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Motivation for feeding in broiler breeder pullets fed different types of restricted high-fibre diets

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ABSTRACT

High-fibre diets have been suggested as alternative feeding strategies that potentially may alleviate the hunger felt by feed-restricted broiler breeders and fulfil their behavioural need for feeding behaviour. The aim of the present study was to investigate the effects of four dietary treatments, differing in fibre types and content, on the motivation for performing feeding behaviour, including both the appetitive and consummatory phase, in broiler breeder pullets. In total, 1200 female breeder chicks of the genotype Ross 308 were housed in 24 pens, six pens of initially 50 birds per treatment. The dietary treatments were: 1) standard feed (Control), 2) feed containing insoluble fibres (Insoluble), 3) feed containing a mix of insoluble and soluble fibres (Mixed) and 4) standard feed supplemented with maize silage (Roughage). Four measures of feeding motivation were obtained: feeding rate, behaviour indicating frustration during thwarted feeding, compensatory feed intake over 5 days and motivation to gain access to fresh litter. The latter was performed at 12–13 weeks of age, whereas the other measures were obtained both at 8–9 and 17–18 weeks of age. Litter quality in the home pens was assessed at age 13 weeks and dry matter content at 5, 13 and 14 weeks of age. Feeding rate was not affected by treatment ($P = 0.26$). Insoluble and Mixed birds showed fewer behavioural transitions, i.e. changes in activity, during thwarted feeding at 17/18 weeks of age than Control birds ($P = 0.004$), indicating less frustration and thus a lower feeding motivation. The compensatory feed intake was lower for Insoluble birds compared to Control birds, indicating that the Insoluble treatment reduced the hunger experienced by the birds. Mixed birds were clearly more motivated to gain access to the fresh litter with more and faster crossings into the litter compartment ($P \leq 0.01$). Although foraging was the predominant behaviour performed in the litter compartment, Mixed birds spent less time on locomotion ($P = 0.002$), more time on comfort behaviour ($P = 0.02$) and more time resting ($P < 0.0001$) than Control birds, suggesting that they were also motivated to gain access to litter for increased comfort. The litter quality in the home pens was poorest in the Mixed treatment ($P = 0.0001$). In conclusion, none of the treatment diets seemed to improve the welfare of broiler breeders markedly during the rearing period, although the Mixed and Insoluble diets may, to some extent, have reduced the feeding motivation.

1. Introduction

Conventional broiler breeders are feed restricted, particularly during the rearing period, to prevent health and reproductive problems. Unfortunately, this practise compromises the welfare of the birds, as the feed restriction causes chronic hunger and frustration due to unfulfilled needs for feeding behaviour (de Jong et al., 2003; EFSA, 2010). Qualitative feed restriction has been suggested as a feeding strategy that potentially may alleviate the hunger felt by broiler breeders and fulfil their behavioural need for feeding behaviour while still keeping the birds from becoming obese (Savory et al., 1996). The idea of qualitative feed restriction is to reduce the quality of the feed in terms of nutrient content by adding non/low-nutritious dietary fibres. Compared to standard feed, a larger ration of the fibre-rich feed can be provided without increasing the nutrient intake (Sandilands et al., 2006). Insoluble types of fibres, such as oat hulls, are often used in qualitative feed rations. However, the addition of soluble types of fibres such as sugar beet pulp (which absorb more water) can further increase intestinal content and gut fill (Hocking et al., 2004).

Compared to quantitative feed restriction, the use of qualitative feed restriction is thought to improve satiety of the broiler breeders, as the intestinal content is increased and the passage time of feed is prolonged (Hocking et al., 2004; Steenfeldt and Nielsen, 2012). Furthermore, time spent feeding increases when a larger amount of feed is to be ingested

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(Zuidhof et al., 1995; Savory et al., 1996; de Jong et al., 2005; Moradi et al., 2013). Therefore, applying qualitative feed restriction increases the chance of meeting the behavioural need of the broiler breeders for performing feeding behaviour. However, it has been questioned to what extent qualitative feed restriction improves welfare. For example, if nutrient requirements and energy needs remain unsatisfied metabolic hunger will still occur even though the gastrointestinal system of the bird is full (Savory et al., 1996). In addition, studies evaluating different types of diluted diets used for qualitative feed restriction show contradicting results (Savory et al., 1996; Savory and Lariviere, 2000; de Jong et al., 2005; Hocking, 2006; Nielsen et al., 2011).

Previous studies have sought to develop methods for quantifying the hunger felt by broiler breeders. Among the methods used are the time taken for a bird to ingest a known feed ration, i.e. the feeding rate (Savory et al., 1993). The expectation is that the faster the feed is ingested, the hungrier the bird is. In the feed intake motivation (FIM) test, the compensatory feed intake over several days of previously feed-restricted birds can be used as an indicator of hunger (Ehlhardt et al., 1997; de Jong et al., 2003). For any test where the birds receive a food reward, there is an initial positive feedback increasing hunger, followed by a move towards satiety, which decreases the bird's rate of responding. Focusing on the appetitive phase of feeding instead of the consummatory phase will eliminate the potential influence on the quantification of hunger that the feedback from feed ingestion may instigate. In the present study, we developed an appetitive foraging motivation (AFM) test. The purpose of this test is to quantify hunger by using an indicator based on the strength of motivation for performing the appetitive phase of feeding, i.e. foraging consisting of ground scratching and ground pecking.

The aim of the present study was to investigate the effects of four dietary treatments, differing in fibre types and content, on the motivation for performing the two phases of feeding in broiler breeder pullets. The daily feed allotment among treatments was adjusted based on weekly weighing of the birds for all treatments to reach a similar growth rate. We expected that the treatment birds would show a lower motivation for feeding compared to the Control birds. More precisely, we hypothesised that compared to the Control birds, the treatment birds would show a lower feeding rate, perform less behaviour indicating frustration in a test where feeding was thwarted and have a lower feed intake in the FIM test. Furthermore, we expected that the treatment birds would work less hard to gain access to foraging material and spend less time on foraging behaviour in the AFM test compared to the Control birds. This study was part of a larger study, comparing the effects of qualitative feed restriction on a range of other welfare and gastrointestinal parameters (Riber et al., submitted; Steenfeldt, in prep.; Tahamtani and Riber, 2020; Tahamtani et al., 2020).

2. Materials & methods

2.1. Animals & housing

A total of 1200 day-old Ross 308 female breeder chicks were acquired via DanHatch A/S from Aviagen, Sweden. The birds were transported to the experimental facilities at AU Foulum, Denmark. Upon arrival, they were individually wing tagged and housed in 24 groups of 50 chicks. The groups were randomly selected, and the weight of the chicks was measured in groups of 12 to ensure that the average weight and the weight variation at the starting point were as equal as possible between all pens. The pens were located in two identical and adjacent rooms with 12 pens in each room. Each pen measured 2 m × 2 m × 2 m (L × W × H) and was covered with wire netting. The initial stocking density was 12.5 birds/m^2. Five birds per pen were sacrificed for experimental purposes at weeks 5, 10 and 15, resulting in a stocking density of around 10.5 birds/m^2 from week 5. The stocking density was kept at approximately 10.5 birds/m^2 by adjusting the back wall of each pen 0.25 m into the pen at the end of 10 weeks of age (2 m × 1.75 m) and again at the end of 15 weeks of age (2 m × 1.50 m). Under commercial conditions in Denmark, the standard stocking density at placement of day-old chicks is 12 (10–13) birds/m^2, but, if kept at the higher density of the range, birds are moved at an earlier age to the production unit earlier.

A light-grey sheet of hard plastic covered the bottom 70 cm of the sides of each pen, preventing visual contact between individuals from neighbouring pens. The floor of the pens was littered with wood shavings (Røde Softspån, Agroform, same type used in practise). When the litter quality degraded to an unacceptable level in the pens, extra litter was added, or the litter was exchanged. Any interventions with the litter included spreading Stalosan Dry (Vilofoss, Fredericia, Denmark) either on top of the old litter or, when the litter was exchanged, on the concrete floor before new litter was added. Stalosan Dry is a slightly acidic hygiene powder product consisting of calcium sulphate, iron sulphate and pine oil, which absorbs water and neutralises ammonia. Interventions were done similarly for all pens, even if some pens contained good quality litter, with the exception that the litter in the Mixed pens was exchanged in week 10 (see description of treatments in the section 'Dietary treatments'), whereas the other pens only had extra litter and Stalosan Dry added.

