# The effect of reduced balanced protein diet on the behavior of female broiler breeders in 2 generations

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**ABSTRACT** The behavior of 2 generations of broiler breeders undergoing a 25% reduced balanced protein (RP) dietary treatment was investigated in the current study. There were 2 treatments for the F0 generation: control (C) breeders fed with standard C diets and RP breeders fed with RP diets. The female progeny of each treatment was again subjected to 2 dietary treatments, resulting in 4 treatments for F1 generation: C/C, C/RP, RP/C, and RP/RP (breeder feed in F0/F1 generation). To maintain the target body weights throughout the trial, breeders on RP diet received on average 10% more feed than C diet breeders. The behavior of the breeders at 8h30 (30 min before feeding at 9h00), 12h00, and 15h30 in weeks 23 and 37 of the F0 generation and in week 6, 11, and 22 of the F1 generation was observed. Litter scratching, feather pecking, and object pecking were occasionally increased by RP diet feeding which indicated feeding frustration. Drinking behavior decreased dramatically by the RP dietary feeding and resulting in a better litter condition which could benefit dust bathing behavior. In addition, feeding the breeders RP diet in the F0 generation decreased litter scratching (week 6) and feather pecking (week 22, 15h30) but increased sitting (week 11, 15h30) and drinking (a tendency in week 6 and a significant effect in week 11) behavior of offspring breeders (F1 generation). In general, breeders fed with reduced balanced protein diets, to some extent, spent less time drinking and their offspring could have an adaptation to the maternal RP diet. The mechanism of this adaptation still needs to be further investigated. In general, positive effects were found by reducing protein level of breeder diets. However, negative side effects such as feeding frustration were also observed, which merit further study.

**Key words:** broiler breeder, offspring, reduced balanced protein diet, behavior, generation

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## INTRODUCTION

Driven by market demand and economic interests, genetic selection towards fast growth and high feed efficiency of broiler chickens resulted in a drastic decrease in time to reach the desirable market body weight (**BW**). When parent stock is fed ad libitum, this desirable rapid growth is accompanied by a voracious appetite which coincides with high BW, excessive mortality, and decreased reproductive performance (Hocking et al., 2002; Mench, 2002; Heck et al., 2004). Broiler breeders need to follow specialized programs of controlled feed intake to avoid overweight and to retain their egg and chick production (Hocking, 1993). On average, the normal feed allocation of broiler breeders is 25 to 33% of the feed amount when fed ad libitum (Savory et al., 1996). However, several reports have indicated the occurrence of welfare problems during these controlled feeding programs. Examples include increased stress as indicated by increased plasma corticosterone concentrations (De Jong et al., 2002) and chronic hunger and frustration as indicated by stereotypic pecking, polydipsia, and aggressive pecking (Savory and Maros, 1993; Hocking et al., 2001).

Despite numerous studies, the welfare problems of parent stock have not yet been completely remedied. Some promising approaches now exist which partially ease hunger and frustration caused by controlled feed intake, thereby alleviating the accompanying behavioral disorder. Inclusion of oat hulls, softwood sawdust, and 50 g/kg calcium propionate in the feed have been reported efficient in controlling the growth rate without negative effects on reproductive performance or rate of lay (Savory et al., 1996; Morrissey et al., 2014). Broiler breeder hens fed diluted diets were observed to drink less and perform less oral-based activities such as stereotypic pecking (Zuidhof et al., 1995). A low protein


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dietary treatment was observed to decrease stereotypic object pecking during the rearing period (Van Emous et al., 2014). This improvement in the welfare of broiler breeders was mostly attributed to a higher feed allocation to maintain the target BW, which prolonged feeding time. However, studies on the effect of dietary treatments with reduced protein level in different periods on broiler breeder behavior are rare. More research is needed to have a comprehensive understanding of the effects of reduced protein diets on the behavior of broiler breeders.

