multiple comparisons between diet treatment and time of day, resulting in a critical alpha of $\alpha = 0.012$.

Data from the frustration test were analysed using the mixed procedure, with diet treatment, week of age, and their interaction as fixed effects, and focal bird nested within pen as a random factor. This model was applied to total number of behavioural transitions, as well as time spent standing, pacing, foraging, and pecking the box. Drinking behaviour was initially analysed as a dichotomous Yes/No variable using a binomial model with a logit link function via the glimmix procedure. Subsequently, instances of drinking behaviour (lasting > 0 s) were analysed using the same mixed model as other behaviours. Due to the low occurrence of behaviours such as pecking objects, feather pecking, toe pecking, preening, comfort behaviours, and aggression during the frustration test, these results are presented descriptively.

During the TI test, TI was successfully induced in all birds with 3 or fewer attempts. The latency to perform head movements and the total duration of tonic immobility in the TI test were analysed using the mixed procedure, with diet treatment, week of age, and their interaction as fixed effects, and pen as a random factor. The likelihood of requiring more than one induction to achieve TI was analysed using the glimmix procedure with a binomial distribution and logit link function, with the same fixed and random effects.

Finally, the number of minor, moderate, and total feather fault bars were analysed using mixed models. These models included diet treatment, feather type, and their interaction as fixed effects, and bird ID nested within pen as a random factor. The occurrence of severe fault bars was analysed using the glimmix procedure with a binomial distribution and logit link function, with the same fixed and random effects.

The normal distribution of the residuals in all the mixed models mentioned above was checked using the univariate procedure and performing a visual examination of the histogram and QQ plot of the residual distribution. Higher-order insignificant interactions were removed through backward stepwise reduction for all models mentioned above. Unless otherwise stated, post hoc pairwise comparisons were performed using Tukey's HSD test (critical alpha $= 0.05$).

3. Results

3.1. Novel objects test

Regarding the latency to approach the novel object in the NO test, there was no effect of diet treatment ($F_{1,64} = 2.26$; $P = 0.14$) despite what looks to be a numerical difference between the two treatments. Control birds took an average of 105.4 s $\pm$ 23.5 (LS means $\pm$ SE) to approach the NO whereas birds which received the diluted diet and roughage had an average latency of 55.6 s $\pm$ 23.2. The week of age and time of day tended to affect latency ($F_{2,64} = 3.11$; $P = 0.051$), with latencies being higher on week 8 of life and particularly in the afternoon of that week (Table 3).

At 6 weeks of age, there was no effect of treatment on the number of birds in each zone during the NO test ($F_{1,6374} = 0.14$; $P = 0.70$; Fig. 1A). There was, however, a general effect of time of day, with all the birds being more likely to approach the NO in the morning, before feeding, than in the afternoon, 4 hours after feeding (estimated odds: 0.26; $F_{1,6374} = 46.68$; $P < 0.0001$).

In week 8 of age, there was an effect of the interaction between diet treatment and time of day ($F_{3,6384} = 46.49$; $P < 0.0001$, Fig. 1B). Birds in the D+R treatment where more likely to approach the NO in the morning than in the afternoon (estimated odds: 0.74; $P < 0.0001$). The same was true for the control birds, being more likely to approach the NO in the morning than in the afternoon ($P < 0.0001$). Nevertheless, this difference was larger in the D+R birds than in the control birds (estimated odds 0.74 versus 0.37 respectively). However, the direct pairwise comparison between D+R and Control birds in the afternoon was not significant ($P = 0.026$; critical alpha Bonferroni corrected to 0.0125). In addition, the birds in the D+R in the morning were more likely to approach the NO compared to all other treatment and time of day combinations (estimated odds range: 0.64 – 1.0; $P < 0.0001$).

In contrast, at 10 weeks of age, there were no observed effects of the fixed factors diet treatment ($F_{1,6173} = 0.08$; $P = 0.78$), time of day ($F_{1,6173} = 2.21$; $P = 0.13$), or their interaction ($F_{1,6173} = 0.82$; $P = 0.36$; Fig. 1C).

Table 3
Latency (s) to touch the novel object during the NO test across diet treatment, week of age and time of day.

