

# Health effects of feed dilution and roughage in Ross 308 broiler breeder cockerels

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**ABSTRACT** To ensure reproductivity and prevent obesity, broiler breeder's growth rate is controlled by quantitative feed restriction. However, feed restriction is associated with chronic hunger, frustration, and abnormal behaviors, thus representing a welfare problem. Feed diluted with insoluble fiber is an alternative, allowing larger amounts of feed and more gut filling, increasing satiety without increasing the caloric intake. Previous research on feed dilution has focused on pullets not cockerels. In addition, the health effects of diluted feed are less explored. The aim of this study was to investigate the effects of feed dilution and roughage on health indicators in broiler breeder cockerels, 5 to 10 wk of age (**WOA**), a very feed restricted period. In total, 200 Ross 308 broiler breeder cockerels were housed in 12 pens (6 pens/treatment), 16 to 17 birds per pen. The treatments were standard feed (Control) and feed diluted with (20%) insoluble oat hulls and 150 g of roughage (lucerne/alfalfa) daily per pen (D + R). The

D + R birds received 20% more feed per day. Body weight, pecking injuries, footpad dermatitis (**FPD**), and litter quality were recorded weekly between 5 and 9 WOA. At 10 WOA all birds were euthanized and subjected to postmortem assessment. D + R birds tended to weigh more than controls ( $P = 0.055$ ), but diet did not affect flock uniformity ( $P = 0.57$ ). The weekly assessment showed no effect of treatment on foot pad lesion scores ( $P = 0.31$ ). However, an effect on FPD was observed postmortem ( $P = 0.04$ ), where Control had slightly better footpad condition compared to the D + R group. Litter had to be completely changed in 2 control pens during the trial, due to quality deterioration. There was no effect of treatment on the weight of specific organs, the length of the gut, the intestinal mucosa, nor on the incidence of wounds on the head ( $P = 0.15$ ). Overall, these results indicate that feed dilution and roughage have neither adverse nor beneficial health effects on Ross 308 broiler breeder cockerels.

**Key words:** broiler breeder, cockerels, feed restriction, feed dilution, health

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## INTRODUCTION

Increased growth rate has been an important criterion for genetic selection in modern broiler chickens for decades (Torok et al., 2011; Zuidhof et al., 2014). This selection also affects the growth rate of the parent stock, the broiler breeders, who have a very high feed consumption if fed ad libitum. In the breeders, the results of ad libitum consumption are reproductive issues, like decreased sexual activity, and health problems, such as obesity,

ascites, and leg pathologies (Mench, 2002; De Jong and Guémené, 2011). Therefore, the growth rate in the broiler breeders is typically controlled by restricted feeding, especially during rearing.

Such quantitative feed restriction has positive effects on important health parameters, through a reduction in obesity and leg problems. On the other hand, severe feed restriction has negative effects on bird welfare including chronic hunger, stress, frustration, aggression, and abnormal behaviors, such as stereotypic object pecking, pacing, and overdrinking (Shea et al., 1990; Mench, 2002; De Jong et al., 2003; De Jong and Guémené, 2011; Nielsen et al., 2023). Thus, alternatives to quantitative feed restriction are needed.

Several studies have tried to mitigate this challenge by providing qualitative feed restriction methods, meaning increasing the amount of feed allocated per bird by

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increasing the fiber content while keeping the energy level similar to standard, commercial feed. The aim of this strategy is to increase satiety due to gut fill, by diluting the feed with non-nutritive ingredients such as fiber (De Jong et al., 2005; Tolcamp et al., 2005; Nielsen et al., 2011). This provides the birds with larger daily feed portions, without receiving more calories. In addition, this increases the time spent feeding and therefore reducing the frustration caused by feeding motivations (De Jong et al., 2005; Moradi et al., 2013). Furthermore, feed dilution is found to reduce long-term stress (Zuidhof et al., 1995), lower the corticosterone response (Harvey et al., 1983; Moradi et al., 2013), and to reduce stereotypic pecking (Hocking et al., 2004; Nielsen et al., 2011). Furthermore, insoluble fibers in the diet have also shown a preventative effect on feather pecking in laying hens (Desbruslais et al., 2021). However, diluents are costly, and it is difficult to find ingredients that do not affect the quality of the feed, the production results or the gut health negatively.

The gastrointestinal tract has digestive, absorptive, metabolic, immunological and endocrinological functions (Perry, 2006). Therefore, the intestinal health of poultry may affect both health and production efficiency (Oviedo-Rondón, 2019). Soluble fibers may affect the microbiota in the poultry gut. In contrast, the insoluble fibers are not degraded extensively by bacterial fermentation in poultry, thereby leading to relatively insignificant alterations in the microbiota (Hetland et al., 2004). Previous studies with insoluble fibers in broiler chickens have shown a positive effect on intestinal health (Kalmendal et al., 2011); however, the effect on broiler breeder cockerels needs to be explored further. Therefore, it is important to investigate how feed dilution affects gut health and the development of gastrointestinal organs, like the gizzard, the gut lining and the length of the gut, in broiler breeder cockerels.