Each pen provided seven water nipples (Ziggity, developed for broiler breeders), which were adjusted in height, as the birds grew, and allowed a water flow of up to 110 mL/min. Water was available 24 h per day for the first 7 days of life and subsequently during the period of light only. Feed was provided by scattering. During the first 3 days, this was mainly done on paper placed underneath the drinking nipples to encourage feeding. The feed was given manually during the first 7 days, and the daily amount allocated was divided in four (days 1 + 2), three (days 3 + 4) or two meals (days 5 + 6+7) per day and scattered on the floor and on paper. During the first 7 days of life, the recommended amounts of feed per bird per day were very close to *ad libitum* intake. From day 8, the birds were fed once a day; a pre-weighed amount of feed was given at 09:00 h from two containers above the pen and thereafter scattered on the floor *via* four out-lets in the roof of each pen. The containers were filled *via* an automatic pneumatic system, which allows different feeds and different amounts to be allocated to each pen. The refilling of the container occurred between 9:30 h and 10:30 h every day in order to separate in time the sound of the filling from feeding. Scatter feeding was used as this method has been introduced commercially to encourage foraging, prolong feeding and improve uniformity of live weight.

During the first 2 days of life, the light schedule was a 23 h light/1 h dark cycle. On day 2, the light hours were reduced by 1 h/day until a light period of 8 h was reached at 16 days of age. Dawn and dusk were included in the dark period and consisted of 20 min each. The light was switched on at 08:00 and off at 16:00. The mean light intensity started at approximately 10 lx. However, due to issues with cannibalism, the light intensity was reduced to approximately 5 − 6 lx at 26 days of age, which is common practise under commercial conditions. The room temperature was set at 33 °C at placement and was gradually reduced to 21 °C by day 28.

At 3 weeks of age, cannibalism developed in a Control pen. As mentioned above, the light intensity was decreased. Birds showing signs of having been pecked were sprayed with hartshorn oil solution (Pyroleum Animale Crudum, Porcivet from Kruuse, Denmark). In addition, one peck stone (extra hard, 10 kg, Vilofoss) per pen was introduced at 4 weeks of age. It was placed centrally in the pen and lasted throughout the study. Initially, the anti-pecking treatment eliminated further pecking, but after 2 weeks new incidences of cannibalism occurred. At 7 weeks of age, the birds in the Control pen affected by cannibalism were culled by CO_2 gassing. A few incidences (n = 1–2 per pen) of cannibalism/peck wounds occurred in four other pens (one Control pen and three Roughage pens). Affected birds were sprayed with the hartshorn oil solution, which brought cannibalism to an end.

At the end of the study, the 19-week-old broiler breeder birds were killed by CO_2 gassing.

2.2. Experimental treatments

Each of the 24 pens were assigned to one of four dietary treatments, such that each treatment had six replicates. This allocation was done in a balanced fashion, so that each of the two rooms in which the pens were located had three replicates per treatment. Furthermore, within each room, the treatments were randomly allocated within three blocks to take into account the potential difference in the physical conditions in the rooms (variations in humidity, temperature, activity by the doors v. in the middle of the barn *etc.*).

The four dietary treatments used were:

1 Control: standard commercial feed as used in on-farm conditions.
2 Insoluble: standard commercial feed diluted with insoluble fibres (oat hulls).
3 Mixed: standard commercial feed diluted with a combination of insoluble fibres (oat hulls) and soluble fibres (sugar beet pulp).
4 Roughage: standard commercial feed and a provision of roughage (maize silage).

Full diet composition information is provided in Table 1. Throughout the study, the amounts of feed in MJ metabolisable energy (ME) allocated per bird and the feeding programme followed the scheme recommended by DanHatch for broiler breeder pullets. During the experimental period of 19 weeks, the daily amounts of feed (and maize silage) given per treatment were evaluated per week in order to follow the growth curve recommended by Aviagen, though modified by DanHatch. The birds were weighed weekly on a pen basis (in subgroups of 12 birds) until week 18. Daily feed allowance was then adjusted based on the growth of the birds in the previous week and to account for reductions in group size due to mortality or birds being removed for testing. Thus, approximately the same amount of daily ME was allocated in all treatments, but due to the differences in fibre content and types, the amount of feed allocated differed between treatments. Compared to Control, Insoluble was on average allowed 15.4% larger amounts of feed (min. 5.9%, max. 37.9%), Mixed 9.1% (min. 4.2%, max. 22.1%) and Roughage 14.2% (min. 8.8%, max. 26.2%).

Until day 7, all groups were fed the same standard starter diet 1. A starter diet 2 was provided from day 8 to day 42 of age, and thereafter a grower diet was provided. In order to adapt the birds to fibre-rich diets, the starter diet 2 contained less added fibre sources compared with the grower diet (Table 1). For the Roughage treatment, the maize silage was given manually once per day at 9:30 h in two flat, round feeders which were removed again every day at 11:30 h. The amount of maize silage given as a start was 5 g per bird per day, increasing gradually to 15 g per bird per day from 15 to 19 weeks of age.

Oat hulls, sugar beet pulp and maize silage were chosen as fibre sources for a number of reasons. Firstly, oat hulls have been shown to successfully alleviate some indicators of hunger positively (Nielsen et al., 2011). While Nielsen et al. (2011) found both negative and positive effects of sugar beet pulp on animal welfare, it was suggested the positive effects may potentially outweigh the negative effects, if the concentration of sugar beet pulp was adjusted (*personal comment*, Dr. Sanna Steenfeldt). Secondly, oat hulls, sugar beet pulp and maize silage are readily available in Denmark at a relative cheap price. Thirdly, it is well-known that domestic fowl find maize silage attractive as a source of feed. Finally, broiler breeders readily eat pellets containing oat hulls and sugar beet pulp (Nielsen et al., 2011).

2.3. Data collection

A number of measures on the motivation for feeding were performed: 1) feeding rate, 2) frustration level elicited by hampered access to feed, 3) feed intake motivation (FIM) and 4) appetitive foraging motivation (AFM). In addition, the litter quality in the home pens was assessed.

2.3.1. Feeding rate, frustration test and feed intake motivation (FIM) test

Both at 8–9 and 17–18 weeks of age, feeding rate was measured, a frustration test was performed and a FIM test was carried out. In each of the two units housing the home pens, 16 test pens, i.e. 32 in total, were placed. The test pens each measured 1 m × 1.65 m, provided four drinking nipples and had a littered floor (wood shavings). The height of the test pens was 65 cm, so a netting was placed on top to prevent the birds from escaping. In each unit, a pair of birds chosen randomly from each home pen was placed in the test pens in the same unit. For the four remaining test pens in each unit, a pair of birds from each of the four treatments was selected from the home pens with largest flock size. As the birds in one Control pen had been culled due to cannibalism, two Control pens provided two pairs of test birds. In total, eight replicates from each treatment group were obtained. The test birds were housed in the test pens for 7 days in total during which time they went through the following procedures:

2.3.1.1. Feeding rate. The test birds were placed on day 0 around noon, i.e. after finishing feeding that day. At the normal feeding time (9:00 h) on day 1, the test birds were allowed access to feed for 2 min in the test pens. The feed provided was the diet allocated to the treatment group that each of the pairs belonged to. This conscious choice allowed for comparison with Nielsen et al. (2011), who had a similar design of a feeding rate test, involving broiler breeders fed diets differing in fibre content. Following that, the test birds were weighed individually, one of the test birds were marked on the back with blue spray paint and the

Table 1
Diet composition information for the starter 1, starter 2 and grower diets used.

Diet/Treatment	Age	Pellet size mm	Metabolisable energy (ME) MJ ME/kg	Protein content g/kg	Added fibre sources
Starter 1					
All treatments	days 1–7	2	11.8	200	n.a.
Starter 2					
Control	days 8–42	3.5	10.8	178	n.a.
Roughage	days 8–42	3.5	10.8	178	n.a.
Insoluble	days 8–42	3.5	9.3	152	300 g oh
Mixed	days 8–42	3.5	9.3	153	191 g oh + 25 g/kg sbp
Grower					
Control	day 42 – week 19	3.5	10.4	145	n.a.
Roughage	day 42 – week 19	3.5	10.4	145	n.a.
Insoluble	day 42 – week 19	3.5	7.3	110	400 g oh
Mixed	day 42 – week 19	3.5	7.5	115	298 g oh + 70 g/kg sbp

oh = oat hulls; sbp = sugar beet pulp.

Table 2
Ethogram used for data collection during the frustration test[1] and the appetitive foraging motivation test[2] (see description later).