Treatment	Week of age	Time of day	LS means	SE	Lower CI	Upper CI
Control	**6**	**morning**	18.40	63.94	0	146.34
		afternoon	100.83	58.37	0	217.63
	8	**morning**	118.83	58.37	2.04	235.63
		afternoon	331.00	58.37	214.20	447.80
	10	**morning**	17.50	58.37	0	134.30
		afternoon	39.33	58.37	0	156.13
Dilution + roughage	**6**	**morning**	40.33	58.37	0	157.13
		afternoon	5.00	58.37	0	121.80
	8	**morning**	58.42	58.37	0	175.21
		afternoon	226.00	58.37	109.20	342.80
	10	**morning**	1.33	58.37	0	118.13
		afternoon	2.83	58.37	0	119.63

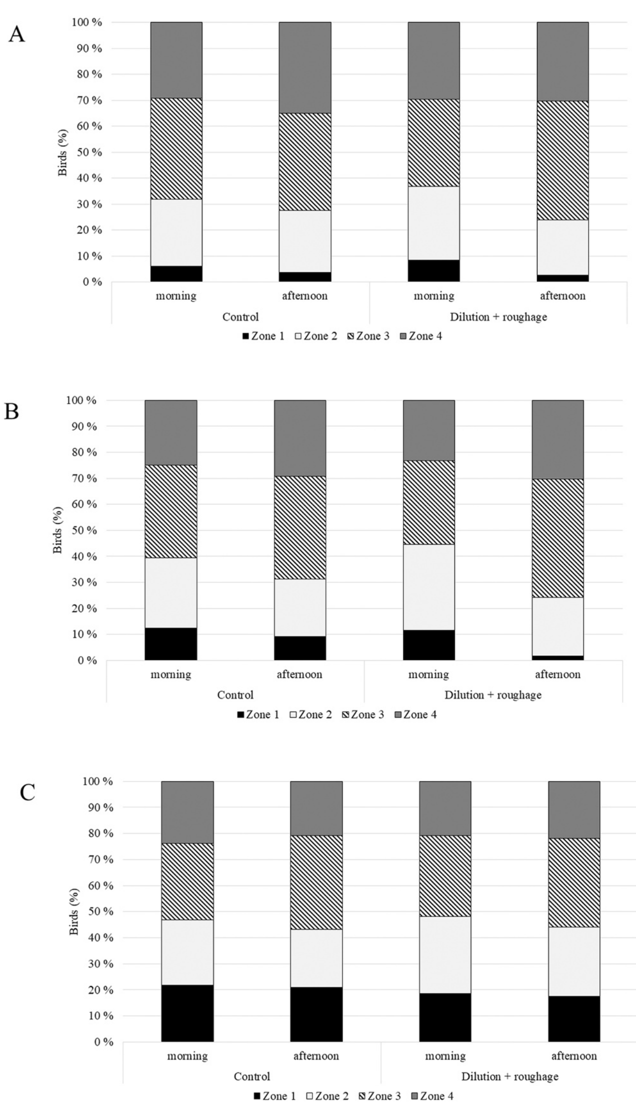

Fig. 1. Distribution of the frequency of birds in each of the four zones (%) during the novel object test at 6, 8 and 10 weeks of age (panels A, B and C, respectively). Higher zones were further away from the novel object.

3.2. *Frustration test*

There was an effect of the interaction between diet and week of age on the number of behavioural transitions during the frustration test ($F_{2,65} = 5.20$; $P = 0.008$). As can be seen in Fig. 2A, the birds from both groups showed a decrease in behavioural frustrations with age ($P < 0.03$). However, the two diets differed only at week 6 of age with Control birds performing more behavioural transitions than D+R birds ($P = 0.001$).

There was also a tendency for an effect of the interaction between diet and week of age on the amount of time spent standing ($F_{2,44} = 3.13$; $P = 0.054$), however, any differences between the diet groups were lost during the post hoc analysis due to the number of pairwise comparisons. Nevertheless, birds from both diet groups performed less standing with age. As Fig. 2B shows, less time was spent standing at 10 weeks of age in the Control birds compared to week 6 ($P = 0.02$), and in the D+R birds compared to week 8 of age ($P = 0.04$).

Pacing behaviour was also affected by the interaction between diet and week of age ($F_{2,66} = 4.38$; $P = 0.02$). Birds from both diets experienced a decrease in time spent pacing from week 6 to week 8 and 10 ($P < 0.0001$). In addition, compared to the D+R birds, Control birds performed more pacing at 6 weeks of age ($P < 0.003$), but they did not differ at 8 or 10 weeks of age ($P > 0.995$; Fig. 2C).

