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Diet dilution and feeding frequency have only minor effects on the behaviour of broiler breeder pullets

Annemarie J.W. Mens *, Ingrid C. de Jong, Johan W. van Riel, Henk Gunnink, Theo van Hattum, Rick A. van Emous

Wageningen Livestock Research, WD, De Elst 1, NL-6708 Wageningen, the Netherlands

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A B S T R A C T

During the rearing period, broiler breeders are feed restricted to prevent the negative impact of fast growth and high body weight on health and reproduction. Feed restriction causes frustration and stress, resulting in stereotypic pecking and hyperactivity. Nutritional strategies have the potential to reduce these welfare issues. Using a 2 × 2 factorial completely randomized block design, pullets were fed with two diet densities and two feeding frequencies during rearing. From 3–23 weeks of age (WOA), pullets received either a standard control diet (CON) or a 16% diluted diet (DIL) containing oat hulls. These diets were provided either once (FO) or twice (FT) a day. After 23 WOA, all pullets received the same standard layer diet once a day. Home pen behaviour was observed by scan sampling at 5, 10, 15, 20, 30 and 39 WOA over eight observation sessions. Furthermore, pullets were subjected to Novel Food Tests (12 and 17 WOA) and Novel Object Tests (5, 10, 15 and 20 WOA). Minor treatment effects were found for foraging and sitting behaviour that varied with age during rearing. Feeding frequency influenced the behavioural patterns of all home pen behaviours ($P < 0.001$; object pecking $P = 0.034$), while diet dilution only affected the expression of foraging behaviour during the day ($P = 0.007$). In all treatment groups, many pullets were observed standing and walking in anticipation of the first meal. After the first meal and before the second meal, more FT pullets were observed standing and walking, while during and after the second meal more FO pullets were observed sitting and performing comfort behaviour. Feeding twice a day resulted in lower daily peaks in drinking behaviour. In the laying period, when all birds received the same layer diet at the same frequency, those who were fed twice daily during rearing foraged more than those who were fed once ($P = 0.028$) and birds that received a diluted diet during rearing tended to drink more than birds that received the control diet ($P = 0.083$). Few treatment effects were found in the Novel Food and Novel Object Tests, indicating that diet dilution and/or feeding frequency, as applied here, did not affect fearful behaviour or the motivation to explore. In conclusion, this study showed that neither diet dilution, twice-daily feeding, or the combination of these, improved broiler breeder welfare during rearing and laying. Some changes in the pattern of home pen behaviour were found that indicated a response to the frequency of food provisioning.

1. Introduction

Broiler breeders (BB) have a high growth potential, since their offspring (broilers) are primarily selected for efficient growth (Zuidhof et al., 2014). Feeding BB pullets ad libitum during rearing will result in high feed intake and high adult body weight, which negatively affects mortality, reproduction, health and welfare (Hocking et al., 2002; Heck et al., 2004). Therefore, BB are feed restricted, especially during rearing. Between 5 and 15 weeks of age (WOA) the restriction level is estimated to be between 40% and 60% of ad libitum intake (Arrazola, 2018). Numerous studies have shown that feed restriction in rearing causes frustration and stress, resulting in stereotypical behaviour such as object pecking and elevated water consumption (E.g., Hocking, 1996; Hocking et al., 2001; de Jong et al., 2002), and hyperactivity (E.g., Savory and Maros, 1993; Arrazola et al., 2020). Feed restriction is, therefore, one of the main welfare challenges for BB (D'Eath et al., 2009; De Jong and Guémené, 2011).

Nutritional strategies can be used to alleviate the frustration and stress caused by feed restriction. In the past 15 years, several studies have applied nutritional strategies such as adjustment to diet

* Corresponding author.
E-mail address: annemarie.mens@wur.nl (A.J.W. Mens).

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composition and alternative ways of distributing the diet. Diet dilution with fibrous content has been found to reduce object pecking in the first half of the rearing period (6 and 10 WOA; de Jong et al., 2005a), spot pecking, and the heterophil to lymphocyte ratio (Hocking et al., 2004). Additionally, it has been found to increase dustbathing behaviour and reduce stereotypic pecking (Nielsen et al., 2011), indicating improved welfare in comparison to non-diluted diets. Furthermore, lower crude protein and amino acid levels are known to reduce stereotypic object pecking during the rearing period (van Emous et al., 2014, 2015). More recently, Arrazola et al. (2019) observed a reduction in feeding motivation, improved feather cover, fewer feather fault bars, and a delayed increase in the basophil-lymphocyte ratio during rearing in pullets fed a diluted diet. Those pullets were also less active and spent less time performing abnormal repetitive behaviours (Arrazola et al., 2020), which is in line with Morrissey et al. (2014), indicating that diluted diets might have a positive effect of BB welfare during rearing. In contrast, Tahamtani et al. (2020) fed broiler breeders different fibre sources, and found that the provision of roughage improved welfare, whereas insoluble fibres had no effect, and a mix of both actually resulted in reduced broiler breeder welfare. Also, de Los Mozos et al. (2017) observed no effects of 15% diluted diets on behaviour.

As well as diet composition, feeding frequency and the feeding system used may also affect behaviour. de Jong et al. (2005b) studied the effect of twice-daily feeding and scatter feeding during the rearing period on behaviour associated with frustration and hunger. Neither method reduced object pecking and hyperactivity, and physiological stress indicators were also unchanged, indicating that these management measures did not improve BB welfare in rearing. Despite the lack of studies on this subject, feeding more portions during the day is more in line with natural eating behaviour patterns of chickens (Collias and Collias, 1967).

In addition to the effects of feed restriction on home pen behaviour and physiological stress indicators, feed restriction also seems to affect fear and the motivation to explore in novel environments, especially with food as a reward. Dixon et al. (2014) showed that BB fed at a commercial restriction level were more motivated to cross a water barrier in order to access a foraging area in comparison to BB that were fed twice or three times that amount, and de Jong et al. (2005b) observed that as restriction levels increased, BB became increasingly active in an open field, suggesting a higher motivation for exploration. Furthermore, restricted-fed BB have been shown to be highly motivated to explore a novel location, even when this novel location is associated with aversive stimuli (Dixon et al., 2013). Similarly, BB fed at a 5:2 schedule showed more interest in a novel object on fasting days in comparison to daily-fed BB (Lindholm et al., 2018) and also Tahamtani and Riber (2020) suggested that hungrier breeders were more likely to approach the novel object. Nielsen et al. (2011) tested the motivational conflict between hunger and fear using a novel food test and found that BB fed diets with high levels of fibres, but with low non-starch polysaccharides, were less motivated to explore novel food, compared to BB fed the control diet, and this was likely caused by higher levels of satiety. The combination of more frequent feeding and the inclusion of fibre in BB diets has not been empirically tested before. Both individual strategies can offset the negative consequences of feed restriction, and the combination of strategies could have an additive positive impact on breeder welfare. Therefore, the objective of this study was to evaluate the combined effect of diet density and feeding frequency on BB behaviour during rearing and laying. Breeders were fed according to the same target body weight with either a standard commercial diet (control) or a diet diluted with 10–20% oat hulls. They were also fed either once or twice daily. We hypothesised that BB fed the diluted diet would suffer less from feeding frustration and hunger compared to breeders fed the control diet. Additionally, we anticipated that BB receiving the combination of a diluted diet and an increase in feeding frequency would show fewer indications of stress, frustration and hunger compared to BB fed once daily and/or the control diet because their feeding behaviour would be distributed more evenly over the light period. Previously it has been shown that feeding a control diet twice daily did not positively affect BB welfare (de Jong et al., 2005b), but as this has been tested only once, we also included a treatment where the control diet was fed twice a day. Home pen behaviour and the responses to novelty in an Open Field Test, Novel Object Test and Novel Food Test were measured as indicators of hunger, frustration and feeding motivation. This paper is part of a larger study therefore the effects of the dietary treatments on performance are described elsewhere (van Emous et al., 2021).