Furthermore, it is important to investigate the effects of diluted feed on litter quality and footpad dermatitis (FPD). FPD is a type of contact dermatitis that causes inflammation and necrotic lesions on the plantar surface of the footpads (Ekstrand et al., 1998; Shepherd and Fairchild, 2010). Wet litter and ammonia irritation from the bedding are the main causative factors (Cengiz et al., 2011; Kyvsgaard et al., 2013). The provision of roughage can stimulate foraging behavior, increasing the scratching and aeration of the litter, thereby improving the quality of the litter. Another possible outcome is that diluted feed may lead to increased water intake, which in turn can affect litter quality negatively. It is, therefore, necessary to investigate the effect of diluted feed and roughage on FPD and litter quality in broiler breeder cockerels.

Effects of diluted feed in broiler breeders have previously been studied in breeder hens, and information on how feed dilution affects roosters is, to our knowledge, lacking. Furthermore, the immediate effect of different feeding strategies on health has not been investigated thoroughly, in hens nor roosters. In the present study, in order to explore the potential of improving rooster

welfare by qualitative feeding strategies, the main aim was to investigate the effects of feed dilution with 20% insoluble oat hulls and daily roughage provision on selected health indicators in broiler breeder cockerels, with weekly investigations of foot pads, pecking injuries, dirtiness, and plumage condition, between 5 and 9 wk of age (WOA), a very feed restricted period in the bird's life. In addition, we performed postmortem investigation on all cockerels at the end of the trial, to examine effects on the gizzard, the gut, the footpads, and prevalence of pecking injuries. Finally, the effect of feed dilution on litter quality was also investigated.

## MATERIALS AND METHODS

### *Ethical Approval*

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

### *Birds and Housing*

The chicks (Ross 308) were hatched at a commercial hatchery, vent sexed and transferred to a commercial rearing facility, where they lived until 5 WOA. The birds were not beak trimmed but were toe clipped. The rearing barn consisted of 8,200 hens and 1,100 roosters, housed in sex separated compartments in the barn. The barn was fully insulated with mechanical ventilation and concrete floor with wood shavings as litter. Management during rearing followed the recommendations of the breeding company and Norwegian regulations (Landbruks-og Matdepartementet, 2006). The flock was managed according to standardized practices with regards to feed, water, ventilation, litter, and lighting (Norwegian Quality Standard, KSL, 2020), with a short dusk period, where the light gradually decreased over 5 min. The cockerels were fed pelleted feed using a spin-feeder once per day. At 5 WOA, 200 cockerels were selected based on a live weight close to the Ross breeding manual's weight recommendation at the age. The birds were caught by trained poultry catchers, crated in pullet transport containers, and transported 2 h in a climate-controlled truck to the animal husbandry experimental research facility, Center for Husdyrforsk, at the Norwegian University of Life Sciences. At arrival, the cockerels were randomly allocated to 12 elevated pens with solid floors, 16 to 17 birds per pen. The pens measured 296 × 60 × 71 cm (length × height × depth). All pens were covered with fresh wood shavings. Water was provided ad libitum from nipple drinker lines (4 nipples per pen). Each pen was equipped with a 70 cm jute rope (ø: 20 mm) hanging by the middle (i.e., 2 tail ends side by side) from the ceiling as environmental enrichment. All pens were situated in the same room. Ventilation, humidity, temperature, and lighting were according to the Ross breeding manual and consistent across treatments. The light period was 8 h, with light intensity of

**Table 1.** Nutritional content for the experimental diets provided.

Feed formula	Age (wk)	ME (MJ/kg)	Protein (g/kg)	Crude fiber (%)	Soluble NSP (%)	Nonsoluble NSP (%)	Daily amount of feed g/bird/d <sup>1</sup>
Starter							
Control	0–5	11.80	168	4.77	3.06	12.73	15–61
Grower							
Control	6–10	11.20	135	4.93	2.59	15.59	62–78
Diluted	6–10	9.10	113	10.97	2.72	28.86	74–94
Alfalfa	6–10	3.58	168	27.10	Total NSP 55%		Ca. 8–9

<sup>1</sup>The daily amount increased according to the weight of the birds. This shows the increase from the first week to the last week.

10 lux. The cockerels were allowed 3 d of habituation period to the experimental facilities before the start of the study. During the first day of the habituation period, all cockerels received the same feed as they had received in the rearing farm (starter control, described in [Table 1](#)). The provision of the treatment diets started on the third day following the birds' arrival.

