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Qualitative feed restriction affects frustration, fear, motivation to explore, and feather fault bars in Ross 308 broiler breeder cockerels

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ABSTRACT

Broiler breeders show behaviours indicative of hunger and frustration stemming from unfulfilled behavioural needs for feeding. This is largely due to quantitative feed restriction which is common practice in the management of these birds, particularly in the rearing period. As an alternative, qualitative feed restriction allows a larger portion of feed to be provided without increasing the caloric intake. While several studies have assessed the effects of qualitative feeding on female broiler breeders, no such assessment has been done on broiler breeder cockerels. The aim of this study was to investigate the effects of feed dilution and roughage on the level of stress, fear and motivation to explore of broiler breeder cockerels. 200 Ross 308 broiler breeder cockerels (between 5 and 10 weeks of age) were housed in 12 pens (6 pens/treatment), 17 birds/pen. The treatments were standard feed (Control) and feed diluted with 20 % insoluble oat hulls and 150 g of alfalfa roughage daily/pen (D+R). The D+R birds received 20 % more feed per day. Novel object (NO) and frustration during thwarted feeding tests were performed in the home pen and the response recorded with video cameras. In addition, four birds from each pen were subjected to a tonic immobility test (TI). All behavioural tests were performed once a week at 6, 8 and 10 weeks of age. Finally, after euthanasia, two feathers from each bird were plucked and macroscopically examined for the presence of fault bars. The control birds showed a tendency to approach the NO faster than the D+R birds ($P = 0.07$) and were more likely to approach at 10 weeks of age ($P = 0.006$). In the frustration test, D+R birds spent less time pecking the feed container ($P = 0.049$), more time standing ($P = 0.01$) and tended to have fewer behavioural transitions ($P = 0.09$) than control birds, which indicates a reduction in frustration levels. In addition, Control birds stayed in TI longer than D+R birds (160.7 ± 15.5 s and 98.1 ± 15.4, respectively. $P = 0.005$). However, D+R had more fault bars compared to Control birds ($P = 0.02$), highlighting that care is needed to interpret these contradicting results. Nevertheless, the results show that the combination of feed dilution and daily roughage can have positive effects on the welfare of broiler breeder cockerels by reducing the sensation of hunger as indicated by fear, frustration, and motivation to explore.

1. Introduction

Broiler breeders, the parent stock of broiler chickens, are routinely kept under quantitative feed restriction, particularly during the rearing period, in order to ensure a healthy weight and reproductive success (de Jong et al., 2002). The birds are fed a smaller amount of feed compared to the ad libitum intake in order to limit the energy consumption and therefore, regulate the weight and body development of the birds. These birds have a growth potential similar to that of their offspring and therefore cannot be fed ad libitum because too high body weight compromises egg production (McDaniel et al., 1981). However, the feed restriction, which can be as severe as 20–25 % of ad libitum intake (Arrazola, 2018; de Jong et al., 2002), often results in hunger, stress, and frustration indicated by increased foraging behaviour, plasma corticosterone concentration and stereotypies (Sandilands et al., 2005). As an alternative, qualitative feed restriction is expected to have positive effects on welfare. The idea is that feed can be diluted with non or low-nutritious fibres which allow for a larger portion of feed to be provided without increasing the caloric intake (Sandilands et al., 2006). The expectation is that qualitative feed restriction can cause satiety in the broiler breeders, as it increases feeding time and intestinal content and the passage time of feed is prolonged (Hocking et al., 2004; Steenfeldt and Nielsen, 2012). However, the results are often conflicting (Sandilands et al., 2006; Savory and Lariviere, 2000). For example,

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Riber et al. (2021) found that dilution using insoluble fibres did not affect time spent foraging in the home pen. Furthermore, the authors suggest that the observed reduction of foraging time on birds fed a diet diluted with both soluble and insoluble fibres was likely due to the unexpected deterioration of the litter quality rather than an actual melioration of hunger (Riber et al., 2021).

The relationship between hunger and a chicken's motivation to avoid fear inducing stimuli and explore novel resources has been examined in previous studies. For example, hunger leads to a strong urge to explore newly available areas in search of food even when these areas were previously associated with severe feed restriction (Dixon et al., 2013). In addition, feed restricted broiler breeders are two times more likely to cross a water barrier, something typically avoided by chickens, than birds fed two or three times this amount (Dixon et al., 2014). In addition, risk taking behaviour such as approaching a novel object (NO) in the home pen is increased in broiler breeders fed on a skip-a-day schedule compared to birds fed daily (Lindholm et al., 2018). However, conflicting results are also found in these fear studies. For example, Hocking et al. (1996) found feed-restricted broiler breeders to be less fearful than *ad libitum* fed birds following a tonic immobility (TI) test. In contrast, (Tahamtani and Riber, 2020) found no differences in responses during the TI test between birds fed quantitative versus qualitative restrictive diets.