Behaviour	Description
Resting[2]	Sitting/lying on the floor while not engaged in other activities.
Standing[1,2]	The focal bird stands on the ground with both feet.
Locomotion[1,2]	Horizontal or vertical movement of body, such as running, walking, jumping and hopping without performing any other type of behaviour.
Drinking[1]	Having the beak in touch with the drinker. Includes the pauses between sips (= bouts*).
Pecking feed trough[1]	Pecking all parts of the feed trough. Includes the pauses between pecks (= bouts*).
Foraging[1,2]	Pecking and scratching the ground. Includes the pauses between each of the described elements (= bouts*).
Pecking object[1]	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[1]	Pecking the feathers, except the tail, of the other bird. Includes the pauses between pecks (= bouts[2]), which often involves following the recipient bird.
Toe pecking[1]	Pecking to the toes or feet of the other bird. Includes the pauses between pecks (= bouts*).
Preening[1]	Manipulating own plumage with the beak. Includes the pauses between each contact between beak and feathers (= bouts*).
Comfort behaviour[1,2]	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*).
Dustbathing[2]	The focal bird is sitting or lying while not engaged in other activities. Resting on the ground, not standing on both feet.
Aggressive behaviour[1]	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.

feed intake was registered. Feeding rate was calculated as the amount (g) of feed consumed by each pair of birds during the two minutes they had access to the feed. When done with the feeding rate and frustration tests on day 1, the test birds were allowed *ad libitum* access to feed for 5 days (days 1–5). At 10:00 h on day 6, the feed was withdrawn, and the test birds were feed restricted for 23 h. On day 7, the feeding rate test was repeated around the normal feeding time (9:00 h). The second test allowed us to investigate whether birds that had been accustomed for several weeks to restrictive feed allowance provided in one meal per day would eat slower (indicating a lower level of hunger) than birds that had been *ad libitum* fed before a 23 h feed withdrawal (Nielsen et al., 2011).

2.3.1.2. Frustration test. Upon returning to the test pen after being weighed at the end of the feeding rate measurement, a transparent lid hampering access to the feed was placed on the feed trough. The behaviour of the test birds was then video recorded by handheld cameras for 5 min. From the video recordings, focal animal sampling using continuous recording was done for both test birds using a predetermined ethogram (Table 2). 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 considered to be an indicator of frustration (Tinbergen, 1951; Roper, 1984). On day 7, the frustration test was repeated completion of the feeding rate measurement. The second test allowed us to investigate whether birds that had been accustomed for several weeks to restrictive feed allowance provided in one meal per day would perform less frustration-related behaviour (indicating a lower level of hunger) than birds that had been *ad libitum* fed before a 23 h feed withdrawal. After completion of the test on day 7, the test birds were culled.

2.3.1.3. Feed intake motivation (FIM) test. For the FIM test, the test birds were allowed *ad libitum* access to feed for 5 days (days 1–5). Water was available only during the period of light (8:00 – 16:00 h). During the test, the birds were fed the diet allocated to their treatment group in a round feed trough (Ø38 cm). In the Roughage treatment, the maize silage was provided *ad libitum* in a trough hanging on a wall and designed to minimise spillage. All test birds were weighed daily around 9:00 h. Daily feed consumption and allocation were registered by weighing the feed troughs before and after filling them up. This included the troughs containing maize silage in the Roughage test pens. A test period of 5 days was chosen based on the recommendation from de Jong et al. (2003) who performed a FIM test over 22 days but suggested, based on their results, only to analyse the first 5 days with *ad libitum* access to feed (days 0–4, corresponding to days 1–5 in our study).

2.3.2. Appetitive foraging motivation (AFM) test

At 12–13 weeks of age, a test of the motivation for performing appetitive foraging was performed. Four test arenas, each consisting of two compartments (0.80 m × 0.80 m × 0.60 m; W × D × H, Fig. 1) with a gap between and wire netting on the top, were placed in each of the two units housing the home pens. The test consisted of four habituation sessions followed by four test sessions, all carried out in the period from 10:00 – 16:00 h in order not to interfere with the feeding or light schedules. The door opening between the two compartments in the test arenas was initially 20 cm wide (H:35 cm). The width of the door opening was designed so that it could be adjusted to narrower widths in order to make it more difficult for the birds to access through the door opening. During the habituation sessions, the door opening remained at 20 cm width, whereas it was decreased daily on the four test days.

2.3.2.1. Habituation sessions. The purpose of the habituation sessions was to habituate the test animals to the test arena and to be isolated from companions. During all habituation sessions, the door opening

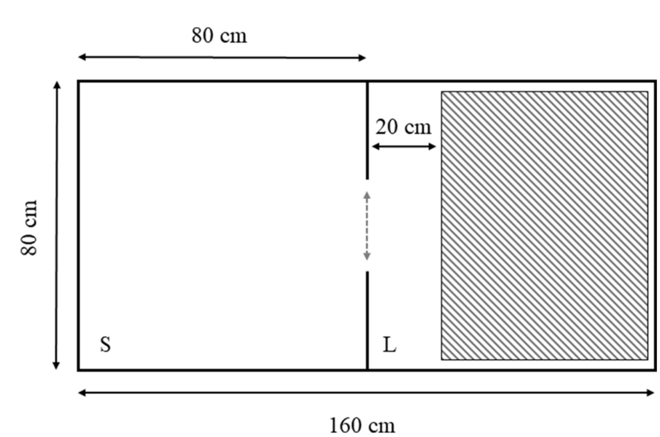

Fig. 1. Schematic design of the arena for the Appetitive Foraging Motivation test. S and L mark the start and litter compartment, respectively. The area with grey stripes represents the position of the litter. The grey dashed arrow marks the door opening whose width was decreased on each test day.

was 20 cm wide. On habituation day 1, six test birds from the same pen were selected pseudo-randomly, and the width of each bird was measured with a digital calliper (Facom 1300e, precision ± 0.01 mm) at the widest point between the shoulders. They were then placed together in the test arena for 30 min for habituation. There was no food, no litter and no water in any of the two compartments. After completion of the habituation period, the birds were fitted with numbered leg bands for easy identification and then released back to their home pen. On habituation day 2, the group of six test birds from each pen was again placed for 30 min in the test arena. On habituation days 3–4, the test birds were placed in the test arena individually for a 10-min habituation period. The floor in the start compartment had no litter, but now wood-shavings were available in the other compartment, now litter compartment.

2.3.2.2. Test sessions. On test days 1–4, each bird was exposed to a 10-min test per day. The start compartment had no litter on the floor, whereas wood-shavings were used as litter in the other compartment. The litter in the litter compartment was placed 20 cm from the door opening, in order for it not to be accessible from the start compartment, e.g. by head inside. As the uniformity of the test birds could be affected by the treatments, a statistical test for differences between treatments in shoulder width was carried out. No difference between treatments was found ($F_{3,115}$ = 0.93; P = 0.43). Thus, within test day we used the same door opening width for all the birds, regardless of treatment. The door opening was continuously decreased from test days 1–4 as follows: test day 1: 14.8 cm (150% average bird width); test day 2: 12.0 cm (122% bird width); test day 3: 9.9 cm (100% bird width); test day 4: 8.0 cm (81.5% bid width). One video camera (CCTV Camera, D1325, Dahua Technology, Hangzhou, China) was fitted above each test arena, allowing a clear view of both compartments. Between each habituation/test session, the arenas were cleaned for droppings.

Data on the behaviour during the 10-min test periods from all of the test sessions were collected from the videos by two observers. The treatments and door widths were balanced between the observers, and observers were blind to the treatment of the birds. The first five videos were scored by the two observers together. During the following days, the observers discussed any uncertainties that came up in the videos to ensure that they had high agreement on how to score the birds. The data collected from the videos included the number of movements into each compartment, the number of failed attempts (shoulders against wall of the door opening), inspections of each of the compartments (head inside) as well as the latency to first crossing into the litter compartment and the total time spent performing the following behaviours while in the litter compartment: resting, standing, locomotion, comfort behaviour, dustbathing and foraging (Table 2).

2.3.3. Litter quality

On the last day of the AFM test, week 13 of age, the litter quality in one location of the home pens was assessed using the Welfare Quality protocol (Welfare Quality, 2009). The location chosen was the centre of the triangle between the peck stone, the water line and the corner with the door. The litter quality was scored on a scale from 0 to 4 where the higher the score, the poorer the quality of the litter: score 0 – completely dry and flaky, i.e. easily moved with foot; score 1 – dry but not easy to move with foot; score 2 – leaves imprint of foot and will form a ball if compacted, but ball does not stay together well; score 3 – sticks to boots and sticks readily in a ball if compacted; score 4 – sticks to boots once the cap or compacted crust is broken (Welfare Quality, 2009). Once visually assessed, a sample of the litter was collected from the selected location. Care was taken to sample an equal amount of litter from the top through to the bottom. Similarly, samples were collected in weeks 5 and 14 of age, although at these ages two samples were taken in the middle of the pen (1 m from the corner of the pen) within the same distance from each of the outer fences (0.5 m). The two samples collected per age were pooled to one, and all samples were kept frozen until analyses of dry matter (DM) content.