## Treatments

Each of the 12 pens was assigned 1 of 2 treatments such that each treatment had 6 replicates. Furthermore, the placement of the pens in the room was done to account for the potential differences in the physical condition in the room (variations in humidity, temperature, activity by the doors vs. the back of the room, etc.). The trial was set up with 4 rows of pens, 3 pens in each row. The control and trial pens alternated throughout the room, so both treatments were represented in all rows and all parts of the room. The 2 treatments were Control, and Dilution + Roughage (**D + R**), both provided as pellets (2.50 mm). The control diet was formulated according to nutritional specifications of a commercial rearing diet. Raw material composition was optimized as similar as possible between control and diluted diet to avoid raw material effects. The **D + R** diet was diluted with 20% oat hulls, reducing metabolizable energy (**ME**) and digestible amino acids content by one fifth, resulting in 20% more feed allowance per bird per day ([Table 1](#)). In all pens, the pelleted feed was given once per day, scattered on the floor of the pen at 09:00. In addition, the **D + R** diet received a total of 150 g lucerne/alfalfa roughage/pen/day provided 15 min after the pelleted feed in metal hay feeders attached to the side of the pen. Feed amounts allocated per bird in the control group were based on Norwegian growth curves for broiler breeder cockerels and recommendations of the breeding company ([Aviagen, 2016](#)).

## Health Registrations

All cockerels in all pens were examined at 5, 6, 7, 8, and 9 WOA. The weekly examination included weighing, investigation for FPD, evaluation of plumage condition, dirtiness, and pecking injuries, along with assessment of the litter quality. All birds were examined and weighed individually. Scoring of the footpads including lesions on the toes was based on the size of the lesions. A score 0

indicates no lesions, score 1 is a very minor lesions, score 2 is a lesions affecting 25 to 50% of the footpad, score 3 is more than 50% of the foot pad and score 4 is a severe lesions affecting the majority of the foot pad and the toes, according to the Welfare Quality Assessment Protocol for Poultry ([Butterworth, 2009](#)). Both footpads and all toes were examined after brushing off litter and fecal material. In cases of discrepancy between the feet, the highest score was recorded for that animal, according to the Welfare Quality Assessment Protocol for Poultry ([Butterworth, 2009](#)). Several of the cockerels had lesions on the toes, but not on the footpads. Such birds were scored on the same scale, but with the size of toe lesion as a determinant for the footpad lesion score. See [Figure 1](#) for an example of a score 2.

Plumage condition was scored for head, back/wings, breast, and tail in a 3-point scale (0–2); 0 = no feather loss, 1 = feather loss 1 to 5 cm in diameter, 2 = feather loss above 5 cm in diameter. Dirtiness was defined as prominent dark staining of the back, wing, or tail feathers, covering at least 25% of the body; not including light



**Figure 1.** An example of a footpad with a score 2, according to the Welfare Quality assessment protocol.

**Table 2.** Scoring of litter quality according to the Welfare Quality Assessment Protocol.

Litter quality	Classification
0	Completely dry and loose
1	Dry but not easy to move
2	Will form a ball if compacted, but ball does not stay well together
3	Sticks readily in a ball if compacted
4	Sticks to boots once the cap or compacted crust is broken

discoloration of feathers from dust. Wounds were evaluated on the head, tail, cloaca, and back. A wound was defined as a prominent mark in the skin, either fresh with blood or with crust. Litter quality was assessed in all pens according to the score described in the Welfare Quality Assessment Protocol for Poultry (Butterworth, 2009), ranging from 0 for dry and loose litter to 4 for solid litter covered with a crust (Table 2). If the litter was scored as a 2 new, fresh litter would be applied on top of the litter to improve the litter quality. If the litter in the pen was scored as a 3 or 4 all litter would be replaced with fresh, unused wood shavings. Recordings were made each time new litter was added or if the litter was completely changed in individual pens.

### Postmortem Assessment

All birds were subjected to postmortem assessment at 9 WOA on 2 consecutive days by 2 experienced veterinarians. The birds were stunned 1 pen at a time, by blunt trauma to the head and euthanized with cervical dislocation, followed by an immediate postmortem investigation. The time frame between culling and dissection was 5 to 45 min. The postmortem included weighing of the whole bird, followed by external examination, including evaluation of the plumage condition, footpads, length of the tibia, wounds, and dirtiness. The internal examination included inspection of the femoral heads and visual inspection of the body cavity to look for pathological conditions. Femoral head necrosis was scored on a dichotomous scale (yes/no) in the case of macroscopically visible lesions in the growth plate, fractures of the neck and head of the femur, brittle cortex or separation of the articular cartilage (McNamee, 2010). The heart, liver, and full gizzard were weighed. The koilin layer and the gizzard mucosa were inspected macroscopically for pathology like detachment of the koilin layer, bleeding, or lesions in the mucosa. The full length of the entire gut, from the start of the duodenum to the cloaca, was measured. The entire intestinal tract was opened to inspect the mucosa for lesions, necrosis, or bleeding. The ceca were inspected for bloating and gas filling.