Several generations of breeders precede the broilers which enter the market: i.e., the pedigree stock, great grandparent, grandparent, and parent stock. It is well documented by Dixon et al. (2016) that environmental (e.g., temperature, stress, toxins, noise) and nutritional (e.g., prenatal malnutrition, supplements, feed restriction) conditions of the parental generation have transgenerational effects on the development, physiology, and behavior of the progeny. Perinatal low protein diet has been associated to negative developmental effects on the learning and motivation behavior of offspring rats (Reyes-Castro et al., 2011). The transgenerational effects of a low protein diet on the behavior of broiler breeders are yet unknown. Behavioral observations can be used as a basis of animal welfare assessment (Dawkins, 2003; Van Beirendonck et al., 2014; Bulens et al., 2015). Therefore, in the current study, 2 successive generations of broiler breeders treated with reduced balanced protein (**RP**) diet were generated and their welfare was assessed by behavioral observation. The effects of an RP diet on the behavior of broiler breeders and their offspring were investigated.

## MATERIALS AND METHODS

The present research was approved by the ethical commission for experimental use of animals of the KU Leuven under accession number P187/2013.

### Experimental Design

The F0 generation was subjected to either standard control (**C**) diet or an RP diet containing 25% reduced dietary crude protein (**CP**) and amino acids (**AA**). The female progeny of each treatment was again subjected to these 2 dietary treatments, resulting in 4 treatments for the F1 generation: C/C, C/RP, RP/C, and RP/RP (letters indicating the breeder feed in F0/F1 generation). The female breeders were raised until week 40 and 44 for the F0 and F1 generation, respectively.

### Birds

The detailed experimental animal information can be found in Li et al. (2018a). Briefly, a total of 160 one-day-old pure line A (a pure broiler breeder male line of an anonymous breeding company at the A position of a 4-way cross which makes up a commercial broiler) female breeders were evenly assigned to 10 floor pens resulting in 5 replicates for the C and RP treatment of the F0 generation. Concurrently, 40 one-day-old pure line A male breeders were raised for semen collection, and artificial insemination was done to produce fertilized eggs. After doing artificial insemination and egg collection for incubation (weeks 35 and 36), 152 and 136 one-day-old female F0 progeny chicks were obtained from the C and RP groups, respectively. The female progeny of the C group was evenly divided into 8 pens with 19 birds per pen and were fed on either C or RP diets. This resulted in 2 treatments (C/C and C/RP group) with 4 replicates each. Similarly, the progeny of the RP group was divided into RP/C and RP/RP groups of the F1 generation with 17 birds in each pen. Thus, breeders in the F1 generation were housed in 16 pens with 4 replicate pens per treatment.

### Husbandry and Management

A detailed description can be found in Li et al. (2018a,b,c). Briefly, a controlled amount of feed was daily allocated to each pen during the rearing period from the third week of age onwards. This feed amount was calculated per treatment on a weekly basis by the number of animals and the required BW profile (Aviagen, 2013). From the start of the laying period onwards, the egg production of the corresponding groups was also taken into account for the feed amount calculation. Light and temperature schedule were followed according to the parent stock management handbook (Aviagen, 2013). At day 3, beak trimming was carried out for each generation and vaccinations were conducted according to the standard commercial vaccination program (Galluvet, Lummen, Belgium).

### Dietary Treatment

The RP diet fed breeders received on average 10% more feed to achieve the similar target BW as the C diet fed birds. The information of BW and feed allocation is available (Lesuisse et al., 2017, Lesuisse et al., 2018a). All breeders had free access to water and were fed ad libitum for the first 2 wk. From the third week onwards, a certain amount of feed was provided at 9h00 on each day and water was available during the light period (Lesuisse et al., 2017, 2018a, 2018b). A 4-phase feeding program was applied for the pullets of both generations during the rearing period. The starter 1 diet was provided for all birds from hatch until day 21, the starter 2 diet from day 22 until 42, the grower diet from day 43 to 105, and the pre-breeder diet from day 106 until 5% of egg production (calculated for each treatment). During the laying period, a breeder diet was given to breeder hens. For each feeding phase, diets were