2.4. Statistical analysis

Statistical analyses were performed using the software SAS 9.3. The data on the weight of feed consumed by each pair of birds, in grams, during the feeding rate test were analysed using the mixed procedure. The model included the fixed factors treatment, age and restricted/*ad libitum* (i.e. a categorical variable for whether the test was performed when the birds were accustomed to feed restriction or they had experienced *ad libitum* access to feed for the past week and then feed restricted 23 h before the test). The live weight of the birds on the day of testing was added to the model as a covariate, and the ID of the pair of the birds nested in pen was added as a random effect to account for the repeated measures. The initial model also included all the possible interactions between the fixed factors. Stepwise model reductions were performed by sequentially removing the higher order interaction terms if they were not statistically significant. As there were no significant interaction terms, the final model contained only the main effects of each fixed factor.

The dependent variables from the frustration test (i.e. number of behavioural transitions and time spent pecking the feed trough, foraging and walking) were analysed using the mixed procedure. The data were square root transformed to fit the model assumptions. The explanatory variables treatment, week of age and feeding schedule (i.e. restricted or *ad libitum*) were used as fixed factors and bird ID nested in test pen as the random effect. Stepwise model reductions were performed by sequentially removing the higher order interaction terms if they were not statistically significant. The occurrence of the other behaviours (i.e. standing, foraging, drinking, object pecking, toe pecking, feather pecking, preening, comfort behaviour, aggressive behaviour) was too seldom to be analysed. Therefore, descriptive statistics are presented for these.

From the data of the FIM tests, we calculated the feed intake in MJ ME relative to the metabolic weight of the birds in each day of the test. The feed intake in MJ ME was calculated taking into account the energy content of the different dietary treatments (see Section 2.2 for details) and an energy content of 10.8 MJ ME/kg of DM maize silage (Kolver et al., 2001) for the birds in the Roughage treatment. The metabolic weight of the pair of birds in each FIM pen was calculated according to Kleiber's law (Kleiber, 1947). These data were analysed with the mixed procedure, using treatment and day of testing as fixed factors and the FIM pen as a random factor. Tukey correction was used for the post hoc analysis of the main effects treatment and day of testing. For the post hoc analysis of the interaction between treatment and age, Bonferroni correction was used instead, as the comparisons of interest were only those within treatment or within days (i.e. 70 comparisons, Bonferroni-corrected critical alpha = 0.0007).

In the AFM test, we were interested in (1) the crossing of the door opening between the start and litter compartments and (2) the behaviour of the birds in the litter compartment. Regarding the crossing of the door opening, two Yes/No variables were created: one relating to whether or not the birds crossed the door from the start compartment into the litter compartment, and another relating to whether or not the birds had any failed attempts to cross the door. The failed attempts included those from the litter to the start compartment as well as from the start to the litter compartment. These two variables were analysed for each door width in turn using a binary glimmix procedure, with the models including treatment as the fixed effect and pen as a random effect. In addition to these binomial variables, the latency to cross the door and the total number of crossings (including only those birds that crossed the door at least once) were analysed using the mixed procedure with the fixed factors treatment, door width and their interaction. The models also included bird ID nested in pen as the random effect. Finally, the number of inspections (towards either compartment) was analysed by sorting into three categories: 0) 0 inspections, 1) 1 or 2

Table 3
Number of transitions, time spent pecking the feed trough (s) and time spent foraging (s) for the four treatments at the two ages (8/9 v. 17/18) and two time points (restricted v. *ad libitum*) during the frustration test (LS means, SE and back-transformed means).

Explanatory variable	Level	LS means	SE	Back-transformed means
Transitions				**(n)**
Treatment*Age (weeks)†	**Control - 8/9**	3.98	0.19	15.8
	Control - 17/18	4.51	0.18	20.3
	Insoluble - 8/9	4.20	0.18	17.7
	Insoluble - 17/18	3.42	0.17	11.7
	Mixed - 8/9	4.23	0.17	17.9
	Mixed - 17/18	3.84	0.17	14.7
	Roughage - 8/9	4.30	0.17	18.5
	Roughage - 17/18	4.16	0.17	17.3
Feeding schedule	***Ad libitum***	4.39^{b}	0.09	19.3
	Restricted	3.76^{a}	0.09	14.2
Pecking the feed trough				**(s)**
Age (weeks)*Feeding schedule	**8/9 *ad libitum***	9.47^{b}	0.47	89.8
	8/9 restricted	13.47^{c}	0.47	181.4
	17/18 *ad libitum*	7.21^{a}	0.46	51.9
	17/18 restricted	8.15^{ab}	0.46	66.4
Treatment	**Control**	9.40^{a}	0.54	88.4
	Insoluble	9.63^{a}	0.50	92.7
	Mixed	9.76^{a}	0.50	95.2
	Roughage	9.51^{a}	0.50	90.5
Foraging				**(s)**
Treatment	**Control**	10.36_{a}	0.74	107.3
	Insoluble	9.86_{a}	0.69	97.2
	Mixed	10.53_{a}	0.69	110.9
	Roughage	8.46_{a}	0.68	71.6
Age (weeks)	**8/9**	8.49_{a}	0.50	72.2
	17/18	11.11_{b}	0.49	123.4
Feeding schedule	***Ad libitum***	10.13_{a}	0.45	102.7
	Restricted	9.47_{a}	0.45	89.7

†Refer to main text for significant differences in the effect of treatment*age interaction on the number of transitions.
[a−c]Different letters within explanatory variable indicate significantly different values ($P \leq 0.05$).

inspections, and 2) 3 or more inspections performed. This variable was analysed using the glimmix procedure with treatment, door width and their interaction as fixed factors and bird ID nested in pen as the random effect. Post hoc tests were performed using Tukey's test (latency to cross and number of crossings) or by Bonferroni corrections of the critical alpha value (Yes/No variables and number of inspections). In the case of an effect of the interaction between treatment and door width, the critical alpha value was corrected to 0.001 (i.e. 48 pairwise comparisons between treatment and door width). For the effect of the main factors treatment or door width, the corrected alpha value was 0.008 (i.e. six pairwise comparisons between four groups).

The data on the behaviours performed in the litter compartment during the AFM test were analysed using the glimmix procedure with a negative binomial distribution and a log link function. The total time spent in the litter compartment, also in the log scale, was used as an offset for the linear predictor. This model, therefore, compares the proportion of time in the litter compartment spent performing a specific behaviour between treatments. Due to low numbers of birds crossing the door when the width was narrower (e.g. only two birds crossed into the litter compartment when the door width was 8.0 cm) the door widths were analysed separately and only for the three widest door openings (i.e. 14.8 cm, 12.0 cm and 9.9 cm wide). The models included treatment as the fixed factor and pen and observer as the random effects. When necessary (i.e. when the conversion criteria were not met), the random statement had to be removed, and pen and observer were attempted as fixed factors instead. Dustbathing behaviour was performed infrequently and, therefore, could not be statistically analysed. Descriptive statistics are presented instead. Resting behaviour also occurred at a low frequency and, therefore, could only be analysed for the door widths 12.0 cm and 9.9 cm. Furthermore, these models included only the Control, Insoluble and Mixed treatments as no birds from the Roughage treatment performed any resting behaviour.

The data on the litter quality at 94 days of age, collected using the Welfare Quality protocol, were analysed using a χ^2 test. Post hoc analysis was also performed using a χ^2 test, and the critical *P*-value associated with these analyses was Bonferroni corrected to $P = 0.008$. The analysis of the DM content of the litter was performed with the mixed procedure, with treatment, age in weeks and their interaction as fixed factors and pen as the random effect. For the post hoc analysis, the critical alpha was Bonferroni corrected to 0.003 (i.e. 18 interesting comparisons of treatments within age).

2.5. Ethical statement

The experiment was carried out according to the guidelines of the Danish Animal Experiments Inspectorate, Ministry of Environment and Food, Danish Veterinary and Food Administration with respect to animal experimentation and care of animals under study.