Feeding regime has also been shown to affect feeding motivation, physiological stress parameters and even feather characteristics such as regrowth weight and length as well as the occurrence of fault bars (Arrazola et al., 2019; de Jong et al., 2002; Riber and Tahamtani, 2020; Tahamtani et al., 2020). However, these results are also often conflicting and/or display minimal effects. In addition, it is important to note that most of the previous research on the effects of qualitative feed restriction on broiler breeders has been performed on broiler breeder pullets or hens. There is, therefore, a large knowledge gap on the effects of feed dilution and daily roughage on broiler breeder cockerels and roosters. In commercial conditions, pullets and cockerels are housed separately during rearing (up to week 15 of age) and are only mixed housed during the laying period. Therefore, it is not impractical for them to be fed different diets.

The aim of the present study was to investigate the effects of the combination of feed dilution with oat hulls and a daily provision of alfalfa roughage on several behavioral indicators of welfare in broiler breeder cockerels. The control diet (Control) was a standard feed for broiler breeders. The behavioural indicators assessed were the level of fear and motivation to explore measured in a TI test and a NO test, frustration response during thwarted feeding and feather fault bars. We hypothesized that the birds receiving a standard control diet would spend less time in TI and keep a shorter distance to the NO in the NO test. Furthermore, we expected Control birds to show more signs of frustration during thwarted feeding, such as more time pecking the feed box, pacing and higher number of behavioural transitions than the birds receiving the diluted feed. Finally, we expected the Control birds to have more feather fault bars than the other birds. This study was part of a larger study, comparing the effects of a combination of qualitative feeding restriction and daily roughage on a range of other parameters including behaviour in the home pen (Vasdal et al. in prep) and health (Kittelsen et al., 2023) of broiler breeder cockerels.

2. Materials and methods

2.2. Animals and housing

200 Ross 308 broiler breeder cockerels from 5 to 10 weeks of age were used in this study and housed as in Kittelsen et al. (2023). The chicks were hatched at a commercial hatchery, sex sorted by vent sexing, toe clipped, and reared at a commercial rearing farm until week 5 of age. The birds were not beak trimmed. In the commercial rearing farm, 1100 cockerels and 8200 pullets were housed in separated compartments. The farm was insulated with mechanical ventilation, and wood shavings covering the concrete floor. All management during rearing followed recommendations of the breeding company and Norwegian regulations (Landbruks- og matdepartementet, 2006). At 5 weeks of age, 200 cockerels were caught at the commercial farm by trained poultry catchers, crated in transport containers, and transported 2 hours in a climate-controlled truck to the animal husbandry experimental research facility, Centre for Husdyrforsøk, at the Norwegian University of Life Sciences. The cockerels were selected based on a live weight close to the Ross breeding manual's weight recommendations at that age.

At the experimental facilities, the birds were housed in 12 pens measuring 296 × 60 × 71 cm (length × height × depth) in groups of 16 or 17 birds per pen. While Aviagen recommends a stocking density of 3–4 birds/m^2 for males during rearing (Aviagen, 2023), Norwegian law allows for up to 15 birds/m^2 (Landbruks- og matdepartementet 2006). Therefore, in our study, we used a mean density of 8 birds/m2, which is commonly used in practice in Norwegian rearing farms. All pens were located in the same room and contained wood shavings as litter and a 70 cm jute rope (ø: 20 mm) hanging by the middle (i.e., 2 tail ends side by side) from the ceiling of the pen as environmental enrichment. Water was provided ad libitum during the day and night period from nipple drinker lines (4 nipples per pen). Ventilation, humidity, temperature, and lighting followed the recommendations of the Ross parent stock management handbook (Aviagen, 2023) and were consistent across pens. The light period was 8 h (from 08:00–16:00) at 10 lux. After arriving in the experimental facility, the birds were allowed 3 days of habituation before the start of the study. During the first day of habituation, all cockerels received the same feed as they had received in the rearing farm. The provision of the treatment diets started on the third day following the birds' arrival.

2.3. Experimental treatments

The 12 pens were assigned to 1 of 2 diet treatments (6 pens per treatment) also taking into account potential differences in the physical conditions in the room such as variations in temperature, humidity, activity by the door vs the back of the room, etc. With this in mind, the room was set up with 4 rows of pens, 3 pens in each room. The control and trial pens alternated throughout the room and both treatments were represented in each row. The two treatments were Control and Dilution + Roughage (D+R). The control concentrate was formulated according to the nutritional specifications of a commercial diet for the rearing period. The composition of the raw materials was optimized to be as similar as possible between the control and diluted concentrates to avoid any effects of the raw materials. The D+R concentrate diluted the control concentrate with 20 % oat hulls, which reduced the metabolizable energy (ME) and digestible amino acid content by 1/5 and resulting in 20 % higher feed allowance per bird per day (Table 1). Pellet size for both concentrates was 2.50 mm. The pelleted feed was given once per day, at 09:00 to all pens by scattering on the floor of the pen. In addition, the D+R pens received 150 g lucerne/alfalfa roughage per pen per day always 15 minutes after the pelleted feed was given. The roughage was placed in metal hay feeders attached to the side of the pens. Feed amounts allocated per bird in the control group were based on Norwegian growth curves for broiler breeder cockerels and recommendations from the breeding company (Aviagen, 2023).

2.4. Data collection

2.4.1. Novel object test

At 6, 8 and 10 weeks of age, the effect of the diet treatments on the explorative behaviour of the birds in the home pens was investigated with the use of the Novel Object test. On test days, a NO test was performed one hour before feeding (8:00) and again 4 hours after feeding (13:00). A novel object, which differed regarding shape and colour every time, was placed inside the pens by the back corner near the door of the

Table 1
Diet composition information for the experimental diets provided. From (Kittelsen et al. (2023).