2. Materials and methods

2.1. Ethical statement

The study was carried out in compliance with the ethical guidelines of the International Society of Applied Ethology (Sherwin et al., 2003). The study design, housing and management were all in accordance with local and European legislation (EU directive 2010/63EU). The protocol for the experiment was approved by the Dutch Central Committee on Animal Testing, Den Haag, The Netherlands (approval number: AVD4010020185007) and the Institutional Animal Care and Use Committee.

2.2. Experimental design

This study was part of a larger experiment, for which the experimental design has been reported in detail in van Emous et al. (2021). Briefly, the pullets were subjected to a 2 × 2 factorial completely randomized block design with two diet densities and two feeding frequencies. Pullets were fed a 5-phase feeding program. All pullets received the same starter-1 diet until 3 WOA. From 3 until 23 WOA, pullets received either a standard control diet (**CON**), according to the recommendation of the breeder company (Aviagen-EPI, 2017), or a diluted diet (**DIL**) containing oat hulls (Table 1) (ABZ Diervoeding, Leusden, The Netherlands). The starter-2 (3–8 WOA), grower (8–17 WOA), pre-breeder-1 (17–20 WOA) and pre-breeder-2 diets (20–23 WOA) were 10%, 20%, 20% and 10% diluted, respectively as compared to the control diet (Table 1). This scheme was applied to minimize the effects of differences in daily amount of feed between phases. From 23 WOA onwards, all breeders received the same standard breeder-1 diet (2850 kcal/kg AMEn; 14.8% CP; 0.58% dig. Lys; 0.56% dig. M+C; 30.0% Ca; 3.2% aP) until the end of the trial at 40 WOA. In addition to the different diets, pullets were either fed once (**FO**) or twice (**FT**) a day from 3 to 23 WOA. The FO-pullets were fed at 08:15 h (100% of the daily feed allowance) and the FT-pullets were fed at 08:15 h (60% of the daily feed allowance) and 12:15 h (40% of the daily feed allowance).

2.3. Housing and management

A total of 960 Ross 308 female broiler breeder chickens (Aviagen-EPI, Roermond, The Netherlands) were allotted to 24 floor pens (2.5 × 2.0 m) in 2 identical climate-controlled rooms in the experimental facility of Wageningen University & Research at day (d) 0 of age, with each 40 chick/pen. This resulted in 3 replicates of the 4 treatments per room. An extra pen was available with 30 chicks to replace dead and culled chicks between d 1 and d 21. In the pens an elevated floor (0.57 × 1.45 m) with plastic slats was available from 6 WOA onwards (the area under the slats was not accessible) and wood shavings were used as bedding in the remaining area (2.0 kg/m^2). The sidewalls of the pens were built of wire mesh so that birds could see birds in other pens, except for the lowest 40 cm which was closed off to prevent interaction between pens on the floor. Feed was provided manually between 0 and 2 WOA in three feed trays, between 2 and 6 WOA in three round feeders and from week 6 onwards in two feeding troughs with a male exclusion system (4.1 m length) in each pen. Males were reared commercially and

Table 1
Dietary ingredients, and calculated and analysed nutrients of the pullet diets (g/kg, as-fed basis).

	Starter-1	Starter-2		Grower		Pre-breeder-1		Pre-breeder-2	
	(0–21 d)	(22–56 d)		(57–119 d)		(120–140 d)		(141–161 d)	
Item		CON[1]	DIL	CON	DIL	CON	DIL	CON	DIL
Ingredient									
Maize	400	405	358.6	380	291.9	390	300.3	390	345.2
Wheat	217.3	254	224.9	200	153.6	233	179.4	233	206.2
Wheat middlings	30.5	30.5	27	150	115.2	100	77	100	88.5
Maize gluten feed	20	30	26.6	120	92.2	77.9	60	77.9	68.9
Sunflower meal	33.7	58	51.4	80	61.5	81.9	63.1	81.9	72.5
Soybean meal	232	159	140.8	21.8	16.7	53.3	41	53.3	47.2
Oat hulls	10	10	123.8	10	239.9	10	238.3	10	124.1
Soya oil	13.2	12.5	11.2	4.5	3.5	12.2	9.4	12.2	10.8
Chalk	17.3	17.6	15.6	18.7	14.4	26.7	20.8	26.7	23.7
Monocalcium phosphate	9.3	10	8.9	3	2.5	3.6	2.8	3.6	3.2
Salt	2.5	2.5	2.7	2.3	1.9	2.6	2.1	2.6	2.4
Sodium carbonate	2.8	3.1	1.9	2.4	1.5	2.5	1.5	2.5	2
Premix rear[2]	4	4	3.5	1	0.8	–	–	–	–
Premix lay[3]	–	–	–	3	2.3	4	3.1	4	3.5
Maxifit[4]	4	2	1.8	–	–	–	–	–	–
L-Lysine	1.5	0.2	0.1	1.5	1	1	0.6	1	0.8
DL-Methionine	1.3	1	0.7	0.9	0.6	0.8	0.4	0.8	0.6
L-Threonine	0.6	0.6	0.5	0.9	0.6	0.5	0.2	0.5	0.4
Calculated content[5]									
AME_n (kcal/kg)	2800	2800	2520	2600	2080	2700	2160	2700	2430
Crude ash	63.7	62.1	60.1	57.7	54.8	64.2	59.8	64.2	62
Crude protein	189.4	166	152.1	137.4	115.9	140	118	140	129
Crude fat	44.7	44	40	39	32.1	45	36.7	45	40.9
Crude fiber	38.8	44.5	73.2	61.1	115.2	56.2	111.1	56.2	83.6
NSP	172.9	178.8	244.5	235.8	355.9	210.8	335.7	210.8	273.3
Dig. Lys	9	6.4	5.8	4.8	3.8	4.9	3.9	4.9	4.4
Dig. Met+Cys	7	6.2	5.5	5.3	4.2	5.3	4.2	5.3	4.8
Dig. Thr	6.3	5.5	5	4.5	3.6	4.3	3.4	4.3	3.9
Dig. Trp	1.9	1.6	1.5	1.1	1	1.2	1.1	1.2	1.1
Na	1.9	2	1.8	2	1.6	2	1.6	2	1.8
K	8.6	7.6	7.2	7.3	6.5	7	6.3	7	6.6
Cl	2.4	2.2	2.3	2.5	2	2.5	2	2.5	2.3
DEB (mEq/kg)	235	220	198	203	180	195	174	195	185
Calcium	10	10	9	9	7.2	12	9.6	12	10.8
Total phosphorus	6.3	6.4	5.8	5.9	4.8	5.5	4.4	5.5	5
Available phosphorus	4.1	4.1	3.7	3.2	2.6	3.2	2.6	3.2	2.9
Analyzed content									
DM	888	881	882	880	888	882	889	882	886
Crude ash	62	56	55	52	50	58	55	58	57
Crude protein	195	166	152	138	113	140	119	140	128
Crude fat	49	46	42	43	41	49	43	49	47
Crude fiber	44	49	71	64	110	55	107	55	77
Total phosphorus	6.4	6.3	5.6	5.5	4.6	5	4.2	5	4.7