3. Results

3.1. Feeding rate, frustration test and feed intake motivation (FIM) test

3.1.1. Feeding rate

There was no observed effect of treatment on the amount of feed consumed by the pairs of test birds during the feeding rate test (LS means ± SE: Control = 72.6 g ± 3.40; Insoluble = 63.7 g ± 3.11; Mixed = 65.5 g ± 3.2; Roughage = 68.8 g ± 3.1; $F_{3,60} = 1.36$; $P = 0.26$). Furthermore, the amount of feed consumed did not differ with age (LS means ± SE: week 8/9 = 60.4 g ± 6.4; week 17/18 = 75 g ± 6.5; $F_{1,60} = 1.37$; $P = 0.25$) or whether the test was performed when the birds were accustomed to feed restriction or if they had experienced *ad libitum* access to feed for 5 days followed by 23 h of feed deprivation (LS means ± SE: restricted = 68.7 g ± 2.9; *ad libitum* = 66.6 g ± 3.0; $F_{1,60} = 0.18$; $P = 0.67$).

3.1.2. Frustration test

There was an effect of the interaction between treatment and age in the number of behavioural transitions performed during the frustration test ($F_{3,117}$ = 4.65; P = 0.004; Table 3). Birds in the Insoluble treatment performed fewer transitions than the Control birds and those in the Roughage treatment at 17/18 weeks of age (P = 0.0027 and P < 0.0001, respectively). There was also a tendency for Control birds to perform more behavioural transitions than the birds in the Mixed treatment at 17/18 weeks of age (P = 0.008). In addition, the birds in the Insoluble treatment performed more transitions at 8/9 weeks of age than at 17/18 weeks of age (P = 0.002), whereas the birds from the other treatment groups did not differ between the ages. There was also an overall effect of the feeding schedule on the number of transitions ($F_{1,124}$ = 25.92; P < 0.0001; Table 3) where all birds performed more behavioural transitions when they had been experienced *ad libitum* access to feed compared to when they had been accustomed to the feed restriction.

Full results for the time spent pecking at the feed trough during the frustration test can be seen in Table 3. There was an effect of the interaction between age and feeding schedule on the time spent pecking at the feed trough ($F_{1,120}$ = 13.60; P = 0.0003) with birds used to feed restriction spending more time pecking the feed trough at 8/9 weeks of age than at 17/18 weeks of age (P < 0.0001). The same was the case when the birds had been fed *ad libitum* for the previous week: younger birds performed more pecking at the feed trough than older birds (P = 0.004). Furthermore, when the birds were younger, they performed more pecking at the feed trough when used to feed restriction than when they had experienced *ad libitum* access to feed (P < 0.0001). However, this difference between feeding schedules was not observed when the birds were older (P = 0.36).

There was no effect of treatment ($F_{3,119}$ = 1.85; P = 0.14) or the feeding schedule ($F_{1,\ 122}$ = 1.43; P = 0.23) on the time the birds spent foraging during the frustration test. However, there was an effect of age ($F_{1,119}$ = 14.02; P = 0.0003) where the older birds spent more time foraging than the younger birds (Table 3).

In regard to time spent walking during the frustration test, there was an effect of the three-way interaction between treatment, age and feeding schedule ($F_{3,114}$ =5.51; P = 0.001; Fig. 2). In general, birds performed more walking during the frustration test when they had been used to *ad libitum* access to feed than when they were used to feed restriction. For example, at 8/9 weeks of age, birds from the Mixed and Roughage treatments performed more walking when they had experienced *ad libitum* access to feed than when accustomed to feed restriction (P = 0.002 and P = 0.03, respectively). Within the test performed after experience with *ad libitum* access to feed, birds from the Roughage treatment at 8/9 weeks of age spent more time walking than the Control birds of the same age (P = 0.026), whereas the other treatments did not differ.

Some behaviours in the ethogram for the frustration test could not be statistically analysed due to low occurrence. These behaviours were standing (mean ± SE: 12.9 s ± 34.4), preening (3.6 s ± 7.3), comfort behaviours (0.4 s ± 0.9), drinking (1.3 s ± 6.7), object pecking (0.05 s ± 0.5), toe pecking (0.02 s ± 0.3) and aggressive behaviour (0.22 s ± 1.15). No occurrence of feather pecking was observed.

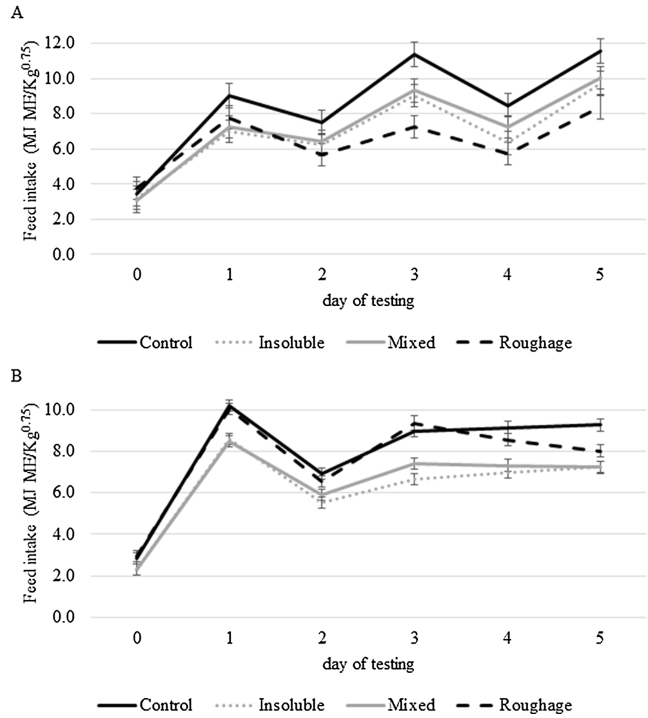

Fig. 3. Relative feed intake by birds from the four treatments during the testing days (days 1–5) in the Feeding Intake Motivation tests performed at 8–9 weeks of age (A) and 17–18 weeks of age (B). Day 0 presents the feed intake the last day before the FIM test was initiated, i.e. when the birds were fed restrictively.

3.1.3. Feed intake motivation (FIM) test

Fig. 3 shows the feed intake in MJ ME corrected for metabolic weight for all treatments during the 5 days of *ad libitum* feeding during the FIM tests. During the first FIM test, performed when the birds were 8–9 weeks of age, there was an effect of the main factors treatment ($F_{3,27}$ = 5.45; P = 0.005) and day of testing ($F_{4,108}$ = 32.89; P < 0.0001, Fig. 3A). Control birds had a higher feed intake per kg of metabolic weight (9.6 ± 0.5 MJ ME/kg$^{0.75}$) compared to birds from the Roughage treatment (6.9 ± 0.4 MJ ME/kg$^{0.75}$; P = 0.003) and Insoluble treatment (7.7 ± 0.4 MJ ME/kg$^{0.75}$; P = 0.04). The Control birds did not differ from the birds fed the Mixed treatment (8.0 ± 0.4 MJ ME/kg$^{0.75}$; P = 0.12). The feed intake also did not differ between

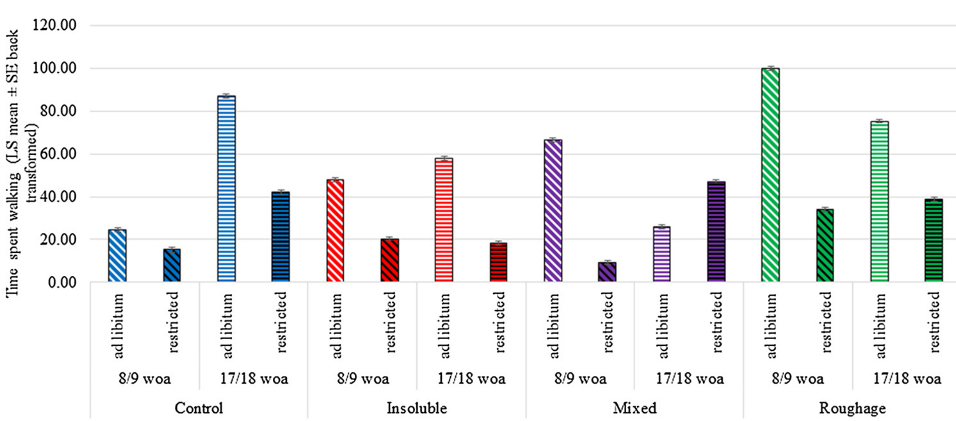

Fig. 2. Time spent walking (back-transformed LS mean ± SE) during the frustration test per treatment, week of age (WOA) and feeding schedule (*ad libitum*/restricted). The different treatments are colour coded (i.e. Control = blue, Insoluble = red, Mixed = purple, Roughage = green). The age is coded with the orientation of the stripes on the bars (i.e. 8/9 weeks of age = diagonal stripes, 17/18 weeks of age = horizontal bars), and feeding schedule is coded with the colour of the stripes on the bars (i.e. *ad libitum* = white stripes, restricted = black stripes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the Roughage, Mixed and Insoluble treatments ($P > 0.05$). In regard to differences between the days of testing, the feed intake of all birds seemed to oscillate between the days, with days 3 and 5 having the highest intake (9.2 ± 0.3 MJ ME/kg$^{0.75}$ and 9.9 ± 0.3 MJ ME/kg$^{0.75}$, respectively) and days 2 and 4 having the lowest intake (6.4 ± 0.3 MJ ME/kg$^{0.75}$ and 6.9 ± 0.3 MJ ME/kg$^{0.75}$, respectively).