### Statistical Analysis

Statistical analyses were performed using the software SAS 9.4 (SAS Institute Inc., Cary, NC). The data on the

live weight measured from wk 5 to 9 were analyzed using the mixed procedure, with the diet treatment, week of age, and their interaction as fixed factors. Pen was the experimental unit and was included in the models as a random effect. Post hoc analyses were performed with the Tukey test (Tukey's HSD test). The critical alpha value was 0.05. Flock uniformity calculated as the coefficient of variation per pen (CV %) using the following formula and analyzed using the same model as the live weight:

$$CV \% = \frac{\text{Pen standard deviation}}{\text{Average pen weight}} \times 100$$

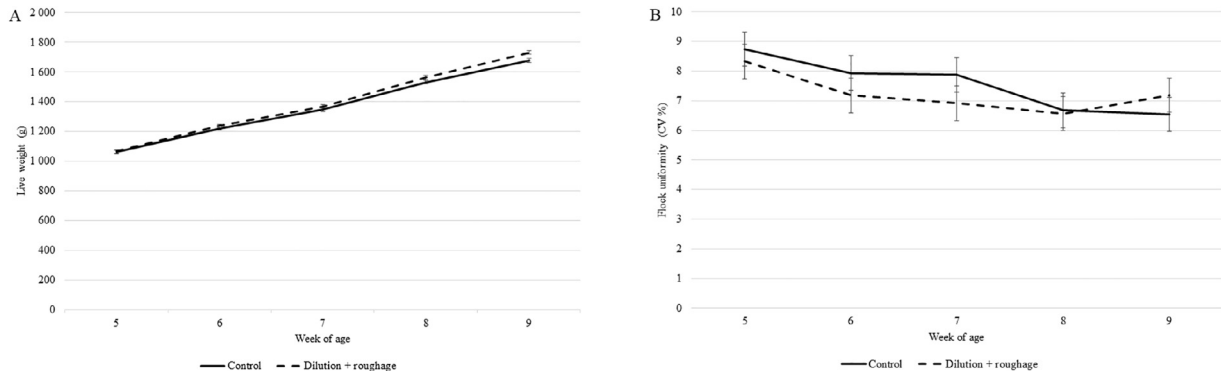
The scores for footpad dermatitis and litter quality during the health assessments were analyzed using the glimmix procedure with an ordered multinomial distribution. As in the mixed model, diet treatment, diet and their interactions were included as fixed factors and pen as a random factor. The prevalence of head wounds was analyzed with a glimmix with binary distribution (Yes/No).

The analysis of the postmortem data was similar to that of the live health assessments, with the exception that week of age was not included in any model, as the dissections were performed during 2 consecutive days. The body weight of the birds dissected on the second day of postmortem was corrected by subtracting the weight of the extra daily feed portion that they received compared to those birds culled the day before. In addition, the models for the weight and/or length of the body parts assessed during the dissections (i.e., liver, gizzard, heart, intestines, and tibia) were corrected for the body weight of the bird by including this variable in the model as a covariate. Finally, the prevalence of pathologies (i.e., alterations of the intestinal mucosa, head wounds, femoral head necrosis, and the presence of gas in the ceca) were analyzed with a binomial distribution glimmix with treatment as a fixed factor and pen as a random factor.