**Table 1.** Ethogram of recorded behavior according to Bokkers and Koene (2003).

| Behavior | Description |
|---|---|
| Litter scratching | Scraping of the litter with the claws |
| Drinking | Obtaining water from the bell shape drinker and swallow the water by raising up its head |
| Sitting | Sitting (including sternal recumbency) without performing other behavior |
| Feather pecking | Peck towards own feathers or the feathers of pen mates |
| Standing | Standing without performing any other activities |
| Walking | Walking or running without performing other behavior |
| Dust bathing | Performed with fluffed feathers while laying down, head rubbed on floor, wings opened, scratching at ground |
| Preening | Grooming of own feathers with beak while standing or sitting |
| Object pecking | Object pecking (including feather pecking, litter pecking, wall pecking, drinker pecking, feeder pecking) while standing, walking, or sitting |
| Aggressive pecking | Pecking directed to the head of a pen mate or sparring while standing, walking, or sitting |

formulated to be isocaloric and with the same raw materials composition. The RP diets had a 25% reduction in CP and all AA compared to the C diets. Comparable to the female breeders, males received a controlled feed amount from the third week onwards and were fed a standard diet during their entire experimental period. For the composition of the different diets in the rearing and laying phases, refer to the study of Lesuisse et al. (2017) and Li et al. (2018a).

### Behavioral Observations

Wide angle action cameras (PNJ CAM AEE SD23, France) were mounted over the cages to have supervision of 2 pens per camera. It was always the same 4 out of 5 pens of each treatment in the F0 generation that were filmed. In the F1 generation, the 4 replicate pens of each treatment were filmed. Behavior of the broiler breeders was observed in 2 wk of the F0 generation (weeks 23 and 37) and 3 wk of the F1 generation (weeks 6, 11, and 22). Video footage was collected on 1 D per week and 3 time points per day. Behavior was recorded at 8h30 (before feeding at 9h00) followed with another 2 recordings at 12h00 and 15h30. Each recording lasted for 20 min of which the middle 10 min were used for behavioral observations. Eight breeders were randomly chosen to conduct the 10 min focal sampling resulting in behavioral observation of 32 breeders per treatment in both generations. One experienced person performed the focal samplings of breeders in the F0 generation. A second experienced person finished the focal samplings of breeders in the F1 generation.

During the focal sampling, behaviors such as standing, walking, drinking, sitting, preening, aggressive pecking, litter scratching, dust bathing, and object pecking (including feather pecking, litter pecking, wall pecking, drinker pecking, feeder pecking) performed by the breeders during the 10 min of footage. The ethogram of recorded behaviors is listed in Table 1 based on Bokkers and Koene (2003) with slight modifications. The proportion of time spent per breeder on the different activities over the 10-min session was calculated.

### Statistical Analysis

All statistical analyses were performed using JMP Pro.12 software (SAS Institute Inc., Cary, NC). Percentages of time spent on each activity were arcsine square root transformed to meet the assumption of normality. For the F0 generation, 2-way repeated measures ANOVAs were conducted for each kind of activity to examine the effect of age, treatment, and their interaction. For the F1 generation, 3-way repeated measures ANOVA was used for every activity to test the effect of F0-diet, F1-diet, age, and their interactions. Repeated measures ANOVA was performed by each week for both generations because age effect and interactions between age and dietary treatments were frequently observed. For all the repeated measures ANOVAs, the time points (8h30, 12h00, and 15h30) of each pen were considered as repeat measurements. Behaviors that showed interaction effects between time points and dietary treatments were further investigated by doing mixed model ANOVAs to examine the treatment effects at each time point. For each mixed model ANOVA tests, pen was nested in groups and considered to be the random term. Differences with $P$ values smaller than 0.05 were considered to be statistically significant. A $P$ value between 0.05 and 0.1 was considered a tendency difference.