	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	0–5	11.80	168	4.77	3.06	12.73	15 – 61
Grower							
Control	6 – 10	11.20	135	4.93	2.59	15.59	62–78
Diluted	6–10	9.10	113	10.97	2.72	28.86	74–94
Alfafa	6–10	3.58	168	27.10	Total NSP 55 %		

[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.

pen, and the response of the birds recorded from that moment using video cameras (Sony Handycam HDR-CX405) attached to the outside of a neighbouring pen, for 10 minutes. After the 10 minutes were completed, the NOs were removed, and the cameras taken away for retrieval of the video files. The two objects presented on the same day had approximately the same size. The NOs were: 75 mm diameter white plastic balls, 135 mm yellow plastic doorstoppers, 15 cm tall green toy plastic rakes, 330 ml coca cola aluminium cans, 21 cm diameter blue plastic frisbee, 30.5 cm tall orange plastic cones. Three pens within each treatment were exposed to one of the objects during the morning test and the other object during the afternoon test and vice versa. During the video analyses, each pen was divided into four areas on the screen. Zone 1 was the NO itself and was recorded when the birds were in physical contact with the NO. Zones 2–4 were the third of the pen (99 cm x 71 cm each) closest, next closest, and furthest from the NO. Approach/avoidance responses were scored every 30 s for the first 5 minutes and every 1 min for the last 5 min by counting the number of birds in each of the 4 zones. The latency for the first bird to touch the NO was recorded.

2.4.2. Frustration test

Frustration during thwarted feeding was assessed 1 day per week at 6, 8 and 10 weeks of age, at 11:00 between the first and second novel object test described above. The test was performed in the home pen. At the start of the test, a plastic transparent box filled with feed and covered with a plastic transparent lid was placed in the home pen. The feed used was the respective feed for each diet treatment, but while the birds could see the feed inside the box, they could not assess it. For 3 days prior to this test, the birds were allowed to habituate to the empty box, which was removed from the home pen the afternoon before the testing day. The behaviour response of the birds to the box filled with feed was video recorded (Sony Handycam HDR-CX405) for 5 minutes. From the video recordings, focal animal sampling using continuous recording was done for 2 randomly selected birds using a predetermined ethogram (Table 2) and using the event-logging software BORIS (Friard and Gamba, 2016). From these data, the total duration spent on each behaviour and the number of transitions between different types of behaviour were obtained from each test bird. Increased occurrence of behavioural transitions is an indicator of frustration (Roper, 1984; Tinbergen, 1951).

2.4.3. Tonic immobility

The effect of the different diets on the fear response of the birds was assessed with the TI test 1 day per week at 6, 8 and 10 weeks of age. A total of 4 birds per pen, resulting in 24 birds per treatment, were tested. The testing order was completely randomised in each of the testing days. The test was performed by two observers between 10:00 and 14:00 h to not interfere with the feeding or the light schedule. The number of birds tested by each observed from each pen was balanced to preclude any effect of observer. To perform the test, a bird was pseudo-randomly selected and carried in the arms of the observer from the home pen to an adjacent room. To avoid disturbing each other, the observers performed the test in opposite ends of the testing room (approx.10 m apart). Tonic immobility was induced by placing the birds on its back on a V-shaped wooden cradle. The observer held the bird in place with on hand on its chest and the other covering the bird's head for 10 s, at which point the observer slowly removed both hands. If the bird righted itself within 5 seconds, the observer attempted to induce again. A maximum of 3 inductions were tried. If a state of TI could not be induced, the bird was returned to the home pen and excluded from the TI duration dataset. Once TI was induced, the observer stood nearby, within sight of the bird but without holding direct eye contact and recorded the latency to perform head movements and the total duration of TI, i.e., until the bird righted itself. Tonic immobility was terminated by the observer if it lasted for longer than 10 minutes. Upon completion of the test, the bird was returned to its home pen.

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

Behaviour	Description
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*).
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 to 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.

2.4.4. Feather fault bars

At 10 weeks of age, the birds were all culled and subjected to a postmortem assessment, the results of which are presented in (Kittelsen et al., 2023). Stunning was performed by blunt trauma to the head followed by euthanasia with cervical dislocation. Stunning was performed one pen at a time on two consecutive days. Immediately after killing the birds, two feathers were plucked from each bird: left primary 8 (P8, the third outmost flight feather), and left scapular 1 (Sc1, central scapular feather)(Arrazola and Torrey, 2019). If the left side feathers were very dirty (for example with blood from the culling), right side feathers were taken instead. The plucked feathers were placed in a plastic bag per bird and the bird ID and pen was noted. The bags with feathers were kept in a freezer (-5°C) for later examination. After thawing, all feathers were macroscopically examined by a single blind observer for the presence of translucent lines perpendicular to the rachis of the feather (i.e., fault bars) by holding them under light and with the aid of a magnifying glass (x 2.25 magnification). The faults were categorised according to length and severity: minor (< 5 mm), moderate (≥ 5 mm), and severe (≥ 5 mm and broken barbules on the fault bar) (Arrazola and Torrey, 2019). Broken and very dirty feathers were excluded from examination.