An interaction between diet and week of age was also found for the time spent foraging ($F_{2,44} = 4.41$; $P = 0.02$). Control birds spent less time foraging at 10 weeks of age compared to the D+R birds ($P = 0.004$). There was no difference between the diets at 6 ($P = 0.99$) or 8 weeks of age ($P = 0.71$; Fig. 2D).

There was an effect of the interaction between diet treatment and week of age on the time spent pecking the feed box in the frustration test ($F_{2,66} = 5.26$; $P = 0.0076$, Fig. 2E). While Control birds spent a similar amount of time pecking the feed box throughout the weeks of the experiment, the birds receiving the D+R feed significantly reduced their time spent on this behaviour from week 6 to week 8 ($P = 0.04$). Furthermore, the two groups significantly differed at 10 weeks of age, with the Control birds spending more than twice as much time pecking the feed box than the D+R birds ($P = 0.04$).

There was no effect of diet treatment ($F_{1,20} = 0.94$; $P = 0.34$); week of age ($F_{2,20} = 1.41$; $P = 0.27$); of their interaction ($F_{2,20} = 0.27$; $P = 0.76$) on the time spent drinking (Mean ± Std Dev = 14.33 ± 32.78 s). There was also no effect of the interaction ($F_{2,44} = 1.00$; $P = 0.38$) or of the diet treatment ($F_{1,44} = 0.01$; $P = 0.97$) on the likelihood of drinking. There was however a general effect of week of age ($F_{2,44} = 4.22$; $P = 0.02$), with birds being less likely to drink at 6 weeks of age (11.11 %) compared to 8 or 10 weeks (40.74 % and 48.15 %, respectively; $P = 0.02$).

Some behaviours in the ethogram for the frustration test could not be statistically analysed due to low occurrence. These behaviours were pecking object (mean ± std dev: 1.5 ± 9.5 s), feather pecking (0.17 ± 1.05 s), preening (4.11 ± 18.5 s), comfort behaviours (0.5 ± 1.2 s) and aggression 0.2 ± 0.7 s). There were no observed occurrences of toe pecking.

3.3. *Tonic immobility*

Diet treatment did not affect the latency to first head movement in the tonic immobility test, either as a main factor (diet: $F_{1,128} = 0.28$;