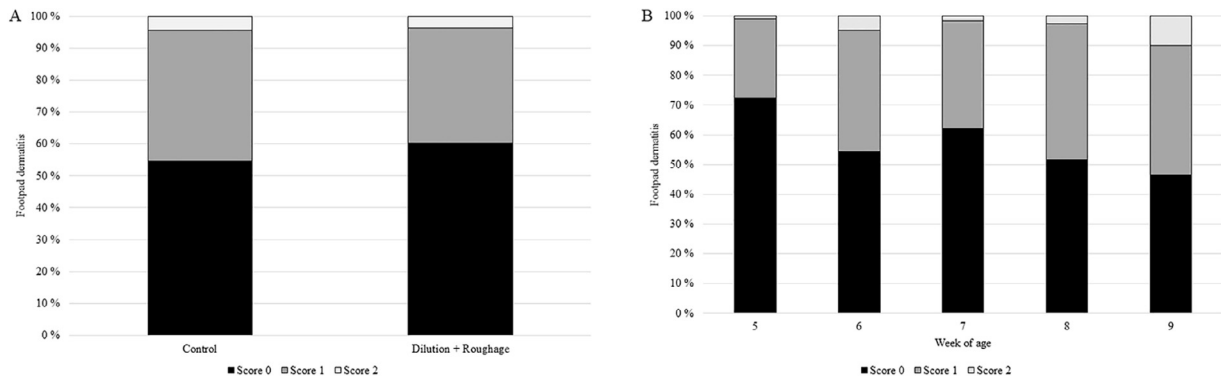
## RESULTS

The results show that, while the birds from both treatments grew from week to week ( $F_{4,929} = 1,189.33$ ;  $P < 0.0001$ ), there was no interaction effect between the diet and the age of the birds ( $F_{4,929} = 1.57$ ;  $P = 0.18$ , Figure 2). There was an overall tendency for the birds in the D + R treatment to weigh more than the control birds at all ages (LS means  $\pm$  SE: D + R = 1,393.20 g  $\pm$  8.3; Control = 1,367.70 g  $\pm$  8.3;  $F_{1,10} = 4.72$ ;  $P = 0.055$ ). There was no effect of diet on the uniformity of the flocks (CV % LS means  $\pm$  SE: Control = 7.55  $\pm$  0.39; D + R = 7.23  $\pm$  0.39;  $F_{1,10} = 0.33$ ;  $P = 0.58$ ; Figure 2). There was, however, an effect of age ( $F_{4,40} = 4.76$ ;  $P = 0.003$ ), with the coefficient of variance reducing with age such as it was smaller at wk 8 and 9 of age compared to wk 5 ( $P < 0.01$ ) (Figure 2).

There was no effect of treatment on the incidence of footpad dermatitis ( $F_{1,930} = 1.02$ ;  $P = 0.31$ ). There was an overall effect of age ( $F_{4,930} = 9.15$ ;  $P < 0.0001$ ), with



**Figure 2.** Live weight (panel A, LS means  $\pm$  SE) and flock uniformity CV % (panel B, LS means  $\pm$  SE) per week for each treatment.

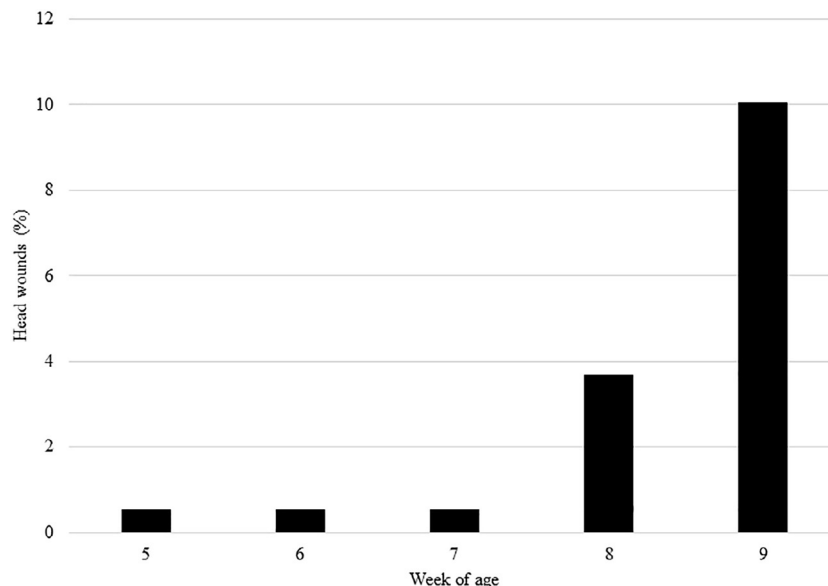


**Figure 3.** Frequency (%) of footpad dermatitis (FPD) scores per treatment (panel A) and per week of age (panel B). Higher scores represent higher severity of FPD.

a higher likelihood of more severe FPD at older ages (Figure 3).

Fresh litter was provided throughout the study to improve declining litter quality (score 2 or higher). In total, fresh litter was provided 10 times to the control pens and 9 times to D + R pens. A complete exchange of litter was done 3 times in control pens, 2 of the times in the same pen. A complete exchange of litter was not necessary in any of the D + R pens.

There was no effect of treatment on the incidence of wounds on the head ( $F_{1,935} = 2.06$ ;  $P = 0.15$ ; Control = 1.05%; D + R = 5.04%). Nevertheless, the expected effect of week of age was observed ( $F_{4,935} = 7.13$ ;  $P < 0.0001$ ), with birds having higher odds of having head wounds at 9 wk compared to the other weeks (odds ratio  $>3.8$ ; Figure 4). No feather loss was observed in any body part. Vent pasting was only observed in 17 cases, 8 of them in Control birds and 9 in



**Figure 4.** Prevalence (%) of head wounds observed during live health assessment across weeks of age.

**Table 3.** Comparison of weight and length (LS means  $\pm$  SE) of body parts measured in the postmortem assessment across diet treatments.