2.5. Statistical analysis

Statistical analyses were performed using the software SAS 9.4 (SAS Institute Inc., Cary, NC). The data on the latency to approach the novel object in the NO test were analysed with the mixed procedure, using diet treatment, week of age, time of day and their interactions as dependent variables and pen as a random effect. The data on the number of birds in each zone during the NO test were analysed per week using the glimmix procedure with an ordered multinomial distribution, diet treatment, time of day and their interaction as dependent variables and pen as a random effect. Bonferroni corrections were used to adjust the value of the critical alpha according to the number of pairwise comparisons between diet treatment and time of day (i.e., Bonferroni corrected critical alpha = 0.0125).

The data from the frustration test were analysed using the mixed procedure with diet treatment, week of age, and their interaction as dependent variables. The focal bird nested in pen was added as a random factor. This model was used for the following behaviours: pecking the box, standing, pacing, foraging and total behavioural transitions. Drinking behaviour was first analysed using a dichotomous Yes/No variable and a binomial model with logit link function using the glimmix procedure. Consequently, the data points when drinking was performed (performed for > 0 s) where analysed using the same mixed model described for the other behaviours. The occurrence of pecking object, feather pecking, toe pecking, preening, comfort behaviours, and aggression during the frustration test was too low to analyse statistically. These results are therefore, presented as descriptive statistics.

The data for the latency to perform head movements and total duration of tonic immobility in the TI test were analysed with a mixed procedure using diet treatment, week of age and their interaction as dependent variables, and pen as a random factor. The likelihood of needing more than 1 induction to achieve TI was analysed using the glimmix procedure with a binomial distribution, logit link function and the same dependent variables and random factors.

Finally, the data on the number of minor, moderate, and total number of feather fault bars were analysed with mixed models using diet treatment, feather type and their interaction as dependent variables and bird ID nested in pen as a random factor. The occurrence of severe fault bars was analysed using the glimmix procedure with a binomial distribution, logit link function and the same dependent variables and random factors as described above.

Backward stepwise reduction of the model was performed by removing higher order insignificant interactions for all models described above. Unless otherwise described above, post hoc pairwise comparisons were performed with the Tukey's test (Tukey's HSD test).

3. Results

3.1. Novel object test

There was a tendency for an effect of treatment on the latency to approach the Novel Object ($F_{1,9}$ = 4.09; P = 0.07), with the average latency for control birds being 30.3 s ± 14.8 (LS means ± SE) whereas the birds in the D+R group had an average latency of 73.2 s ± 15.1. While there was only a tendency for an effect of the interaction between week of age and time of day ($F_{2,52}$ = 2.85; P = 0.07), there was a significant effect of week of age ($F2_{,52}$ = 13.32; P < 0.0001). All birds, regardless of diet treatment, took significantly longer to touch the NO at 8 weeks of age (LS means ± SE: 112.2 s ± 16.1) compared to week 6 (24.9 s ± 15.5) and week 10 of age (17.5 s ± 15.5). As a main factor, time of day did not affect latency to touch the NO ($F_{1,52}$ = 0.12 = P = 0.73).

At 6 weeks of age, there was no effect of the main factors treatment ($F_{1,5893}$ = 0.12; P = 0.73), or time of day ($F_{1,5893}$ = 0.54; P = 0.46) on the distribution of the birds in the zones of the Novel Object test (Fig. 1A). Similarly, there was no effect of treatment ($F_{1,5863}$ = 0.25; P = 0.61) or time of day ($F_{1,5863}$ = 1.11; P = 0.29) on the NO results from week 8 of age (Fig. 1B). However, at 10 weeks of age we observed an effect of the interaction between treatment and time of day ($F_{1,5774}$ = 45.65; P < 0.0001). While there was no difference between the treatments in the morning (i.e. before feeding; P > 0.012), in the afternoon (i.e. 4 hours after feeding) the control birds were more likely to approach the NO compared to the birds which received the experimental diet (estimated odds: 0.59; P = 0.006; Fig. 1C).

3.2. Frustration test

There was a tendency for birds in the Control diet group to perform more behavioural transitions than the birds fed the D+R diet ($F_{1,22}$ = 3.07; P = 0.09). There was also an effect of age ($F_{2,42}$ = 4.48; P = 0.02), with the number of behavioural transitions during the frustration test decreasing from 6 to 10 weeks of age (P = 0.01; Fig. 2A).

There was an effect of treatment on the time birds spent pecking at the box during the frustration test ($F_{1,22}$ = 4.32; P = 0.049), with the Control birds spending more time on this behaviour compared to the birds fed the D+R diet (LS means ± SE. Control: 147.7 s ± 15.3. D+R: 103.2 s ± 14.9). There was no effect of age ($F_{2,43}$ = 0.75; P = 0.47), or the interaction between treatment and age ($F_{2,43}$ = 2.32; P = 0.11).

There was also an effect of diet treatment on the time spend standing ($F_{1,22}$ = 7.70; P = 0.01) with birds in the D+R group spending more time standing (LS means ± SE: 76.3 s ± 6.8) than those in the Control group (LS means ± SE: 49.1 s ± 7.0). There was no effect of age ($F_{2,43}$ = 0.21; P = 0.81) on time spent standing.