1Density diets: CON = control diet; DIL = diluted diet. [2]Provided per kilogram of complete diet: vitamin A, 10,050 IU; vitamin B1, 3.0 mg; vitamin B2, 12.1 mg; vitamin B3, 48.2 mg; vitamin B4, 281.4 mg; vitamin B5, 15.1 mg; vitamin B6, 4.0 mg; vitamin B9/B11, 1.6 mg; vitamin B12, 0.03 mg; vitamin D3, 2513 IU; vitamin E, 40.2 mg; vitamin H, 0.2 mg; vitamin K3, 3.0 mg; iron, 64.3 mg; copper, 5.0 mg; manganese, 30.2 mg; zinc, 30.2 mg; iodine, 1.5 mg; selenium, 0.4 mg. [3]Provided per kilogram of complete diet: vitamin A, 10,050 IU; vitamin B1, 3.0 mg; vitamin B2, 10.1 mg; vitamin B3, 30.2 mg; vitamin B4, 301.6 mg; vitamin B5, 16.4 mg; vitamin B6, 4.0 mg; vitamin B9/B11, 2.0 mg; vitamin B12, 0.03 mg; vitamin D3, 1508 IU; vitamin E, 100.5 mg; vitamin H, 0.3 mg; vitamin K3, 3.0 mg; iron, 100.5 mg; copper, 15.1 mg; manganese, 100.5 mg; zinc, 20.1 mg; iodine, 2.0 mg; selenium, 0.3 mg. [4]Provided per kilogram of complete diet: vitamin B1, 0.8 mg; vitamin B2, 1.6 mg; vitamin B3, 8.0 mg; vitamin B4, 80.4 mg; vitamin B5, 4.0 mg; vitamin B6, 0.8 mg; vitamin B9/B11, 0.4 mg; vitamin B12, 0.006 mg; vitamin C, 30.2 mg; vitamin E, 10.1 mg; vitamin H, 0.002 mg; vitamin K3, 0.8 mg; iron, 24.1 mg; manganese, 20.1 mg; iodine, 0.08 mg. [5]CVB matrix values (CVB, 2011) were used for diet formulation.

introduced into the pens (3 males/pen) when the breeders were 20 WOA. A separate feeding trough (60 cm) was available for males (from week 20 onwards) positioned at a minimum height of 50 cm, to prevent female access to the feed. Water was supplied by drinking cups above the litter floor until week 6 and positioned above the slatted floor from 6 week onwards. Outside each pen, one nest box (88 × 36 cm) was placed adjacent to the slats, which was available from 22 WOA onwards.

During the experiment, all birds of the different treatments were maintained on the same target body weight (BW). Feed allocation was adjusted to the predetermined body growth curve during the rearing and laying period according to breeder guidelines (Aviagen-EPI, 2017). Feed was provided ad libitum from 0 to 2 WOA, and from week 2 onwards pullets were fed restricted amounts of mash feed daily. During the laying period (23 WOA onwards), feed was provided at 08:15 h. Water was provided between 08:15 and 15:30 h during rearing and between 08:15 and 16:30 h during laying (23 WOA onwards). Males received a commercial male diet (2600 kcal/kg AMEn; 13.0% CP; 0.45% dig. Lys; 0.5% dig. M+C; 1.0% Ca; 0.3% aP). Males and females were fed at the same time.

Room temperature was maintained at 35 °C during the first 2 days, and from d 3 onwards temperature was gradually reduced until it reached 20 °C at 4 WOA. The pullets were reared following a photoperiod of 24 L:0D (40 lx) for the first 3 days, which was gradually reduced to a photoperiod of 8 L:16D (5 lx) at week 3 (between 07:45 and 15:45 h). Breeders were photostimulated with 10 h light per day at 21 WOA (40 lx), and day length gradually increased by 1 h (22 – 24 weeks) or 0.5 h (25–26 weeks) per week to a photoperiod of 14 L:10D at week 26. This was maintained until the end of the study at 40 WOA, with lights on from 02:45–16:45 h. Pullets were not beak trimmed and were vaccinated according to a standard, commercial protocol (Aviagen-EPI, Roermond, the Netherlands).

2.4. Behavioural observations

Home pen behaviour was observed by live instantaneous scan sampling once per hour of each pen at 5, 10, 15, 20, 30 and 39 WOA. At each age, observations were carried out over one day by two observers, both of whom observed one room per session. Observers switched rooms between observation sessions. Overall, the total number of observations throughout the experiment were balanced and performed by three pre-trained persons. On each day, the observations were distributed across eight sessions throughout the light period (starting at 07:45, 08:45, 09:45, 10:45, 11:45, 12:45, 13:45, 14:45 h). The first session was 30 min before feeding (07:45 h) and sessions were repeated each hour until the lights went off (during rearing). Each round began with five minutes of habituation time per room during which time the observer walked slowly between the pens. Drinking, standing, sitting, walking, foraging, object pecking and comfort behaviour was scored by counting the number of birds performing these different behaviours according to the ethogram previously described by de Jong et al. (2005a) (Table 2). Eating and drinking was only scored when feed and water was available, otherwise pecking at feeders and drinkers was scored as object pecking. During the availability of feed and water, object pecking was defined as pecking at the pen or equipment. Males were excluded from the observations.

2.5. Behavioural tests

2.5.1. Novel food test

The Novel Food (NF) Test aimed to test the conflict between fear and hunger and was adapted from Nielsen et al. (2011). The test was executed at 12 and 17 WOA. Feeders and feed were originally novel to the pullets at 12 weeks but not at 17 WOA, when the same feeders and feed were used. The test was performed at three timepoints during the day. During each test two pens per treatment, one per room, were tested resulting in 8 pens per timepoint. All pens were tested once per test day. The order was randomized and predetermined in advance and all pens were tested again at 17 WOA. The pullets were presented with two feeders per pen (yellow round feeder, 40 cm in diameter) filled with 500 g feed (mixed grains). The pullets were allowed to eat for two minutes, after which the feeder was removed from the pen. The latency of the first pullet to approach the feeder and the number of pullets at the feeder after one and two minutes were recorded. Leftover food from both feeders in each pen were weighed to measure the feed intake. All observations were performed live and by two trained observers.

Table 2
Ethogram of behavioural measurements (adapted from de Jong et al., 2005a).

Behaviour	*Description*
Eating	Pecking at and/or ingesting the feed in the feeder
Drinking	Pecking at and/or ingesting water from the drinking nipples
Standing	Standing without performing foraging, comfort behavior, or pecking
Sitting	Sitting without performing foraging, comfort behavior, or pecking
Walking	Walking, running without performing foraging, comfort behavior, or pecking
Foraging	Pecking, scratching the litter
Comfort	All comfort behavior without dusthbathing, such as preening, autopecking, nibbling, stroking, wing flapping, stretching
Dustbathing	Dustbathing without performing foraging, comfort behavior, or pecking
Object pecking	Pecking at parts of the cage of the wall
Bird pecking	All pecking at other birds

Table 3
Effects of diet density, feeding frequency and their interactions on the home pen behaviour (expressed as % of chickens showing a particular behaviour) during the rearing period at 5, 10, 15 and 20 WOA (back transformed means).