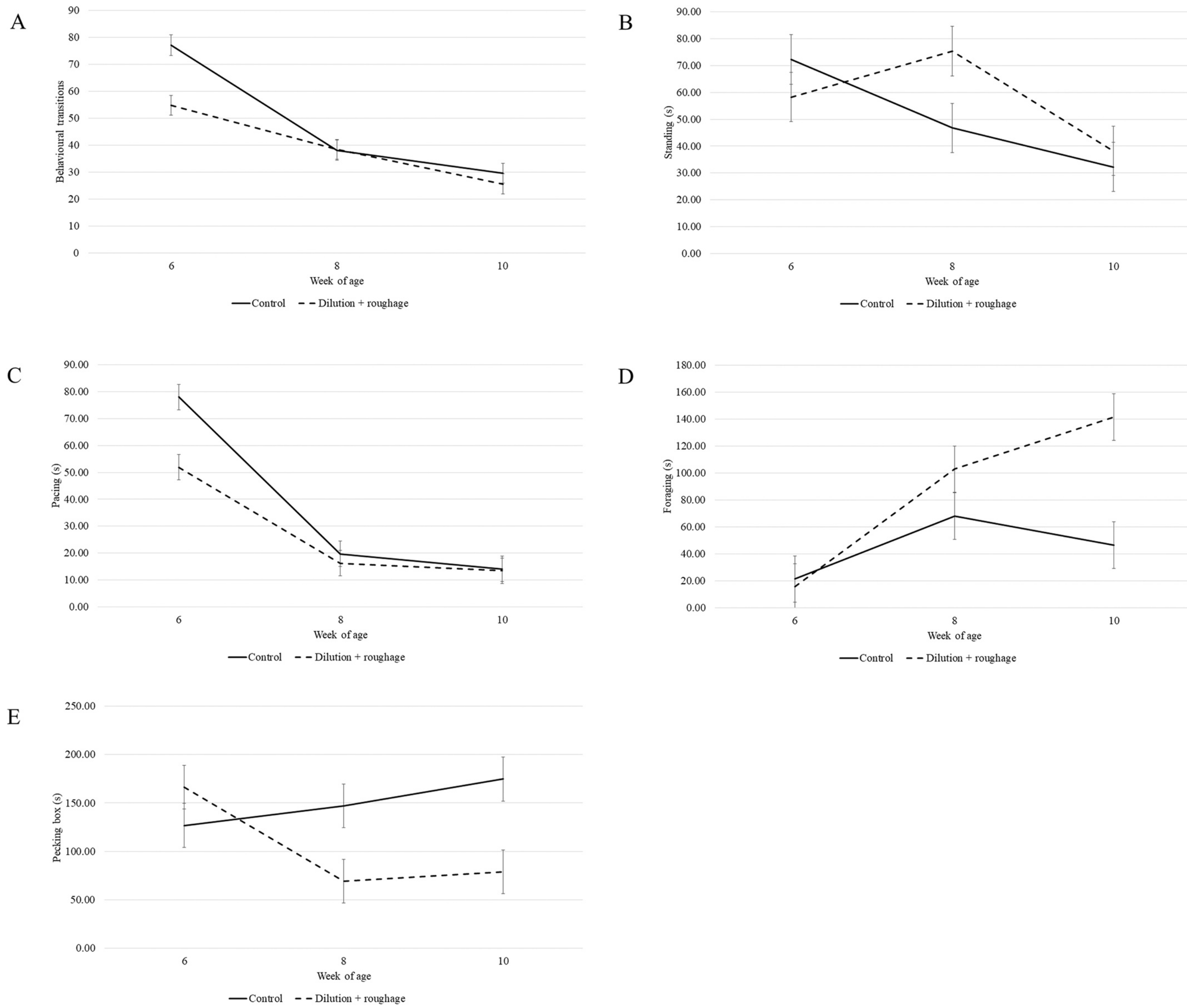

Fig. 2. Results from the behaviours assessed during the frustration test for the two diet treatments and three weeks of age (LS means ± SE). The behaviours assessed were the number of behavioural transitions (panel A), and time spent standing (panel B), pacing (panel C), foraging (Panel D) and pecking the box (panel E).

P = 0.6) or in interaction with age ($F_{2,128}$ = 0.96; P = 0.38). Control birds had a mean latency of 36.0 s ± 2.8 (LS means ± SE) while mean latency for the D+R birds was 33.9 s ± 2.7. For both diet treatments, latency tended to increase with age ($F_{2,128}$ = 2.98; P = 0.055). Regarding the duration of TI, there was no effect of diet treatment ($F_{1,120}$ = 1.58; P = 0.24), age ($F_{2,120}$ = 0.25; P = 0.77) or their interaction ($F_{2,120}$ = 0.36; P = 0.70). Mean TI duration for the Control and D+R birds was 68.8 s ± 6.9 and 56.6 s ± 6.8, respectively (LS means ± SE).

There was a tendency for an effect of diet treatment on the number of attempts necessary to induce tonic immobility ($F_{1,128}$ = 3.84; P = 0.052). Birds fed on diluted feed and roughage were more likely to require more than one attempt to induce tonic immobility compared to

Table 4
Results from the assessment of feather fault bars (F and P values, LS means and Standard Error) for the effects of diet, feather type and their interaction.

	Total		Minor		Moderate		Severe	
Diet	$F_{1,227}$ = 0.02	P = 0.66	$F_{1,115}$ = 0.03	P = 0.87	$F_{1,228}$ = 0.76	P = 0.38	$F_{1,111}$ = 0.01	P = 0.91
	LS means	**SE**	**LS means**	**SE**	**LS means**	**SE**	**%**	**Odds ratio**
Control	4.4	0.3	1.29	0.13	2.71	0.23	21.93	0.9
D+R	4.5	0.28	1.26	0.13	2.99	0.22	20.17	
Feather type	$F_{1,227}$ = 83.91	*P < 0.0001*	$F_{1,114}$ = 15.36	*P = 0.0002*	$F_{1,228}$ = 59.64	*P < 0.0001*	$F_{1,111}$ = 23.64	*P < 0.0001*
	LS means	**SE**	**LS means**	**SE**	**LS means**	**SE**	**%**	**Odds ratio**
Wing	6.3	0.3	1.62	0.12	4.1	0.22	39.17	36.22
Scapular	2.6	0.3	0.93	0.13	1.6	0.23	1.77	
Diet*Feather type	$F_{1,227}$ = 0.19	P = 0.66	$F_{1,114}$ = 0.37	P = 0.54	$F_{1,228}$ = 0.74	P = 0.39	$F_{1,111}$ = 0.00	P = 0.98

those that received the standard control feed (odds ratio = 2.4). There was no observed effect of age on this parameter of the TI test ($F_{2,128}$ = 1.31; P = 0.27).