Time spent pacing was affected by the interaction between diet and age ($F_{2,40}$ = 4.32; P = 0.02). As can be seen on Fig. 2B, the time pacing for the control birds decreased from week 6 to weeks 8 and 10 of age (P < 0.003), whereas this behaviour was consistent in the treatment birds and did not differ from week to week (P > 0.05). The time spent pacing did not differ between the treatment groups at any age (P > 0.05). Time spent foraging was not affected by diet treatment ($F_{1,64}$ = 0.23; P = 0.63), age ($F_{2,64}$ = 1.70; P = 0.19) or their interaction ($F_{2,64}$ = 1.21; P = 0.30).

There was no effect of diet treatment ($F_{1,42}$ = 0.06; P = 0.80), week of age ($F_{2,42}$ = 1.26; P = 0.29) or their interaction ($F_{2,42}$ = 2.59; P = 0.09) on the likelihood to drink during the frustration test. Similarly, there was only a tendency for an effect of treatment on the time spent drinking ($F_{2,17}$ = 17.6; P = 0.06), where any significant differences were lost during pairwise comparisons (P > 0.05).

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: 0.48 s ± 1.33), feather pecking (0.11 s ± 0.49), toe pecking (0.04 s ± 0.22), preening (4.6 s ± 13.59), comfort behaviour (0.75 s ± 1.44), and aggression (0.99 s ± 2.83).

3.3. Tonic immobility

There was no effect of the interaction between treatment and age on the latency to first head movement under the tonic immobility test ($F_{2,132}$ = 2.82; P = 0.06). There was however an effect of dietary

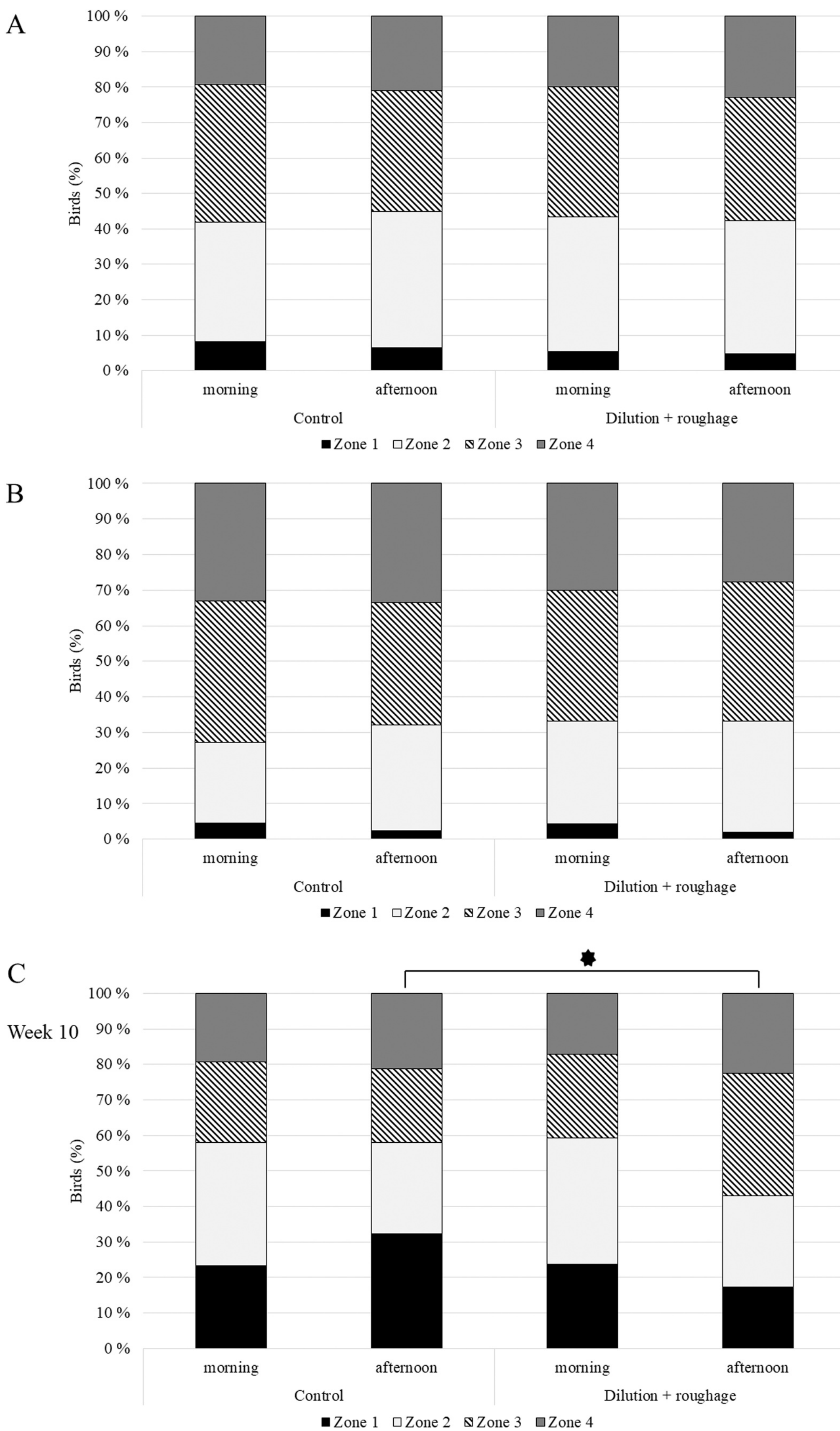

Fig. 1. Distribution of the birds in each of the four zones (%) during the novel object test at 6, 8 and 10 weeks of age (panels A, B and C, respectively). Higher zones are further away from the novel object.