		Age															P-value						
		5 weeks			10 weeks			15 weeks			20 weeks			*Mean*			Diet	Fee-ding	Age	Diet* Feeding	Diet* Age	Fee-ding* Age	Diet*Feeding*Age
	Feeding	Diet Con	Dil	*Mean*	Diet Con	Dil	*Mean*	Diet Con	Dil	*Mean*	Diet Con	Dil	*Mean*	Diet Con	Dil	*Mean*							
Drinking	Once	4.7	4.9	4.8	5.3	5.9	4.6	8.0	7.3	7.6	7.7	7.9	7.8	6.3	7.3	6.3							
	Twice	4.7	3.7	4.2	6.5	6.7	6.6	9.3	9.3	9.3	10.1	11.0	10.5	6.4	7.1	7.2							
	Mean	4.7	4.2	4.5	5.9	6.3	6.1	8.6	8.2	8.4	8.8	9.3	9.1	6.8	6.7	6.8	0.79	0.38	0.031	0.96	0.79	0.14	0.75
Standing	Once	13.5	14.8	14.1	10.6	12.0	11.3	9.6	11.4	10.4	13.4	13.6	13.5	11.7	12.9	12.3							
	Twice	14.5	15.7	15.1	13.1	12.5	12.8	14.2	11.3	12.7	14.3	12.8	13.5	14.0	13.0	13.5							
	Mean	14.0	15.2	14.6	11.8	12.2	12.0	11.7	11.3	11.6	13.9	13.2	13.5	12.8	12.9	12.9	0.71	0.085	0.80	0.52	0.69	0.59	0.55
Sitting	Once	10.5	11.2	10.8	11.2	12.8	12.0	1.4	1.5	1.4	2.0	1.2	1.8	4.4	4.3	4.4							
	Twice	4.5	5.9	5.1	6.3	7.1	6.7	1.6	1.3	1.5	1.9	1.6	1.8	3.1	3.1	3.1							
	Mean	6.9	8.1	7.5	8.4	9.6	9.0	1.5	1.4	1.5	2.0	1.6	1.8	3.7	3.7	3.7	0.29	< 0.001	0.005	0.65	0.49	0.005	0.85
Walking	Once	12.8	7.1	9.6	6.7	7.4	7.0	4.2	3.9	4.0	3.5	2.9	3.2	6.0	5.0	5.5							
	Twice	18.4	16.3	17.3	8.2	5.8	6.9	4.1	4.4	4.3	4.4	3.3	3.8	7.4	6.2	6.7							
	Mean	15.4	10.9	13.0	7.4	6.5	6.9	1.1	4.2	4.2	3.9	3.1	3.5	6.6	5.5	6.1	0.15	0.018	< 0.001	0.84	0.27	0.001	0.040
Foraging	Once	14.4	14.2	14.3	15.0	11.2	13.0	39.9	44.8	42.3	32.3	36.2	34.2	23.7	23.8	23.8							
	Twice	16.9	14.5	15.7	14.7	14.7	14.0	37.0	42.1	39.5	24.1	30.9	27.4	22.1	23.8	23.0							
	Mean	15.6	14.4	15.0	14.8	12.8	14.7	38.5	43.4	40.9	28.0	33.5	30.7	22.9	23.8	23.4	0.97	0.076	< 0.001	0.91	0.009	0.005	0.38
Object pecking	Once	4.7	5.7	5.2	3.1	2.4	2.7	6.0	3.5	4.6	7.0	7.1	7.0	5.0	4.3	4.6							
	Twice	8.4	6.3	7.3	3.0	2.3	2.6	5.4	4.9	5.1	10.6	8.0	9.2	6.2	4.9	5.5							
	Mean	6.3	6.0	6.1	3.0	2.3	2.7	5.7	4.1	4.8	8.6	7.5	8.0	5.5	4.6	5.1	0.17	0.33	< 0.001	0.88	0.48	0.16	0.055
Comfort	Once	5.1	5.1	5.1	3.5	3.2	3.3	2.2	2.5	2.3	3.9	3.4	3.7	3.5	3.4	3.5							
	Twice	3.7	4.4	4.0	2.7	3.2	3.0	1.8	2.1	2.0	3.6	3.9	3.7	2.9	3.3	3.1							
	Mean	4.4	4.7	4.5	3.1	3.2	3.1	2.0	2.3	2.2	3.7	3.6	3.7	3.2	3.4	3.3	0.13	0.13	0.010	0.36	0.91	0.58	0.98

2.5.2. Novel object test

The Novel Object (NO) test was based on the Welfare Quality protocol for loose housed poultry (Welfare Quality®, 2009). At 5, 10, 15, 20 and 40 WOA the test was carried out one hour after the second feeding (12:15 h) with each test day a different NO (rubber duck, coloured block, golf ball, cane and coloured rod respectively). The NO was placed in the middle of the pen and was removed after three minutes. Every 30 s the number of pullets/hens within a 25 cm radius around the NO was counted. In addition, the latency of the first hen to touch the object and of the first 3 pullets/hens to approach the NO within a 25 cm radius were recorded. All observations were performed live by two trained observers.

2.6. Statistical analysis

Data were analysed using Genstat statistical software (Genstat, 2018). A statistically significant difference was declared at $P < 0.05$, and $0.05 \leq P < 0.10$ was considered a tendency. Pen was considered to be the experimental unit and analyses were separated for the rearing period (0–23 WOA) and the laying period (23–40 WOA).

Response variables of home pen behaviour were analysed using generalized linear mixed models (GLMM), with a binomial distribution, where the dispersion parameter phi was also estimated. Treatment (i.e. diet density and feeding frequency), age of the birds, observation session, and multiple interactions between these variables were included as fixed effects in order to assess the effect of treatment, age and time of the day. Room and pen were included in the model as random effects. Only significant interactions are reported. Least square differences were compared, using Fisher Unprotected LSD adjustments for multiple comparisons. Results of the home pen behaviour observations during the rearing period are reported both at the level of observation day (i.e. whether there are any overall effects of treatment), as well as per session per observation (i.e. reporting the expression of the various behaviours during the day for the four treatment groups). Due to the lack of differences in session per observation, means per session are not reported for the laying period and only the results of the complete observation day are shown.

Within the NF-test, the feed intake was expressed as a feed intake rate, calculated as the intake per second per pullet. Regarding the NF-test, the latency to reach the feeder (on the logscale), the feed intake rate (on the logscale) and the percentage of pullets (binomial distribution) at one and two minutes were tested with a GLMM model, where the fixed effects of age and treatment (including interactions) were included, and pen was included as a random effect.

The NO-test carried out at week 20 was excluded from the analysis as extreme short latencies for all treatments were observed, due to disturbances. Therefore, the results of week 20 are not reported. At week 15, the latency to first contact of the novel object sometimes exceeded the three-minute limit. In these cases, a score of 3 min was registered. Latency to first contact was analysed as a binomial trait, expressed as the percentage of the maximum latency of 3 min. The latency of 3 pullets close to the novel object was analysed on the logscale. Both traits were analysed with pen included as a random effect in the model. The repeated counts (every 30 s) of pullets close (<30 cm) to the Novel Object were analysed with a repeated measures mixed model, where the correlation of repeated measures (within pen) was estimated with a power function.