During the second FIM test, which occurred when the birds were 17–18 weeks of age, there was an effect of the interaction between treatment and day of testing ($F_{12,101} = 1.88$; $P = 0.04$). As shown in Fig. 3B, on days 1, 3 and 4 of testing, the feed intake of the Control birds and those from the Roughage treatment was higher than that of the birds from the Insoluble and Mixed treatments ($P \leq 0.0003$). On the second day of testing, Control birds had higher feed intake compared to the birds from the Insoluble treatment ($P = 0.0005$) but did not differ from those in the Mixed or Roughage treatments ($P > 0.0007$). Furthermore, the birds from the Roughage treatment did not differ from those in the Mixed or Insoluble treatments on day 2 ($P > 0.0007$). On day 5 of testing, the Control birds had a higher feed intake compared to the birds from the Insoluble and Mixed treatments ($P < 0.0001$) and tended to have higher feed intake compared to the birds from the Roughage treatment ($P = 0.003$). No other treatment differences were observed on day 5. In general, the feed intake seemed to be highest on the first day of testing, lowest on the second day, where after it increased again on day 3 and stabilised on days 4 and 5.

3.2. Appetitive foraging motivation (AFM) test

There was no effect of treatment on the probability of the birds crossing the door when the width was 14.8 cm (i.e. 150% of the average shoulder width; $F_{3,115} = 1.36$; $P = 0.25$) with 88.4% of the birds crossing this width and accessing the litter compartment. There was also no effect of treatment on the probability of crossing the door at 12.0 cm width (122% shoulder width; $F_{3,115} = 2.43$; $P = 0.07$) with 81.9% of the birds crossing this width. However, there was an effect of treatment when the door width was 9.9 cm (100% shoulder width; $F_{3,115} = 5.39$; $P = 0.002$). Birds from the Mixed treatment were approximately 2.4 times more likely to cross the door (88.9%) and enter the litter compartment compared to the birds from the Control, Insoluble and Roughage treatments (43.3%, 44.4% and 38.9%, respectively; $P \leq 0.001$). The Insoluble and Roughage treatments did not differ from Control or from each other ($P > 0.008$). Only two birds (1.5%) crossed the door when the width was 8 cm (81.5% shoulder width). Both birds were from the Mixed treatment.

There was an effect of treatment on the latency to cross the door ($F_{3,74} = 3.68$; $P = 0.01$) with birds from the Mixed treatment crossing the door significantly faster compared to Control birds and those from the Roughage treatment (LS means ± SE: Control = 70.9 s ± 19.1; Insoluble = 52.8 s ± 18.7; Mixed = 27.2 s ± 17.0; Roughage = 66.8 s ± 19.1; $P < 0.05$). No other treatments differed from Control or from each other ($P > 0.05$). There was no effect of door width on the latency to cross ($F_{3,188} = 0.44$; $P = 0.73$). Looking at the birds that did cross the door into the litter compartment, there was an effect of the treatment ($F_{3,118} = 9.03$; $P < 0.0001$) and of the door width ($F_{3,227} = 29.19$; $P < 0.0001$) on the number of crossings they performed during the test. Birds from the Mixed treatment made fewer crossings (back-transformed LS means ± SE: 1.54 ± 1.2) compared to Control birds (2.46 ± 1.2; $P = 0.004$) and birds from the Roughage treatment (3.0 ± 1.2; $P < 0.0001$). Furthermore, the number of crossings performed by the birds from the Insoluble treatment (2.14 ± 1.2) tended to differ from those of the Mixed and Roughage birds ($P = 0.07$). The Insoluble and Roughage treatments did not differ from Control in the number of crossings ($P > 0.05$). In regard to door width, the birds performed more crossings at 14.8 cm (back-transformed LS means ± SE: 4.0 ± 1.1) compared to 12.0 cm (2.7 ± 1.1) and 9.9 cm (1.5 ± 1.1; $P \leq 0.0001$). Furthermore, more crossings were performed at 12.0 cm compared to 9.9 cm ($P < 0.0001$).

Only two out of 138 birds (1.5%) had failed attempts to cross the door when the width was 14.8 cm. There was no effect of treatment on the probability of birds having failed attempts to cross the door at 12.0 cm width ($F_{3,115} = 0.36$; $P = 0.8$) where 7.3% had failed attempts to cross the door. There was, however, an effect of treatment on the probability of birds having failed attempts to cross the door at 9.9 cm ($F_{3,115} = 3.13$; $P = 0.03$) with birds from the Mixed treatment tending to have fewer failed attempts (22.2%) compared to the birds from the other treatments (Control: 53.3%; Insoluble: 52.8%; Roughage: 52.8%; $P = 0.01$). Furthermore, the Insoluble and Roughage treatments did not differ from Control or from each other ($P > 0.008$). There was also an effect of treatment on the probability of having failed attempts to cross the 8.0 cm wide door ($F_{3,114} = 3.92$; $P = 0.01$) with birds from the Mixed treatment being approximately 1.7 times more likely to have failed attempts (77.8%) compared to birds from the Insoluble and Roughage treatments (37.1% and 36.11%, respectively; $P \leq 0.004$). Furthermore, Mixed treatment birds tended to be more likely to have failed attempts compared to Control birds (40%; $P = 0.01$). The Insoluble and Roughage treatments did not differ from Control or from each other ($P > 0.008$).

There was an effect of the interaction between treatment and door width on the number of inspections the birds performed ($F_{15,400} = 3.25$; $P < 0.0001$; Fig. 4). When the door width was set to 9.9 cm, the birds from the Mixed treatment were less likely to perform inspections compared to Control birds and those from the Roughage treatment ($P \leq 0.0002$) and tended to perform fewer inspections compared to the birds from the Insoluble treatment ($P = 0.04$; Fig. 4C). Furthermore, birds from the Mixed treatment performed more inspections when the door width was 8.0 cm compared to 12.0 cm and 9.9 cm ($P < 0.0001$) and tended to perform more inspections at 14.8 cm ($P = 0.002$).

When the door width was 14.8 cm, the average time spent foraging in the litter compartment was 66.2% and did not differ between treatments ($F_{3,98} = 0.98$; $P = 0.4$). There was also no effect of treatment on time spent foraging when the door width was 12.0 cm (average = 57.3%; $F_{3,87} = 1.07$; $P = 0.4$) or 9.9 cm (average = 67.0%; $F_{3,50} = 1.14$; $P = 0.34$).

In regard to locomotion, there was an effect of treatment on the proportion of time spent on this behaviour when the door width was 14.8 cm ($F_{3,117} = 5.23$; $P = 0.002$). The birds from the Mixed treatment spent less of their time in the litter compartment on locomotion (6.1%) compared to the birds from the other treatments (Control = 13.3%; Insoluble = 12.6%; Roughage = 14.5%; $P \leq 0.02$). The Insoluble and Roughage treatments did not differ from Control or from each other ($P > 0.05$). There was also an effect of treatment when the door width was 12.0 cm ($F_{3,87} = 3.85$; $P = 0.01$) where birds from the Mixed and Insoluble treatments tended to spend less time (5.8% and 5.7%, respectively) on locomotion compared to Control birds and birds from the Roughage treatment (10.9% and 11.3%, respectively; $0.06 < P < 0.09$). The birds from the Roughage treatment did not differ from the Control birds ($P = 1.0$). There was no observed effect of treatment when the door width was 9.9 cm (average = 8.5%; $F_{3,50} = 1.86$; $P = 0.14$).

As regards standing, no effect of treatment was found on the proportion of the time spent standing in the litter compartment when the door was 14.8 cm wide (average = 18%; $F_{3,117} = 1.76$, $P = 0.16$), 12.0 cm wide (average = 16.5%; $F_{3,88} = 2.32$; $P = 0.08$) or 9.9 cm wide (average = 16.7%; $F_{3,68} = 2.46$, $P = 0.07$).