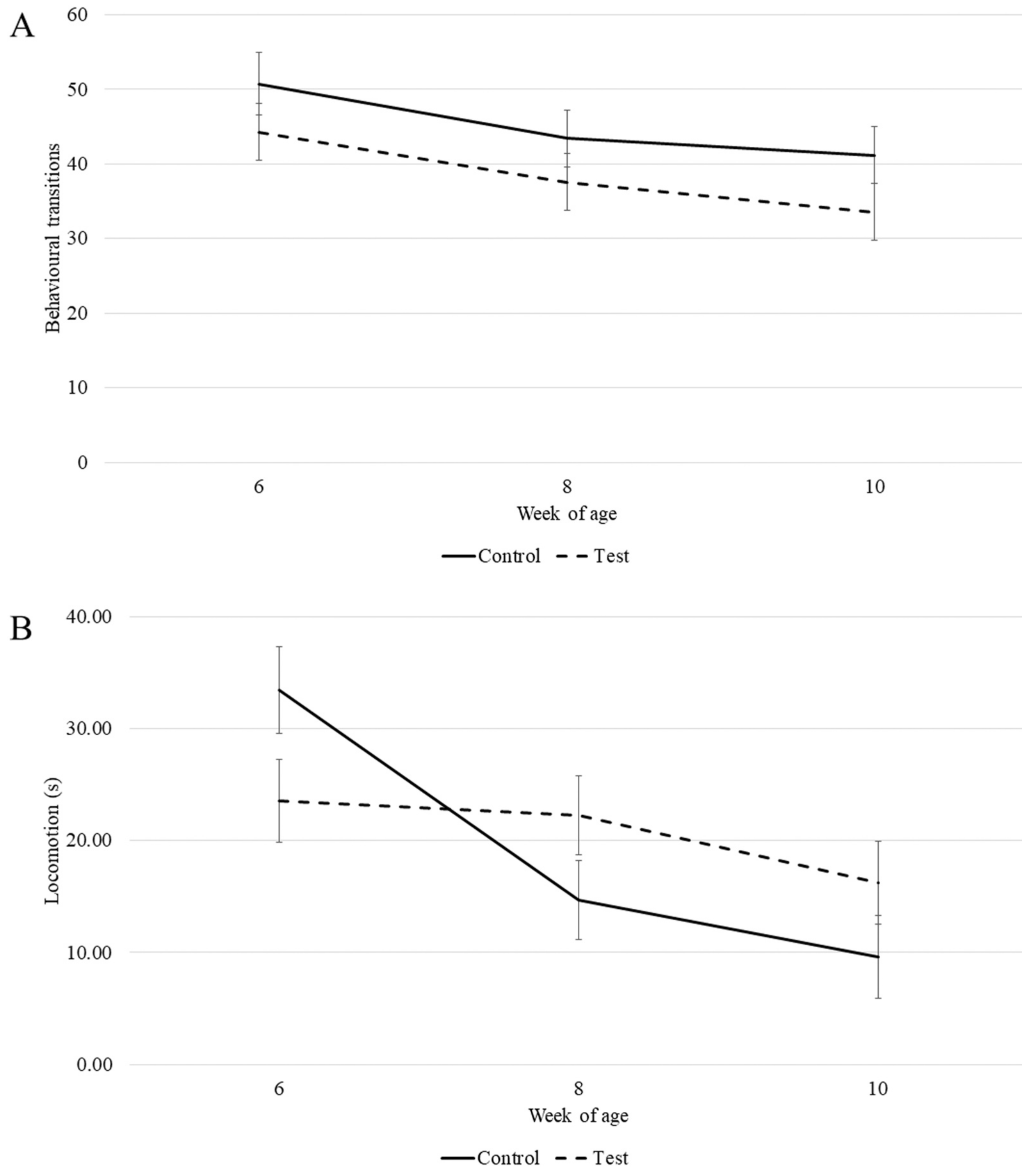

Fig. 2. Number of behavioural transitions (panel A) and time spent in locomotion (panel B) for the two diet treatments and three weeks of ages during the frustration test (LS means ± SE).

treatment on this latency ($F_{1,132}$ = 9.31; P = 0.0028), with a higher mean latency for the birds in the control group (LS means ± SE = 50.04 ± 3.45 s) compared to birds in the treatment group (LS means ± SE = 35.12 ± 3.46 s). In addition, the results show a general effect of week of age irrespective of diet ($F_{2,132}$ = 3.92; P = 0.02) with latency increasing with age when comparing the first TI test at week 6 (LS means ± SE: 34.66 s ± 4.19) with the last one at week 10 of age (LS means ± SE: 54.39 s ± 4.28).