3. Results

3.1. Home pen behaviour during rearing

3.1.1. Average effects per observation day

Table 3 shows the percentage of birds engaged in drinking, sitting, standing, walking, foraging, object pecking and comfort behaviour during rearing, averaged over all observation sessions for a particular age. Diet dilution did not affect any of the home pen behaviours, nor did the interaction between diet and feeding frequency. Pullets fed twice a day tended to show more standing behaviour than pullets fed once per day ($\Delta = +1.2\%$; $F_{1,\ 19.4} = 3.29$; $P = 0.085$), but no other effects of feeding frequency on home pen behaviour were found. A significant interaction between age and feeding frequency was found for sitting ($F_{3,\ 77.0} = 4.64$; $P = 0.005$), and for foraging behaviour both an age * feeding frequency ($F_{3,\ 58.3} = 4.66$; $P = 0.005$) and age * diet interaction ($F_{3,\ 58.3} = 4.20$; $P = 0.009$) were found. At 5 and 10 WOA ($\Delta = -5.7\%$ and $\Delta = -5.3\%$ resp.) fewer FT pullets were observed sitting compared to FO pullets, but this difference was absent at 15 and 20 WOA where for both treatments a very low proportion of sitting birds was observed (mean of 1.8% and 3.7% resp.). The proportion of pullets showing foraging behaviour was higher at 15 and 20 WOA (mean of 40.9% and 30.7% resp.) in comparison to 5 and 10 weeks of age (mean of 15.0% and 14.7% resp.). At 5 and 10 weeks of age, more CON pullets showed foraging as compared to DIL pullets ($\Delta = +1.2\%$ and $\Delta = +2.0\%$ resp.), whereas the opposite was found at 15 and 20 WOA ($\Delta = -5.0\%$ and $\Delta = -5.5\%$ resp.), and, FT foraged more at 5 and 10 WOA ($\Delta = +1.4\%$ and $\Delta = +1.0\%$ resp.) whereas FO foraged more at 15 and 20 WOA ($\Delta = +2.8\%$ and $\Delta = +6.9\%$ resp.). As the pullets aged, drinking increased (from 4.5% to 9.1%; $F_{3,\ 42.2} = 3.25$; $P = 0.031$), object pecking first decreased ($\Delta = -3.5\%$) then increased from 15 WOA onwards ($\Delta = +3.2\%$; $F_{3,\ 21.0} = 13.74$; $P < 0.001$). Comfort behaviour decreased until 15 WOA ($\Delta = -2.4\%$), after which the proportion of pullets showing comfort behaviour increased again ($\Delta = +1.5\%$; $F_{3,\ 20.8\ =\ 4.87}$; $P = 0.01$). For only walking behaviour, a three-way interaction was found between pullet age, diet density and feeding frequency ($F_{3,\ 59.8} = 2.95$; $P = 0.040$). As the pullets grew older, time spent walking reduced, although treatment differences were observed at the earliest age, as at 5 weeks of age FT and FO-CON showed more walking than FO-DIL. At 10 weeks of age, there was no change in the proportion of FO-DIL pullets walking, whereas this behaviour was reduced in the other treatment groups (Table 3).

3.1.2. Expression of behaviour during the day in rearing

No main effect of diet or feeding frequency, nor an interaction between feeding frequency and diet on home pen behaviour in rearing was found. However an interaction between observation session and feeding frequency strongly affected the expression of all behaviours during the observation day (observation session effect; $F_{7,\ 22.2} = 2.23 – 16.81$; $P < 0.001$ for all behaviours except object pecking $P = 0.034$; Fig. 1). A high percentage of pullets in all treatments were observed standing and walking before the first meal. After the first meal and before the second meal, more FT pullets were observed standing and walking, while during and after the second meal more FO pullets were sitting and performing comfort behaviour. Additionally, drinking patterns differed, as less FT pullets performed drinking behaviour after the first meal compared to FO pullets, and an increase in drinking after the second meal was observed in FT in comparison to FO pullets. Notably, FO resulted in higher peaks of sitting and drinking than FT. Overall, minor differences in object pecking were observed as only CON-FO pullets showed a peak in this behaviour after the first meal compared to those in the other treatments. Both an observation session * feeding frequency and an observation session * diet interaction were found for foraging, with more foraging behaviour performed by CON pullets between 10 h and 12 h compared to DIL pullets ($F_{7,\ 141.4} = 2.94$; $P = 0.007$). Furthermore, more foraging behaviour was performed by FT pullets at 9 h and 10 h as compared to FO pullets, however the opposite was found between, and at, 11 h and 13 h ($F_{7,\ 141.4} = 7.92$; $P < 0.001$).

3.2. Home pen behaviour during the laying period

Table 4 shows the effects of the nutritional treatments and interactions on home pen behaviour during the laying period. Feeding the BB twice a day during rearing resulted in more foraging behaviour in the

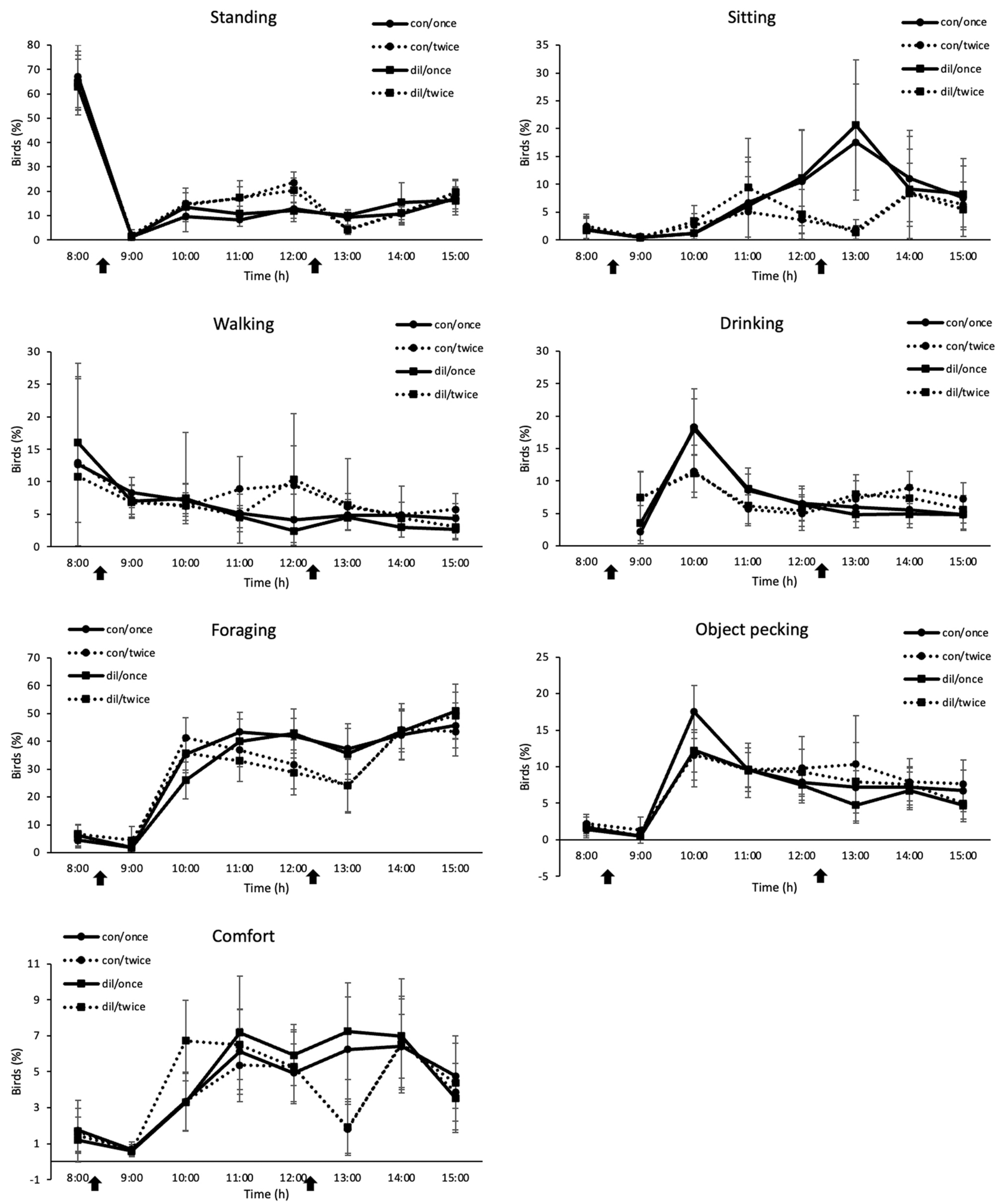

Fig. 1. Influence of feeding once (solid line) or twice a day (dotted line), normal (rounds) and diluted (squares) diets on behavioural patterns of standing, sitting, walking drinking, foraging, object pecking and comfort during the rearing period (5–20 WOA) (predicted means). Arrows indicate feeding moments (08:15 h and 12:15 h).