3.4. Feather fault bars

The results for the analysis of fault bars are found on Table 4. Diet treatment did not affect any of the parameters assessed (P > 0.05). However, all the parameters differed between feather types, with wing feathers having more fault bars of all levels of severity and more total number of fault bars than scapular feathers (P < 0.05).

4. Discussion

The aim of this study was to assess the effects of a qualitative feeding restriction strategy of both feed dilution and daily provision of roughage on some indicators of hunger, stress, and frustration in fast growing Hubbard M77 broiler breeder cockerels. The results showed only limited signs of improved welfare of the qualitative feed restriction compared to the quantitative restriction.

The Novel object test measures the birds' motivation to explore a novel stimulus which can be a source of feed versus their motivation to avoid a novel stimulus which can be a source of danger (Gray, 1987). In other words, this test illustrates the approach-avoidance conflict of the birds: a long distance from the NO indicates that fear of the NO is generally higher than the motivation to explore the NO. As hunger increases, the birds' motivation to search for and acquire food starts to overpower their intrinsic fear of novelty, and so the birds engage with stimuli they would otherwise avoid. The results from the present study showed that the D+R did not significantly differ from the Control birds in their latency to approach the NO or on their distance relative to the NO in weeks 6 or 10 of age. A diet treatment difference was only found on week 8 of age, where the difference between the morning test (approx. 23 hours since the last feeding) and the afternoon test (4 hours after feeding) was larger for the D+R compared to the Control birds. However, the results also showed that the D+R birds were more likely to approach the NO in the morning than the Control birds and did not differ from the Control birds in the afternoon. Therefore, these findings introduce doubt regarding whether hunger was reduced in the few hours after feeding the D+R diet or was increased in the several hours after feeding relative to the commercial standard feed. In a recent study of Ross 308 cockerels, D+R birds did in fact differ from Control birds in the afternoon of week 10 of age, indicating a reduction of hunger at that age point (Tahamtani et al., 2024). Furthermore, the Ross 308 cockerels fed the qualitative restriction diet also tended to have longer latency to approach the NO (Tahamtani et al., 2024), which the Hubbard M77 cockerels in the present study did not show. This may suggest that qualitative restriction feeding has a higher potential to affect Ross 308 than Hubbard M77 cockerels. However, further research is needed to confirm these findings. In addition, a recent study of the effects of oat hull dilution of feed on broiler breeder pullets showed that, while some small behavioural indicators of improved welfare were detected, there was no effect of diet dilution on neuroendocrine parameters of the central control of appetite (Dixon et al., 2024). Birds fed a diluted diet did not differ from the commercially fed control birds in the expression of genes for hypothalamic neurons that drive feeding and promote energy storage (i.e. orexigenic agouti-related protein neurons) or those that inhibit feed intake and increase energy expenditure (i.e. anorectic pro-opiomelanocortin neurons). In contrast, the authors report that broiler breeders fed ad libitum had a 12 times lower gene expression for the neurons that drive feeding and a 1.5 times higher expression for the neurons that inhibit feed intake compared to control birds (Dixon et al., 2024). This suggest that a feed dilution with 40 % oat hulls, as was done by Dixon et al. (2024), or 20 %, as in the present study, does not increase gut fill enough to affect the expression of these genes and, therefore, to impact satiation. Further studies would need to confirm this.

The results from the frustration test were less controversial. The birds fed a standard restriction diet showed some signs of increased frustration compared to the D+R birds, including more behavioural transitions and more pacing behaviour and less foraging. In addition, the D+R birds showed a general reduction in time spent pecking the box with age, whereas no such reduction was observed for the control birds. Nevertheless, these results were not consistent throughout the study, with some behavioural differences being detected at different weeks of age (e.g. number of behavioural transitions, pacing, foraging, pecking at the box). It is not totally unexpected, however, that age differences may appear, considering that different challenges occur along the rearing period. Around weeks 4–8 of age, for example, is a period when farmers may observe variable feed:water ratios due to the transition from the starter feed to the grower feed, reducing light daylength, increasing water intake and changing feed allocations (James Bentley, pers. communication). In the present study, the experimental diets were introduced during week 6 and the light schedule was managed as in on-farm conditions, as described in the methods section. Unfortunately, the size of the present study, particularly the size of the pens, was too small to allow for collection of detailed data on the water consumption of the birds. Future research should aim to fill this gap.