Organ	Control			Dilution + Roughage			P value
	LS means	SE	% body weight	LS means	SE	% body weight	
Liver (g)	33.04	0.62	1.97	33.08	0.62	1.91	1.0
Gizzard <sup>1</sup> (g)	60.44	1.34	3.60	60.88	1.34	3.52	0.8
Heart (g)	8.59	0.28	0.51	8.32	0.28	0.48	0.5
Intestines <sup>2</sup> (cm)	181.62	2.20	na	184.47	2.20	na	0.4
Tibia (cm)	54.06	0.56	na	53.90	0.57	na	0.8

<sup>1</sup>Full gizzard.

<sup>2</sup>The full length of the intestines from the start of the duodenum to the cloaca.

D + R birds. Furthermore, there was only 1 observed occurrence of toe pecking, on a Control bird.

Over the course of the experiment, 7 birds (4 control and 3 D + R) were euthanized due to health issues. These included wing fractures which occurred during transport, stunted growth, lameness, skin infections, and subcutaneous emphysema. Therefore, a total of 193 birds were dissected for the postmortem assessment. The data from these birds show that there was a tendency for the birds in the D + R group to be heavier (LS means g  $\pm$  SE: 1,839.07  $\pm$  16.75) than the control birds (LS means g  $\pm$  SE: 1,788.54  $\pm$  16.70;  $F_{1,10} = 10.2$ ;  $P = 0.06$ ). Nevertheless, there was no effect of diet on the weight of the liver ( $F_{1,10} = 0.00$ ;  $P = 0.96$ ), the gizzard ( $F_{1,10} = 0.05$ ;  $P = 0.82$ ), or the heart ( $F_{1,10} = 0.46$ ;  $P = 0.51$ , Table 3). Likewise, diet did not affect the length of the intestines ( $F_{1,10} = 2.24$ ;  $P = 0.16$ ), or of the tibia ( $F_{1,10} = 0.04$ ;  $P = 0.84$ ), Table 3.

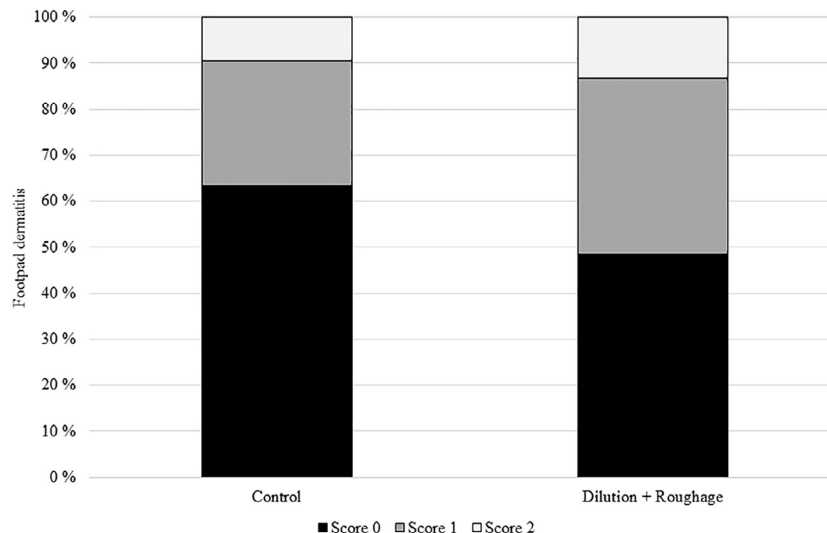
There was a significant effect of diet on the postmortem FPD ( $F_{1,180} = 4.19$ ;  $P = 0.04$ ). As can be seen in Figure 5, birds from the control group were slightly more likely to have lower FPD scores (better footpad condition) compared to those in the Dilution + Roughage group (odds ratio = 1.79; CI = 1.02–3.14).

Regarding the other pathologies assessed, diet did not affect the intestinal mucosa ( $F_{1,181} = 1.18$ ;  $P = 0.28$ ), head wounds ( $F_{1,181} = 0.91$ ;  $P = 0.34$ ), femoral head

necrosis ( $F_{1,181} = 0.27$ ;  $P = 0.60$ ), or gas filling of the ceca ( $F_{1,181} = 2.18$ ;  $P = 0.14$ ; Table 4). Finally, dirtiness was observed only once, in a control bird.