laying period compared to feeding once a day during rearing ($\Delta = +2.4\%$; $F_{1, 37.0 = 5.22}$; $P = 0.028$). Furthermore, dilution of the diet during rearing tended to result in more drinking behaviour in the laying period compared to the CON diet during rearing ($\Delta = +1.8\%$; $F_{1, 37.0 = 3.2}$; $P = 0.083$). Age of the birds affected drinking, sitting, object pecking and comfort behaviour. At 39 WOA, BBs drank ($\Delta =$ - 5.2%, $F_{1, 37.0} = 37.0$; $P < 0.001$) and sat ($\Delta =$ - 4.3%, $F_{1, 37.0} = 21.9$; $P < 0.001$) less, but increased object pecking ($\Delta = +1.9\%$, $F_{1, 37.0} = 11.2$; $P = 0.002$) and comfort behaviour ($\Delta = +4.4\%$, $F_{1, 37.0} = 26.6$; $P < 0.001$) compared to 30 weeks of age. No other effects of diet density, feeding frequency or

Table 4
The average effect of diet density, feeding frequency and their interaction on drinking, standing, sitting, walking, foraging, object pecking and comfort behaviour (%) during the rearing period at 30 and 39 WOA (back-transformed means).

		Age									P-value				
		30 weeks			39 weeks			Mean							
		Diet			Diet			Diet							
	Feeding	Control	Diluted	*Mean*	Control	Diluted	*Mean*	Control	Diluted	*Mean*	Diet	Feeding	Age	Diet*Feeding	Feeding*Age
Drinking	Once	16.7	19.8	18.2	13.2	14.8	13.9	14.8	17.1	15.9					
	Twice	19.6	21.7	20.6	14.0	14.8	14.4	16.6	18.0	17.3					
	Mean	18.1	20.7	19.4	13.6	14.8	14.2	15.7	17.5	16.6	0.083	0.16	< 0.001	0.63	0.45
Standing	Once	14.5	12.3	13.4	14.0	12.5	13.2	14.3	12.4	13.3					
	Twice	12.2	11.3	11.7	15.0	14.9	15.0	13.5	13.0	13.3					
	Mean	13.3	11.8	12.5	14.5	13.6	14.1	13.9	12.7	13.3	0.25	0.92	0.125	0.47	0.096
Sitting	Once	14.3	12.8	13.5	9.2	9.9	9.5	11.5	11.3	11.4					
	Twice	15.1	13.6	14.3	10.1	9.2	9.6	12.4	11.2	11.8					
	Mean	14.7	13.2	13.9	9.6	9.5	9.6	11.9	11.2	11.6	0.53	0.73	< 0.001	0.77	0.74
Walking	Once	6.2	5.4	5.8	6.2	5.6	5.9	6.2	5.5	5.9					
	Twice	4.9	6.0	5.4	7.0	6.9	6.9	5.9	6.4	6.2					
	Mean	5.5	5.7	5.6	6.6	6.2	6.4	6.1	5.9	6.0	0.81	0.58	0.262	0.42	0.34
Foraging	Once	17.9	17.3	17.6	17.8	15.7	16.7	17.9	16.5	17.2					
	Twice	14.5	14.0	14.2	16.0	14.6	15.3	15.2	14.3	14.7					
	Mean	16.2	15.5	15.8	16.9	15.1	16.0	16.5	15.3	15.9	0.27	0.028	0.927	0.92	0.38
Object pecking	Once	2.1	3.0	2.5	3.7	4.0	4.2	2.8	3.7	3.2					
	Twice	2.1	2.4	2.3	4.7	4.2	4.1	2.9	3.2	3.0					
	Mean	2.1	2.7	2.4	3.8	4.5	4.1	2.8	3.5	3.2	0.26	0.76	0.002	0.57	0.81
Comfort	Once	6.7	7.3	7.0	11.9	12.7	12.3	9.0	9.7	9.3					
	Twice	8.4	6.8	7.5	11.1	11.3	11.2	9.7	8.8	9.2					
	Mean	7.5	7.0	7.3	11.5	12.0	11.7	9.3	9.2	9.3	0.92	0.64	< 0.001	0.38	0.38

their interactions were found.

3.3. Behavioural testing

3.3.1. Novel food test

The Novel Food test (NF-test) was performed twice during the rearing period. All pullets were able to reach the feeder and eat at the same time during the test. Feeding the pullets once per day tended to result in a longer latency to eat compared to the FT pullets (Fig. 2; $\Delta = +65$ s; $F_{1, 20.0}$; $P = 0.096$). No other effect of dietary treatment or any interactions were found on parameters measured in the NF-test. Different responses were observed between age groups. At 17 WOA birds in all treatments had much lower latencies to approach the feeder ($\Delta = -356$ s; $F_{1, 18.0}$; $P < 0.001$) and a higher feed intake rate ($\Delta = 0.03$ s; $F_{1, 18.0}$; $P < 0.001$) than at 12 weeks of age.

3.3.2. Novel object test

An interaction was found between feeding frequency and observational timepoint for the number of hens located within 25 cm of the novel object at 30 s scans within the 3-min observation period (Fig. 3). This interaction indicates that for FT more birds were close to the NO at both 120 and 150 s during the test (0.67 and 0.65 for 120 and 150 s, respectively) than for FO (1.62 and 1.30 for 120 and 150 s, respectively; $F_{5, 376.6} = 2.34$; $P = 0.041$). No other treatment effects were found for the number of birds close to the object at the different time points. No effects were found for feeding frequency or interaction between diet density and feeding frequency for the latency to touch the object. There was an age effect ($F_{3, 83.0} = 3.84$; $P = 0.013$) and a tendency was found for an interaction between diet density and age ($F_{3, 83.0} = 2.39$; $P = 0.075$). Latency to touch was longest at 15 WOA (123 s), followed by 5 WOA (100 s), 39 WOA (68 s), while the pullets at 10 WOA were the fastest to touch the NO (24 s). All pullets showed a similar response in the NO-test and were very quick to approach the NO at all ages during rearing. The mean latency of at least three birds to approach the NO within a radius of 25 cm was 10 s at 39 WOA, while at earlier ages the pullets almost immediately approached the NO. In addition, the latency of at least 3 birds to approach the NO within a radius of 25 cm increased with age for FT birds ($P = 0.09$). When comparing this latency per age, only at 39 WOA a treatment effect was found ($P = 0.046$); hens fed the control diet once a day had a lower latency (3 s) as compared to the other treatments (14 s on average).