Similarly, there was no interaction effect on the duration of TI ($F_{2,137}$ = 2.17; P = 0.12), but there was a general effect of diet ($F_{1,137}$ = 8.22; P = 0.005). Birds fed the standard control diet stayed in TI for an average of 160.7 ± 15.5 seconds (LS means ± SE) while the birds fed the treatment diet of diluted pellets and roughage stayed in TI for an average of 98.1 ± 12.4 seconds (LS means ± SE). An effect of age across both diet treatments was also observed for duration of TI ($F_{2,137}$ = 5.58; P = 0.005) with the duration increasing from week 6 of age (LS means ± SE: 79.44 s ± 18.83) to weeks 8 and 10 (P < 0.05; LS means ± SE: 164.94 s ± 18.83 and 143.78 s ± 19.03, respectively).

There was no significant effect of the interaction between diet and age ($F_{2,128}$ = 0.06; P = 0.04), or of the main effects diet ($F_{1,128}$ = 0.43; P = 0.51) and age ($F_{2,128}$ = 0.06; P = 0.94) on the number of inductions to successfully achieve tonic immobility.

3.4. *Feather fault bars*

The results of the assessment of feather fault bars are presented on Table 3. An effect of the diet treatment was only detected on the total number and on the number of minor fault bars, with control birds presenting fewer fault bars than the birds in the D+R group. An effect of feather type was however found for all types of bars (minor, moderate and severe) as well as the total number of fault bars. In all cases, wing feathers presented more fault bars than scapular feathers. There was no interaction effect between diet treatment and feather type in the fault bars assessment.

4. Discussion

The purpose of this study was to examine the effect of a qualitative feed restriction strategy on behavioural and physiological indicators of hunger, fear and stress on male broiler breeders during rearing. In the

Table 3
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	$F_{1,116} = 5.95$	*P = 0.02*	$F_{1,234} = 6.67$	*P = 0.01*	$F_{1,117} = 2.94$	P = 0.09	$F_{1,116} = 1.72$	P = 0.19
	LS means	**SE**	**LS means**	**SE**	**LS means**	**SE**	**%**	**Odds ratio**
Control	5.60	0.35	1.53	0.14	3.47	0.29	53.95	0.60
D+R	6.81	0.35	2.04	0.14	4.18	0.29	46.05	
Feather type	$F_{1,115} = 58.72$	*P < 0.001*	$F_{1,234} = 7.62$	*P = 0.006*	$F_{1,117} = 32.27$	*P < 0.0001*	$F_{1,116} = 43.90$	*P < 0.0001*
	LS means	**SE**	**LS means**	**SE**	**LS means**	**SE**	**%**	**Odds ratio**
Wing	7.95	0.33	2.06	0.14	4.80	0.26	86.84	14.40
Scapular	4.47	0.34	1.51	0.14	2.90	0.27	13.16	
Diet*Feather type	$F_{1,115} = 0.01$	P = 0.91	$F_{1,234} = 0.98$	P = 0.32	$F_{1,117} = 0.40$	P = 0.53	$F_{1,116} = 0.95$	P = 0.33

Novel Object test, the birds in the Control group were expected to engage in more risk prone behaviour due to their increased level of hunger and approach the NO more and faster than the birds in the D+R group (Lindholm et al., 2018). The results presented here did follow the expected trend to some degree. Control birds tended to approach the NO faster than the birds in the D+R group. Furthermore, Control birds were more likely to be close to the NO than D+R birds in the afternoon test (i.e., 4 hours after feeding) compared to the morning test (i.e., 23 hours since the last feeding). This was as predicted, as the birds in both groups were expected to be hungry in the morning before the daily feeding, whereas the level of hunger was predicted to be lower in the afternoon particularly in the D+R birds which received a larger portion of food. However, these results were only observed at 10 weeks of age, and not at 6 or 8 weeks. The proportion of birds, from both diet groups, in zone 1 of the NO test was larger in week 10, with an average of 24 %, compared to weeks 6 and 8 (average of approx. 6 % and 3 % respectively). This can reflect an increase in hunger level within these weeks. Indeed, feed restriction in cockerels is strictest from week 8–15 of age (Aviagen, personal communication), which supports the notion that the control birds were hungrier after week 8 of age and therefore a difference between the two groups was only noted in week 10. One might also suspect that the size of the birds could have an effect here. The birds get larger with age, and the D+R birds tended to be heavier than the Control birds (Kittelsen et al., 2023). This might have resulted in more birds closer to the NO simply due to reduced space allowance. However, this is unlikely as the diet effect observed was only in the afternoon of week 10. If bird size was a confounding factor, one would expect the same results in the morning of that same day. Another possible, though less likely, explanation is that the birds became habituated to the test set up, even though a new novel object was used for every test. Nevertheless, the increased likelihood of control birds to be closer to the NO in the afternoon of week 10 compared to D+R, does suggest an alleviation of hunger in the D+R birds up to at least 4 hours after feeding.