An effect of treatment was found on the proportion of time spent on comfort behaviour when the door width was 14.8 cm ($F_{3,98} = 3.38$; $P = 0.02$) with the birds from the Mixed treatment spending a larger proportion of time on this behaviour (3%) compared to Control birds (0.9%; $P = 0.02$). There was also a tendency for birds from the Insoluble treatment to perform more comfort behaviour compared to Control (2.4%; $P = 0.08$). There was no difference between the other treatments (Roughage = 1.7%; $P > 0.1$). No effect of treatment was found when the door width was 12.0 cm ($F_{3,107} = 1.56$; $P = 0.2$;

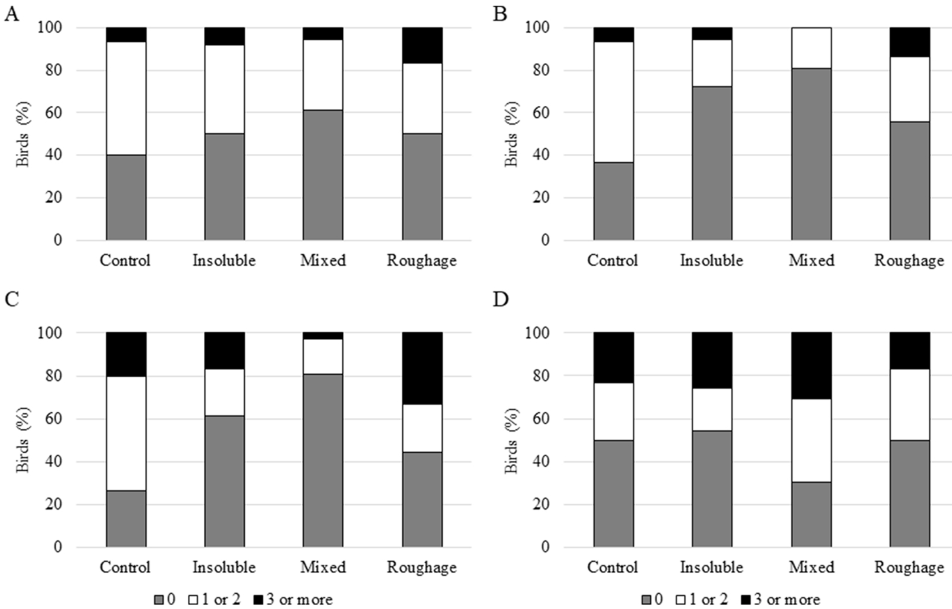

Fig. 4. Percentage of birds in each category of number of inspections across treatments and for each door width (A = 14.8 cm, B = 12.0 cm; C = 9.9 cm and D = 8.0 cm) in the AFM test.

average = 2.5%) or 9.9 cm ($F_{3,50}$ = 2.46; P = 0.07; average = 1.8%).

When the door width was 14.8 cm, resting behaviour was only performed in the litter compartment by the birds from the Mixed treatment (8.8% of birds performed it) who spent 5.3% of their time in that compartment on resting behaviour. When the door was 12.0 cm wide, resting was not performed by birds from the Roughage treatment. However, in a model including only the Control, Insoluble and Mixed treatments, there was an effect of the treatment on the time spent resting ($F_{2,83}$ = 11.03; P < 0.0001) with the Control birds spending less time resting (0.5% of the time) compared to the birds from the Insoluble (9.0% of the time; P = 0.004) and Mixed treatments (23.4% of the time; P < 0.0001). The Insoluble and Mixed treatments did not differ (P = 0.42). Finally, at 9.9 cm door width, there was no effect of treatment on the time spent resting ($F_{2,56}$ = 0.03; P = 0.97; Control: 5.05% of the time; Insoluble: 5.5% of the time and Mixed: 7.8% of the time). Resting was not performed by the birds in the Roughage treatment.

Concerning dustbathing, only birds from the Mixed treatment performed this behaviour when the door width was 14.8 cm (5.8% of the birds performed it and spent 0.1% of the time dustbathing). When the door width was 12.0 cm, there was no effect of treatment on the proportion of time spent dustbathing (average = 3.9%; $F_{3,88}$ = 1.64; P = 0.18). At a door width of 9.9 cm, dustbathing was not performed by the Control birds, but it was performed by the birds in the Insoluble (25% of the birds performed it and spent 1.7% of the time dustbathing), Mixed (6.25% of the birds performed it and spent 0.1% of the time dustbathing) and Roughage (15.4% of the birds performed it and spent 0.2% of the time dustbathing) treatments.

3.3. Litter quality

There was an effect of treatment on the quality of the litter at 94 days of age (i.e. last day of AFM test; χ^2 = 33.33, df = 9; P = 0.0001; Fig. 5) with the Roughage treatment having significantly better litter quality compared to the Mixed and Insoluble treatments (χ^2 = 12.00, df = 2; P = 0.0025 and χ^2 = 12.00, df = 3; P = 0.0074, respectively). Furthermore, the Mixed treatment also had worse litter quality compared to Control (χ^2 = 11.00, df = 2; P = 0.0041). There was also a tendency for the litter quality in Control to be better than in the Insoluble treatment (χ^2 = 8.0, df = 2, P = 0.018) but worse than in the

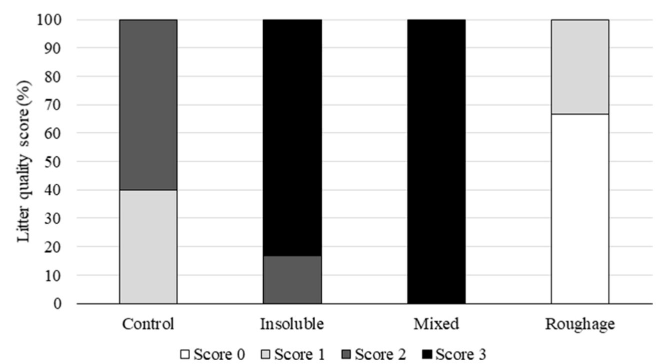

Fig. 5. Distribution (%) of the different litter quality scores across treatments at 13 weeks of age, i.e. last day of the Appetitive Foraging Motivation test. Higher scores refer to worse litter quality.

Roughage treatment (χ^2 = 7.0, df = 2, P = 0.03). There was no observed difference in litter quality between the Insoluble treatment and the Mixed treatment (χ^2 = 1.1, df = 1, P = 0.30). No pen was observed to have a litter quality score of 4.

There was an effect of the interaction between treatment and age on the proportion of DM in the litter samples ($F_{6,38}$ = 10.54; P < 0.0001). At 5 weeks of age, there was a tendency for the litter in the Roughage treatment to contain more DM (64.5%) compared to Control (56.5%; P = 0.005). The Mixed and Insoluble treatments did not differ from the Control (Mixed: 57.3%; Insoluble: 61.2%; P > 0.003). At 13 weeks of age, the Roughage treatment had a higher percentage of DM (71.3%) compared to all the other treatments (Control: 56.4%; Mixed: 39.6%; Insoluble: 45.2%; P < 0.0001). The litter in Control also contained more DM compared to the litter in the Mixed and Insoluble treatments (P ≤ 0.0003). The DM content in the litter of Mixed and Insoluble treatments did not differ (P = 0.05). At 14 weeks of age, approximately 3 days after all the litter was removed and fresh litter placed in every pen, differences between the treatments were already present. The litter in the Roughage treatment contained more DM (64.7%) compared to the other treatments (Control: 54.6%; Mixed: 46.7%; Insoluble: 49.7%; P ≤ 0.0009). The DM of the litter in Control did not differ from that of the Insoluble treatment (P = 0.09) but tended to be higher than that of the Mixed treatment (P = 0.008). There was no difference between the

Mixed and Insoluble treatments at 14 weeks of age (P = 0.3).

4. Discussion

It is generally assumed that feeding rate increases with the level of feeding motivation, and this has previously been shown in broiler breeders (Sandilands et al., 2005, 2006; Nielsen et al., 2011). Although not statistically different, the birds from the Insoluble, Mixed and Roughage treatments in the present study consumed 12.3%, 9.8% and 5.2% less feed, respectively, compared to Control birds in the 2-min test. However, as pointed out by D'Eath et al. (2009), there are a number of concerns involved in using feeding rate as a measure of hunger. Among the ones relevant for our study is that the increased competition for food among animals fed a restricted quantity of feed likely results in animals learning a feeding strategy that speeds up the feeding rate (Nielsen, 1999). Thus, a lower feeding rate may not necessarily be an expression of less hunger felt by the birds. Another challenge when investigating feeding rate in birds fed diets differing in fibre content is that this may instigate different feeding patterns, which may influence feeding rate. Diets may also differ in palatability. Alternatively, a diet novel to all birds may be used in a feeding rate test. However, this is not without disadvantages either. For example, selecting a diet that is equally different to birds from all treatments is difficult and feeding patterns may not change readily.