4. Discussion

This study aimed to improve BB welfare during rearing, by either dilution of the diet, increasing the feeding frequency, or the combination of diet and feeding frequency. Contrary to our expectations, diet dilution, feeding twice-daily, and the combination of these had only minor effects on home pen behaviour and responses within the novel feeding and novel object tests. It is likely that the applied treatments were not sufficient to overcome hunger and frustration due to feed restriction during rearing. The behavioural observations carried out during rearing showed that in all treatments, relatively high proportions of object pecking and low proportions of sitting behaviour were observed, indicating stress and frustration in all treatment groups (Savory and Maros, 1993; Savory and Lariviere, 2000; de Jong et al., 2005b; Sandilands et al., 2005; van Emous et al., 2015).

Treatment had little effect on behaviour during the NF- and NO-tests. A shorter latency to approach the novel feeder is suggested to indicate a higher level of hunger (Nielsen et al., 2014). Feeding twice a day resulted in a tendency towards a shortened latency, indicating that these pullets were more motivated to explore the novel feeder and to overcome their fear, and suggesting that they are hungrier than pullets fed once a day. However, it could also indicate stronger feeding motivation due to their experiences with smaller portions which may have resulted in increased feed competition between the pullets. Due to their

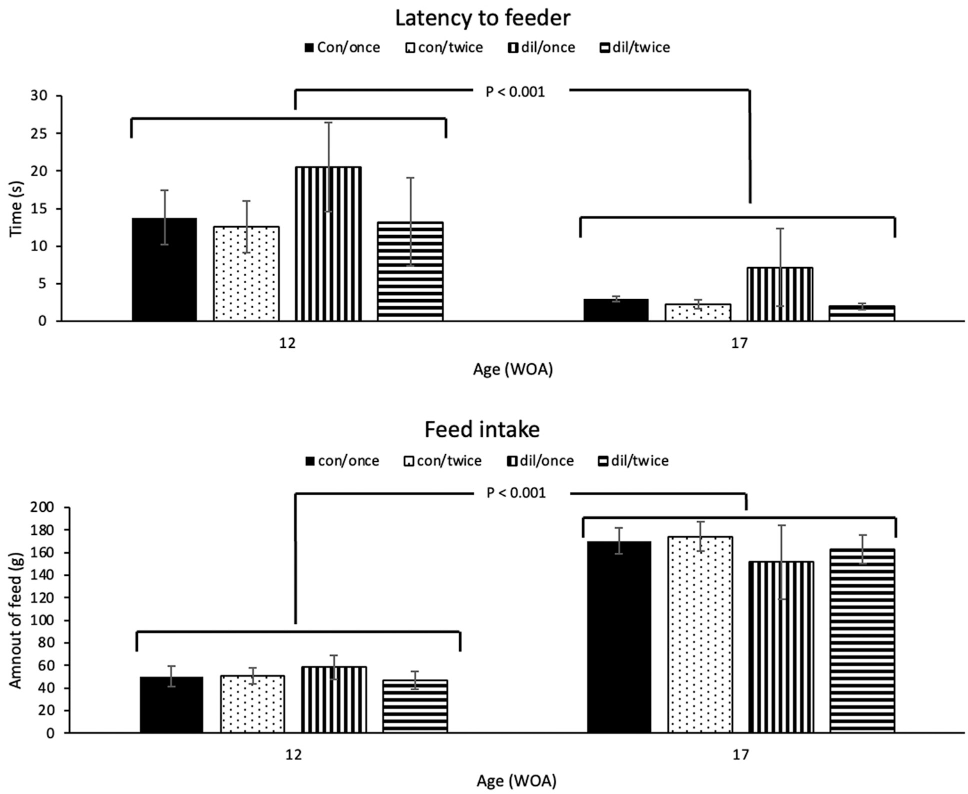

Fig. 2. Latency to approach the feeder (upper panel) and feed intake (lower panel) for pullets fed once or twice per day and/or the diluted (DIL) and control (CON) diet (predicted means). For significant differences, see text.

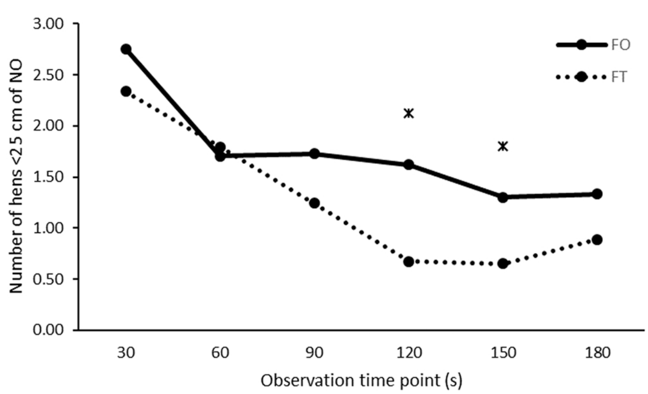

Fig. 3. Mean number of pullets either fed once (FO, solid line) or twice (FT, dashed line) a day approaching the novel object (NO) within 25 cm at several time points. * indicates a significant difference $P < 0.05$.

experiences, FT birds might have understood more quickly that the novel feeder had feed in it, whereas the birds fed only once a day might have been more skeptical of the feeder because they never get extra feed other than their one portion a day. Feeding twice a day created a more complex daily structure, which may have taught the birds to cope with novelty, which is supported by the response to the NO. The balance between motivation to explore and fear of novelty was also tested in the novel object tests. It has been suggested that hungrier breeders are likely to approach the NO faster (Lindholm et al., 2018; Tahamtani and Riber, 2020). The absence of treatment effects on the latency to approach the NO in the current study indicates that pullets in all of the treatment groups may have been equally hungry. The results of our NO-test also showed that during testing, fewer birds approached the NO with time, which indicating a loss of interest over time. However, the birds that were fed twice a day seemed to lose interest sooner than the birds fed only once a day. This is difficult to explain; it might indicate a higher focus on feed and thus a decreased motivation to explore non-feed objects in pullets fed twice a day, but this merits further study. We observed a high interest in the novel object in general, indicated by short latencies to approach the object, which is in contrast with results by van den Oever et al. (2021), who did not find much interest for the NO at 22 WOA. Interestingly, at 39 WOA the breeders in the current study were still quick to approach the NO (mean latency of 10 s), despite the feed allowance no longer being severely restricted. This could indicate a learned response from the rearing period. Both in our study and in Tahamtani and Riber (2020), repeated testing of BB pullets with a NO during the rearing period resulted in no age effects, indicating equal levels of hunger at the different ages or equal levels of boredom. Thus, the minor differences in responses to the fear and motivational tests in the present study aligned with the observations of home pen behaviour and indicate no significant improvement in breeder welfare as a result of the applied treatments. The minor differences found may even suggest higher feeding motivation in pullets fed twice a day, although we should be very careful with this conclusion.