## DISCUSSION

The main purpose of this study was to examine the effects of a diluted feed and daily roughage provision during rearing on selected health indicators in broiler breeder cockerels (Ross 308). There was a tendency for the birds in the D + R treatment to weigh more than the control birds during the trial. It is unclear if this is related to the diluted feed or to the roughage, or the combination of the 2. Lucerne does not provide high nutritional value for birds (Pres and Fritz, 1963) and the fiber used in the pelleted feed was insoluble, therefore it is unclear what caused the observed effect on the birds' weight. Nevertheless, the results show that this tendency in weight difference took several weeks to appear and did not start immediately. In a similar study of qualitative feeding restriction in breeder Ross 308 pullets, birds fed an extra daily provision of maize roughage had a steeper growth curve compared to control birds, while those that were fed a 20% diluted pelleted feed had weighed less than the control birds (Riber et al., 2021). In the present study, alfalfa was provided as roughage instead of maize, since lucerne has lower



**Figure 5.** Frequency (%) of footpad dermatitis observed during the postmortem assessment at 10 wk of age. Higher scores represent higher severity of FPD.

**Table 4.** Prevalence (%) of pathologies assessed during postmortem across diet treatments.

Pathology	Prevalence (%)		P value
	Control	Dilution + Roughage	
Intestinal mucosa <sup>1</sup>	35.40	27.90	0.28
Head wounds	47.92	37.11	0.34
Femoral head necrosis	30.21	26.80	0.6
Gas in the ceca	10.42	26.80	0.14

<sup>1</sup>Lesions, necrosis, or bleeding.

caloric content (Pres and Fritz, 1963), which can account for a smaller effect size on live weight in the present study compared to the pullet study by Riber et al. (2021) where maize was provided. In Norway, alfalfa is provided as environmental enrichment in commercial production. Birds rather play with alfalfa than eating it. This was confirmed by the present trial where the birds ingested only the leaf fraction leaving the stipe untouched. The nutritional contribution of alfalfa is therefore considered minimal. Our hypothesis that a feed dilution of 20% leads to a higher feed allowance of the same energy proportion might have been too optimistic for the cockerels. According to measured body weights, an increase in feed allowance between around 17 to 18% to follow the same weight curve as the control group might be more realistic. However, we cannot exclude that there was a difference in feed digestion between the 2 treatments and that this was the cause of the weight differences observed.

There was no effect of treatment on flock uniformity in our study, which is important in a commercial setting. This result is in contrast to Zuidhof et al., who found high fiber treatment groups to be least uniform in terms of weight compared to standard, commercial breeder feed (Zuidhof et al., 2015). Furthermore, we found flock uniformity to increase with age in both groups, which was a surprise, since it is not uncommon for broiler breeder cockerels to have a nonuniform growth (Liu et al., 2021).

There was no effect of treatment on the incidence of footpad dermatitis during the weekly investigations. This is in line with previous research which has found that birds from diluted, insoluble treatments to not differ from control birds in terms of footpad dermatitis (Tahamtani et al., 2020). However, there was an overall effect of age on FPD, with a higher likelihood of footpad lesions at older ages. This is in line with research from broiler chickens, where the footpad health deteriorates with age (de Jong et al., 2012). Additional litter was added to the pens when the litter quality scored 3 or 4. This litter improvement was performed as many times in the control pens as in the D + R pens. However, in 2 control pens litter had to be completely changed during the trial due to severely poor litter quality. The FPD would likely be more severe in some pens, regardless of treatment, if no actions had been taken to improve the litter quality. It is not possible to say if the wet litter was a result of increased water intake or water wastage, however both can be a sign of frustration or hunger in feed restricted broiler breeders. Unfortunately, it was

not possible to measure water intake in the individual pens. This should, therefore, be included in future studies, as a source of valuable information.

Contrary to the weekly observations, there was a significant effect of diet on the FPD examined postmortem, where birds from the control group were slightly more likely to have lower FPD scores compared to those in the D + R group. A higher feed intake will inevitably result in more feces and a higher load on the litter, which may explain this finding. Arrazola et al. found that inclusion of soybean hulls alleviated stress and acted as an intestinal filler but resulted in a higher intestinal water content, wetter litter, and foot lesions compared to control diets (Arrazola, 2018; Arrazola et al., 2019). However, this is in contrast to a study by Hocking (2006), who found high-fiber diets to decrease water intake in broiler breeder hens and thereby to reduce litter moisture (Hocking, 2006). A more recent study suggests that the type of fiber in the diet is a factor for litter quality. In their study, Riber and Tahamtani (2020) found that provision of a diet including both soluble (sugar beet pulp) and insoluble (oat hulls) fibers to broiler breeder pullets resulted in worse litter condition compared to a control diet while a diet with only insoluble fiber did not. Furthermore, they found that litter condition was improved by provision of maize roughage compared to a control without roughage (Riber and Tahamtani, 2020). This is contrary to our study, with roughage and insoluble fiber. Nevertheless, it must be emphasized that no severe lesions were observed during the present study. The highest score noted was 2, which is a moderate lesion.