To overcome these challenges, we performed a second hunger test, which examines longer-term compensatory feed intake, i.e. the FIM test (Ehlhardt et al., 1997; de Jong et al., 2003). In this test, the feeding rate does not influence the results, as the test is performed over several days. Furthermore, de Jong et al. (2003) proposed that the difference in gut capacity between birds fed on different levels of restriction, which in the present study would be different dietary treatments, is most probably corrected in the FIM test when using the relative feed intake per kilogram metabolic weight in the analysis. The first FIM test at 8–9 weeks of age confirmed our hypothesis that the Control birds had a higher feed intake in MJ ME than the experimental birds, with the exception that the birds from the Mixed treatment had a feed intake in between the Control birds and the birds from the Roughage and Insoluble treatments, not being different from either of them. During the FIM test at 17–18 weeks of age, the treatment effect depended on day of testing, but the feed intake by Control birds were on all testing days among the highest, and it was consistently higher than the feed intake by the birds in the Insoluble treatment. Thus, the results from our study indicate that the Insoluble treatment did reduce the level of hunger experienced by the birds and that this treatment did it more consistently than the treatments Roughage and Mixed. Quite similar results were found by Nielsen et al. (2011). We speculate whether the poor litter condition in the home pens of the Mixed treatment caused a feed loss, resulting in a higher feed restriction level, which would explain why the birds showed a higher than expected compensatory feed intake in the FIM test. The growth curves of the four treatment groups did, however, not differ until week 13 of age, from where they started slowly to differentiate (Riber et al., *in prep*). This was mainly due to Mixed birds gaining less weight than the birds in the other treatments, but from week 17 the other treatments also started to differentiate. Thus, only the tests performed in weeks 17–18 could potentially have been affected by different growth rates. In week 17 of age Mixed birds were notably lighter, whereas the differences between Control, Insoluble and Roughage birds were minor. Compared to Control birds, Roughage birds weighed 3.7% more, Insoluble birds 1.4% less and Mixed birds 9.7% less in week 17 of age.

The restriction level varies throughout the rearing period of broiler breeders. In terms of amount of feed ingested, feed restriction is at its most severe level around age 10–16 weeks (Arrazola, 2018) where restrictively fed female broiler breeders are allocated down to four times less than *ad libitum* fed individuals will eat (de Jong et al., 2002; Savory et al., 1996). According to Arrazola (2018), the feed restriction level in terms of amount of feed ingested is quite similar in the two periods where we conducted the feeding rate, frustration and FIM tests, being around 47% in weeks 8/9 age and 51% in weeks 17/18 of age. Of importance is also the level of feed restriction in terms of nutrient intake, which has been suggested to be most severe around weeks 5–7 of age where female broiler breeders are allocated around 20% of the *ad libitum* nutrient intake (van Emous, unpublished data). For weeks 8–9 and 17–18 of age the levels are suggested to be 22% and 46%, respectively (van Emous, unpublished data). Thus, the relative capability of ingesting feed may be considered similar for our two test periods, but the sensation of hunger was likely higher during weeks 8/9 than 17/18 of age. This may explain why we found no stabilisation of feed intake as the test days progressed during the FIM test at 8/9 weeks of age, whereas the feed intake within treatment reached the same level on test days 3, 4 and 5 during the FIM test at 17/18 weeks of age.

The differences in feed restriction in terms of nutrient intake at weeks 8/9 and 17/18 of age may also explain, why we mainly observed treatment effects in the frustration test at 17/18 weeks of age. Regardless of treatment, the alleviating effect may have been insufficient to have notable effect on the hunger sensation in weeks 8/9 of age. At 17/18 weeks age, birds in the Insoluble and Mixed treatments performed fewer behavioural transitions than Control birds. Increased occurrence of behavioural transitions is typically observed during conflict behaviour such as displacement activities where an action is performed out of its normal context, while the bird is in a state of stress, frustration or uncertainty, e.g. due to thwarting of the behavioural expression of a high priority motivational state (Tinbergen, 1951; Roper, 1984). Thus, the lower frequency of behavioural transitions by birds from the Insoluble and Mixed treatments indicates that they were less frustrated and thus may have been less motivated for feeding compared to the Control birds.

We developed the AFM test with the purpose of quantifying hunger based on the appetitive phase of feeding behaviour as previously suggested by Dixon et al. (2014). This way the common problems typically implicated in motivation tests involving ingestion of feed, i.e. the influence which ingestion of feed has on the feeding motivation and the challenge of which feed type to use when the treatment groups are familiarised with different feed types, can be avoided. The AFM test developed required limited training of the birds and involved performance of explorative behaviour normally associated with foraging behaviour. The cost of accessing the resource (fresh litter) was squeezing through a narrow door, which has previously been used in a test of motivation to access a nest box by laying hens (Cooper and Appleby, 1996). Practically, the design of the AFM test apparatus, habituation and test sessions appeared appropriate. The habituation sessions seemed sufficient, as escape attempts were rarely observed. The design of the test apparatus seemed to promote explorative behaviour, and, importantly, the door widths used during the test sessions resulted as desired in a declining proportion of test birds gaining access with decreasing door width. Clearly, the narrowest door width, 8.0 cm, was too narrow for the vast majority of the birds to be willing/able to squeeze through, and the two widest door widths (14.8 cm and 12.0 cm) were sufficient for the majority of the birds to gain access without too much effort. Only 1.5% and 7.3% of the birds had failed attempts to cross the door when the width was 14.8 and 12.0 cm, respectively. The most interesting door width was the second narrowest, i.e. 9.9 cm, corresponding to the shoulder width of the birds. This door width seemed to separate the birds most motivated to pass from those not as highly motivated.

Evidently, the motivation to gain access to the litter compartment was the possibility of foraging in fresh litter, as the majority of the time in the litter compartment was spent foraging. Nevertheless, we doubt that the level of motivation to perform appetitive foraging was explicitly linked to the level of hunger the birds felt. The idea behind the AFM test is that when ingestion of feed is thwarted, then the motivation for the appetitive phase of feeding is increased. Indeed, this was shown by Dixon et al. (2014) who tested the motivation to gain access to litter by crossing a water barrier in broiler breeders being feed restricted at different levels. Therefore, we expected that the treatment birds would work less hard to gain access to the litter compartment and spend less time on foraging behaviour during the AFM test compared to the Control birds. Surprisingly, we found that those most motivated to gain access to the litter compartment were the birds from the Mixed treatment. However, the litter quality in the home pens was markedly worse in the Mixed pens compared to the Control pens. Indeed, the litter in the Mixed pens was moist and compact; ideally, it should have been loose and friable for optimal foraging and dustbathing. Therefore, the higher motivation for foraging in the Mixed treatment may have been instigated by the lack of suitable foraging material in the home pens more than by an increased level of hunger. Furthermore, the results from the AFM test may not only reflect the motivation for foraging. During the AFM test, birds from the Mixed treatment spent less time on locomotion, more time on comfort behaviour and more time on resting in the litter compartment than Control birds. The birds from the Insoluble treatment, who also to some degree suffered from a deteriorated bedding in the home pens, showed some of the same behavioural differences, although to a minor extent.

5. Conclusions

Minor treatment effects at 17/18 weeks of age were found in the frustration test with the birds in the Insoluble and Mixed treatments showing less frustration and thus a lower feeding motivation than Control birds. The FIM test indicated that the Insoluble treatment reduced the hunger experienced by the birds. In the AFM test, birds from the Mixed treatment clearly showed an increased motivation to gain access to the litter compartment. However, the behaviour of those birds who gained access suggested that the birds from the Mixed treatment were also motivated to gain access for other reasons than just the possibility of performing foraging behaviour. This was likely linked to the poor litter quality in the home pens in the Mixed treatment, suggesting that the increased risk of deteriorated bedding associated with diets containing soluble fibres may compromise welfare by impairing resting comfort. Thus, the AFM test developed appears valid for measuring appetitive foraging motivation, but attention should be paid to the opportunities provided in the home pens for performing the appetitive and consummatory phases of feeding behaviour. Based on the results presented in this paper, none of the treatment diets significantly improved the welfare of broiler breeders during the rearing period, although the Mixed and Insoluble diets may, to some extent, have reduced the feeding motivation. The knowledge gained from the present and previous studies suggests that qualitative feed restriction is insufficient for a complete prevention of hunger in broiler breeders to be gained.

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