In contrast with our expectations, dilution of the diet minimally influenced the behaviour of the pullets, as has also been found by Tahamtani et al. (2020). Only an effect of diet dilution on foraging behaviour was found, while previous studies found changes suggesting improved welfare such as reduced object pecking (de Jong et al., 2005a), spot pecking (Hocking et al., 2004), stereotypic behaviour (Nielsen et al., 2011), stereotypic pecking (van Emous et al., 2014, 2015) and physiological parameters such as the basophil to lymphocyte ratio (Arrazola et al., 2019). Several of these studies also added fibers as well as an appetite suppressant, which might explain the inconclusive effects on behaviour. In the current study, diet dilution interacted with pullet age to influence foraging behaviour which is probably due to the differences in feed restriction level. During the first half of the rearing period, the pullets of the DIL group performed less foraging behaviour, while the reverse effect was found in the second half of the rearing period. Thus, the small effects of DIL on foraging behaviour disappeared in the second half of rearing, indicating that diet dilution had no effect on hunger and frustration. It is possible that foraging behaviour increased in the second half of the rearing period, because of the relative smaller meal size. Furthermore, the 10–20% dilution applied in this study might have been not enough to reduce or overcome the frustration and hunger caused by feed restriction in the rearing period. Another explanation could be that the breeders used in this research are genetically different from the birds used in studies 10–25 years ago. As selection for growth efficiency has continued over the last few decades (Hartcher and Lum, 2020), the birds' genetic makeup has changed significantly, resulting in a relatively higher feed restriction applied by farmers in rearing, and as a consequence the dilution of the diet had little effect.

In our study we aimed to increase possible positive effects of diet dilution by increasing the feeding frequency as well. However, feeding a diluted diet twice a day did not result in any positive effects on home pen behaviour compared to the other treatments. Despite the lack of interaction between dilution and feeding frequency, solely increasing the frequency had some effects on behaviour. During the rearing period, birds that were fed twice a day sat less, and walked more compared to the FO pullets until 10 weeks of age. An increase in walking behaviour in pullets fed twice a day was also found by de Jong et al. (2005b). An increase in walking reflects an increase in activity, which can be caused by frustration (Savory and Maros, 1993; Hocking, 1996; de Jong et al., 2002), or can be anticipatory behaviour towards a meal. Increasing the number of meals per day, however, appears to result in a redistribution of activity rather than specifically affecting hunger, since there was no effect of feeding frequency on time spent on nearly all behaviours. A study with precision feeding of BB pullets in which meals were limited according to the weight of the pullet, have shown that pullets received on average 10 daily meals in the feeding station (Zuidhof, 2018), indicating that when given the opportunity, birds may prefer to eat more meals per day than is commonly provided during the layer period. However, the average success ratio (meals:visits) in that study was 17%, thus many unnecessary visits to the feeder were performed, showing a high motivation to look for feed which is also indicated by our own observations. Other studies with precision feeding observed more aggression (Girard et al., 2017a), less restlessness and standing and more object pecking, but no elimination of feeding motivation (Girard et al., 2017b). Although our study found more standing behaviour in FT pullets, all studies show effects of meal frequency on behaviour. There are, however, obvious differences between the management and feeding systems, which could also explain the differences in results. Feeding frequency also affected foraging behaviour, although effects were dependent on the age of the chickens. Foraging behaviour is considered an important welfare related behaviour for chickens. It is part of their natural repertoire, and therefore fulfils behavioural needs. Moreover, foraging is associated with feed-searching related behaviour and counteracts abnormal or undesirable behaviour. Time spent on foraging cannot be spent on other, abnormal behaviours such as object pecking or feather licking. On the other hand, an essential part of foraging is searching for food, thus an increase could imply hunger. The results of our study indicate that birds are increasing foraging behaviour in an attempt to find feed. Since little to no feed can be found in the litter, an increase in foraging could lead to more frustration and stress. In line with our results, de Jong et al. (2005b) found that birds who were fed twice a day showed less foraging behaviour during early rearing. It is possible that the distribution of meals throughout the day increases the level of satiety, which decreases the need to forage. However, in the second half of the rearing period we found the opposite effect, suggesting that satiety might be increased in the pullets fed once a day.

Home pen behaviour was affected by time of day. Chickens have a stable daily pattern in which the first part of the light period is mostly used for eating, drinking, oviposition and foraging, while the second part is used for grooming and resting (van Emous et al., 2015). Feeding frequency had a major effect on the daily distribution of all home pen behaviours. Before the first meal, a high percentage of pullets, independent of their treatment, was observed standing. Towards the second meal, the FT pullets showed more standing behaviour than the FO pullets; however, this was a much lower proportion than before the first meal (65% all pullets vs 22% FT pullets), furthermore more walking and standing were observed. Both observations indicate anticipation of the meal, as has been found by de Jong et al. (2005b). Similarly, FT pullets showed more walking and less foraging before the second meal than FO Pullets and drinking was strongly related to the time of feeding. Interestingly, our pens were separated by wire which only blocked the birds' line of vision for 40 cm from the ground up, meaning the FO pullets could hear and to some extent see the FT pullets eating. This did not appear to cause more frustration for the FO pullets, since they were relatively inactive during the second meal of the FT groups.

In both the rearing and the laying period, age was an important factor affecting several home pen behaviours, in isolation or in combination with the feeding strategies. Our findings indicate that behavioural patterns change over time and reflect the importance of conducting observations at a range of ages when studying birds in longitudinal experiments, as has been also shown in other studies (Arrazola et al., 2020). The results of our study show that most behaviour indicative of frustration and hunger was expressed during the second half of the rearing period, since object pecking behaviour increased, while sitting and comfort behaviour decreased, a pattern that was not observed in Arrazola et al. (2020).

Despite the small effects of the nutritional treatments applied during the rearing period, some effects on behaviour during the laying period were observed. Feeding strategy influenced foraging behaviour in lay, since the birds that were fed twice a day during the rearing period showed less foraging behaviour in the laying period, and this was also found in late rearing. Experiences and behavioural patterns learned during the rearing period can affect behaviour in later life, thus this finding might show the importance of behavioural ontogeny and learning instead of reflecting persistent effects of the feeding strategies. Contrary to our results, diet dilution with calcium propionate and oat hulls by Sandilands et al. (2005) and alternative diets with soybean hulls by Morrissey et al. (2014) resulted in differences in behaviour during rearing, which faded away during the laying period once breeders were fed the same diet. Others also found no effects of nutritional treatments during the rearing period on layer behaviour (van Emous et al., 2015), or found effects that were likely attributed to differences in feeding time (de Jong et al., 2005a). In the current study, the latency for 3 birds to approach the NO within a 25 cm radius at 39 WOA was affected by treatment. Since the hens that were in the CON-ONCE treatment group showed a lower latency to approach compared to the other groups (3 s vs 14 s), there appears to be some carry-over effects on fear and motivation to explore the novel object. Unfortunately, these results were not confirmed by the other observations.

In contrast to our hypothesis, the nutritional strategies had no clear effects on breeders' welfare and behaviour, both in the rearing and laying period. The increase of one meal to two daily meals would already be a big change in practice. However, to be effective, the feeding frequency could be increased even more, which should be further studied. Furthermore, increasing the dilution of the diet which is more in line with the level of feed restriction could be a more successful strategy. Potentially nutritional strategies could be a simple but effective solution to increase broiler breeder welfare, which needs more research to be proven.

5. Conclusions

In conclusion, feeding diluted diets and/or feeding twice a day did not have any obvious benefits to BB welfare. The present study showed that diet dilution with 10–20% oat hulls, increasing the feeding frequency to twice per day, or a combination of the two resulted only in minor effects on the welfare of broiler breeders in rearing, since behavioural indicators of stress, fear, motivation and frustration were not affected or showed minor changes. Increasing the feeding frequency affected behavioural patterns during the day which could indicate anticipation for the second meal. Lastly, the nutritional treatments applied in rearing had only slight effects on home pen behaviour during the laying period which aligned with the results found during the rearing period.

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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