The Tonic Immobility test, a commonly used fear test for poultry, yielded no significant differences between the two groups of birds in latency to first head movement or in the total duration of TI. The only difference between treatments observed was a tendency for the D+R birds to require more attempts to induce TI compared to the Control birds, suggesting there was little to no effect of the diet treatment on the overall affective state of the birds. In contrast, Ross 308 birds receiving the D+R diet had longer latencies for first head movement and shorter overall duration of TI but did not differ in the number of attempts to induce TI from control birds (Tahamtani et al., 2024). These divergent results further highlight the difference between the response of birds of different genetic lines, even if of similar growth rates. It is also important to note that there is an overall lack of literature on the normal behaviour of M77 broiler breeders. A simple online search yields mostly studies relating to fertility, sperm quality and other health aspects (e.g. Barbarestani et al., 2024; Colles et al., 2011). There are also studies that do not openly report the specific hybrids used in the study (e.g. Arrazola et al., 2022). There is, therefore, still a large knowledge gap in relation to less commonly used hybrids of broiler breeders, particularly the male lines.

Finally, there were no observed differences between the two diet treatments on the results from the assessment of feather fault bars. Feather fault bars, the translucent malformations perpendicular to the rachis of the feather, are often used as an indicator of acute stress events (Arrazola and Torrey, 2019). However, the results of the assessment of this indicator are, thus far, rather inconsistent. In Ross 308 pullets, while daily roughage provision reduced the number and severity of fault bars, feed dilution with 20 % oat hulls did not lead to any differences compared to control birds (Tahamtani et al., 2020). In further contrast, Ross 308 cockerels, receiving the same D+R diet as the M77 cockerels in the present study, presented more fault bars than birds fed the standard restriction diet (Tahamtani et al., 2024). This inconsistency in results is worrying, considering how similar the two birds are. In the future more focused and repeated studies on the effects of feed restriction on feather fault bars are needed.

It is also important to highlight that the cockerels in the present study and in Tahamtani et al. (2024) received pelleted feed via scatter feeding, which is the standard management strategy during the rearing period of broiler breeders in Norway. This feeding method might yield different results compared to, for example, mash feeding using troughs. Pelleted feeding became popular, particularly for broiler chickens, when it was found that growth, feed conversion and performance efficiency were favoured with pellets over mash (see for example Amerah et al., 2007; Lanson and Smyth, 1955; Savory, 1974). Scattering the pellets in the litter has been shown to increase feed intake time and reduce the time spent object pecking as the birds spend more of their time searching the litter for the feed (de Jong et al., 2005). Indeed, scatter feeding, particularly of high-value feed items, is often seen as an enrichment strategy (Pichova et al., 2016; Wood et al., 2021). However, broiler breeders that are fed mash are typically fed in feed troughs to reduce feed wastage. This is particularly important when the quality of the litter is poor as it is harder for the birds to search for and find feed, even pelleted, in wet litter (Riber et al., 2021). The larger portion of feed tested in the present study was expected to aid broiler breeder welfare not just by increasing gut fill, but also by further meeting behavioural needs for performing feeding behaviour (Riber, 2020). A larger portion size would likely contribute to meeting this behavioural need whether the feed is mash or pelleted. However, scattering it in the litter is expected to further promote this, compared to using feed troughs. It would be important, therefore, to investigate whether the results from qualitative feeding restriction strategies using scattered feeding are reproduced when mash feeding is used instead.

We conclude that a combination of feed dilution with 20 % oat hulls and daily provision of roughage did not significantly improve M77 broiler breeder cockerel welfare during rearing according to the indicators assessed here. Furthermore, the present study contributes to the growing literature suggesting that qualitative feed restriction, while promising in theory, in practice fails to deliver strong and consistent improvements to animal welfare. The results from the three behavioural tests and the assessment of fault bars performed here did not align with each other, or with the results from the previous studies on broiler breeder cockerels and pullets.

CRediT authorship contribution statement

Vasdal Guro: Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Kittelsen Kathe E.:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Tahamtani Fernanda:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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