Interestingly, the results from the Tonic immobility test are in the opposite trend. Control birds spent more time in TI compared to D+R birds. TI is known to be an anti-predator response where birds perform a "death-feigning" behaviour (Thompson et al., 1981). It can, therefore, be expected that a reduction in the duration of TI may signify a higher propensity towards risk taking instead of avoiding a potential predator (Forkman et al., 2007). Indeed, previous studies of broiler breeder pullets found feed-restricted birds to spend less time in TI compared to ad libitum-fed birds (Hocking et al., 1996; Savory et al., 1993). On the other hand, TI is known to increase with the level of stress (Jones, 1992; Zulkifli and Azah, 2004). Therefore, a lower TI duration in this context may instead signify an overall improvement of the affective state, and welfare, of broiler breeder cockerels under qualitative feeding restriction. There are also important differences between these two fear tests, the Novel Object and Tonic Immobility test, that suggest that different responses may be expected. The NO test measures fear of novelty in the home environment whereas the TI test, as mentioned before, measures fear responses following capture, restraint, and transport by a perceived predator. It can be suggested that the risk of approaching a NO in the home environment is perceived as low enough that the hungrier Control birds face that risk in the chance of finding a novel source of feed. In comparison, in a predation context, it is possible that the D+R birds are less hungry, and therefore less stressed and in a generally more positive affective state, and for this reason perceive the TI test as less fear inducing. There is, therefore, a need for caution when interpreting TI results in this context.

The results of the frustration test support this suggestion that the D+R birds were less hungry and less stressed than the Control birds. In the face of thwarted feeding, the D+R birds spent less time pecking the lidded feed box and more time standing than the Control birds. In addition, D+R birds tended to perform fewer behavioural transitions, an indicator of restlessness and frustration. The D+R birds received a larger portion of feed than the Control birds, which means that they ate more and for longer, both of which likely led to a decrease in hunger during the frustration test. However, pacing behaviour, another well-established indicator of frustration, was not affected by dietary treatment. Perhaps the relative restrictive dimensions of the home pens had an effect on this as the birds had 4 times less room to move along one axis of the pens compared to the other.

The results of the assessment of feather fault bars showed more fault bars in the feathers of D+R birds compared to Control birds. This direction of effect was also seen when considering only the minor fault bars (< 5 mm), but not when considering the moderate and severe faults (≥ 5 mm and ≥ 5 mm with broken barbules on the fault bar, respectively). Feather fault bars, the translucent malformations perpendicular to the rachis of the feather, have been suggested as an indicator of welfare in broiler breeders. Arrazola and Torrey (2019) showed that exposure to acute unpredicted stress increased the number of fault bars in broiler breeder pullets. In another study, broiler breeder pullets fed a daily portion of maize roughage had fewer and less severe fault bars compared to control birds fed the conventional restrictive diet (Tahamtani et al., 2020). This could be due to a sex effect due to different energy partitioning between broiler breeder males and females. For example, female birds dedicate a lot of energy towards egg production (van Emous et al., 2020). Likewise, it is possible that cockerels and pullets have different energy budgets for feathering. Nevertheless, the results of the present study are somewhat surprising, especially considering the results from the other parameters presented here. Fault bars are a result of acute stress events: punctuate phenomena triggered by factors occurring during a short period of time (see review by Jovani and Rohwer, 2017). The best accepted hypothesis for the mechanisms of fault bar formation is a sudden contraction of the musculature around the soft feather follicle during feather growth (Jovani and Rohwer, 2017; Maderson et al., 2009). Examples of such events are capture, handling and predation (King and Murphy, 1984). As such, it can be understood that feather fault bars are indicators of acute stress events whereas the NO, TI and frustration tests are perhaps better indicators of more holistic/chronic stress. Further studies on the relationship between fault bars and other fear responses are needed. It is important to note also that the current study was performed only between weeks 5 and 10 of age of the cockerels, whereas many broiler breeder pullet studies such as Tahamtani and Riber (2020) were performed for the entire rearing period. Perhaps a clearer picture of the effects of qualitative feeding restriction on broiler breeder cockerels would be found in longer studies of this paradigm.

As briefly touched upon in the introduction, the literature on the effects of qualitative feeding restriction on broiler breeders is often contradictory. The actual source of so much contradiction is not known, but the present authors suggest that, overall, the positive effects of qualitative restriction in the levels researched thus far are minimal and therefore less consistent. We expect that, for larger and more consistent effects to be detected, the qualitative diet must be less restricted than is has been tested so far, with for example the combination of feed dilution and roughage seen here.

5. Conclusion

This study presents the first results on the effects of a qualitative feed restriction diet on the behaviour of broiler breeder cockerels. As mentioned earlier, previous research on qualitative feed restriction has focused on the female birds, or pullets, with cockerels being overlooked. The present study begins to close that knowledge gap, suggesting that a diet including feed dilution and daily roughage can have positive effects of welfare as observed via the behavioural indicators of hunger and fear investigated here. This diet seems to cause a longer period of satiety compared to a conventional diet resulting in lower frustration during thwarted feeding and lower motivation to explore in a novel object test. This diet also caused a reduction in time spent in tonic immobility, which may suggest lower stress and an overall better affective state of these birds compared to the control birds. Nevertheless, care is required when interpreting the results from the assessment of feather fault bars, as the present results were somewhat contradictive in relation to the other tests reported here and the previous literature. More research on the relationship between these welfare parameters and on the effects of feed dilution on broiler breeder cockerels is needed.

Ethical statement

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

CRediT authorship contribution statement

Kathe E Kittelsen: Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Guro Vasdal:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Fernanda Tahamtani:** 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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