Feather coverage score is considered a welfare indicator that is related to feather pecking activity (Girard et al., 2017). Pecking injuries to the comb were observed in some pens during the weekly health observations, and during the postmortem investigations. Recent research has showed a preventative effect of insoluble fibers on feather pecking in laying hens (Desbruslais et al., 2021). In the present study, there was no effect of treatment on the incidence of wounds on the head, however all birds had higher odds of having head wounds at 9 WOA compared to the other weeks, regardless of treatment. No severe injuries were observed and no pecking injuries on other body parts were seen. Aggression in male broiler breeders is a common problem, and it is often directed toward females. This has previously not been found to be a function of feed restriction, but more an overall male broiler breeder aggression issue, which is not found in layer strain males nor in game strain males (Millman and Duncan, 2000). Feed restriction in broiler breeders has been found to lead to gentle feather pecking redirected toward the tail and vent leading to a decreased feather coverage in broiler breeders (Nielsen et al., 2011; Van Emous et al., 2014), however, this was not found in our study. The cockerels in our study were young birds, and we would expect more aggression later in life than at this stage.

The postmortem investigation on wk 10 showed that there was a tendency for the birds in the D + R group to

be heavier than the control birds. Insoluble fibers have been shown to affect the anatomy of the gastrointestinal tract (Jiménez-Moreno et al., 2019). However, there was no effect of diet on the weight of the gizzard, nor on the liver or heart in the present study. This is in contrast to previous research, that have shown moderate amounts of insoluble fiber, especially oat hulls, to increase gizzard weight in young female Cobb broiler chicks and broiler breeder pullets (De Los Mozos et al., 2017; Jiménez-Moreno et al., 2019). The mentioned study with broiler chicks lasted only a few weeks, while the study with broiler breeder pullets lasted 19 wk. This difference may indicate that the treatment is more important than the duration for the development of the gizzard. In a study by Hetland and Svihus (2001) inclusion of oat hulls was found to increase gut weight due to increased feed consumption (Hetland and Svihus, 2001). However in the present study there was no ad libitum feeding, which may explain the lack of effects on the weight of the internal organs, even though the gizzards were weighed with their contents inside. Furthermore, diet did not affect the length of the intestines, a result also found by Jiménez-Moreno (Jiménez-Moreno et al., 2019). Suboptimal digestion may lead to the excess of nutrients in the hindgut (Brown et al., 2012). This may in turn lead to disruption of gut microbiome-host equilibrium, causing metabolic, pathogenic or sterile inflammation (Kogut et al., 2018). Several of the birds showed macroscopic signs of mild inflammation in the duodenum mucosa which was not explored with further histology. Nevertheless, there was no difference between the cockerels fed standard diet and the broilers fed diluted feed and therefore no indications of suboptimal digestion related to diet in our study, which was to be expected since insoluble fibers are not found to negatively affect microbiota in poultry (Hetland et al., 2004). Further studies should explore the effects of insoluble fiber and roughage on gut passage time and microbiota in broiler breeder cockerels.

During postmortem investigation, several of the birds exhibited signs of femoral head necrosis (FHN) irrespective of diet treatment. This finding was surprising, since no birds had exhibited signs of lameness or reluctance to walk during the weekly observations. FHN is defined as lesions in the growth plate, fractures of the neck and head of the femur, brittle cortex or separation of the articular cartilage (McNamee, 2010) and may be related to bacterial infections, like *E. coli* or *Staphylococcus aureus*. The rearing farm from which the birds originated had experienced *E. coli* problems during the first week of life of the flock. Even though the birds were clinically healthy upon arrival at the research facility, it is likely that they carried the bacteria with them, which later on resulted in the observed FHN during the postmortem assessment. Another possible explanation is that the size of the pens restricted the physical activity of the birds. Physical activity is important for leg health in broiler chickens. No bacterial samples were obtained, so a conclusion as to the cause of the observed FHN cannot be made.

In conclusion, the birds receiving diluted feed and roughage tended to weigh more than control birds at the end of the study, but there was no diet effect on flock uniformity. Furthermore, there was no effect of diet on the internal organ weights nor any negative effect on the gastrointestinal tract. The weekly assessment of the footpads showed no effect of treatment, however, there was a significant effect on FPD, observed postmortem, where Control birds had slightly lower scores compared to the D + R group. No severe lesions were found at any time during the trial. There was no negative effect of treatment on the other selected health indicators measured weekly and during postmortem at 9 WOA. Overall, these results indicate that feed dilution has neither adverse nor beneficial health effects in Ross 308 broiler breeder cockerels. It must be noted that the study only included 200 birds, therefore the results must be interpreted with caution and further studies would be needed to explore the health effects on Ross 308 cockerels fed diluted feed in a commercial setting.

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## DISCLOSURES

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

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