## RESULTS

### The Behavior of Breeders in the F0 Generation

The results of the F0 generation can be found in Table 2 and Figure 1. In week 23, no differences were seen between C and RP breeders in the time spent litter scratching, feather pecking, standing, dust bathing, and aggressive pecking. For drinking, C breeders tended to spend more time at 8h30 ($P = 0.062$) and performed significantly longer at 12h00 ($P = 0.001$) compared with RP breeders, as well as on preening behavior at all time points ($P = 0.001$). In contrast, RP breeders performed more walking behavior than C breeders ($P = 0.012$). RP breeders sat significantly more at 12h00 compared to C breeders ($P = 0.047$). RP

**Table 2.** *P*-values of the effects of reduced balanced protein diet on the behavior of broiler breeders in the F0 generation in week 23 and 37.

| | Timepoints | | | | | |
|---|---|---|---|---|---|---|
| Behavior/age | 8h30 | 12h00 | 15h30 | Treatment | Treatment * time | Time |
| Week 23 | | | | | | |
| Litter scratching | – | – | – | ns | ns | <0.001 |
| Drinking | (0.062) | 0.001 | – | 0.045 | (0.093) | <0.0001 |
| Sitting | ns | 0.047 | ns | 0.007 | 0.027 | 0.014 |
| Feather pecking | – | – | – | ns | ns | 0.043 |
| Standing | – | – | – | ns | ns | 0.001 |
| Walking | – | – | – | 0.012 | ns | ns |
| Dust bathing | – | – | – | ns | ns | ns |
| Preening | – | – | – | 0.001 | ns | 0.003 |
| Objective peck | (0.073) | ns | (0.096) | ns | 0.046 | <0.001 |
| Aggressive peck | – | – | – | ns | ns | 0.010 |
| Week 37 | | | | | | |
| Litter scratching | ns | ns | ns | ns | 0.003 | <0.001 |
| Drinking | – | – | – | ns | ns | 0.012 |
| Sitting | – | – | – | ns | ns | 0.037 |
| Feather pecking | – | – | – | ns | ns | ns |
| Standing | – | – | – | ns | ns | 0.005 |
| Walking | – | – | – | ns | ns | 0.013 |
| Dust bathing | – | – | – | (0.063) | ns | (0.089) |
| Preening | – | – | – | ns | ns | 0.013 |
| Objective peck | – | – | – | ns | ns | 0.004 |
| Aggressive peck | – | – | – | ns | ns | ns |

"–" means that the analyses were not run. ns = no significant difference.

breeders were also observed to have a tendency to spend more time on object pecking at 8h30 ($P = 0.073$), whereas there was an opposite tendency at 15h30 ($P = 0.096$). In week 37, no differences between the 2 treatments were observed in all behaviors except that C breeders tended ($P = 0.063$) to dust bathe more than RP breeders.

## The Behavior of Breeders in the F1 Generation

***Litter Scratching*** The results are listed in Table 3 and Figure 2. In week 6, dietary effects of both generations (F0-diet and F1-diet) were found in litter scratching behavior of the breeders. Specifically, for the F1-diet effect, RP diets fed breeders (C/RP and RP/RP breeders) were observed to spend more time on litter scratching compared to C diets fed breeders (C/C and RP/C breeders) at 12h00 ($P = 0.003$). In terms of the F0-diet effect, feeding RP diets in the F0 generation (the RP/C and RP/RP breeders) significantly decreased the litter scratching behavior of breeders in the F1 generation compared to C dietary treatment in the F0 generation (C/C and C/RP breeders) regardless of the time of the day ($P = 0.031$). No difference was seen between dietary treatments in weeks 11 and 22.

***Drinking*** The results can be found in Table 3 and Figure 2. In week 6, there was a tendency of both the F0-diet ($P = 0.051$) and F1-diet ($P = 0.072$) effects on drinking behavior. In week 11, significant F0-diet ($P = 0.041$) and F1-diet ($P < 0.001$) effects were observed as well. In detail, for the F1-diet effect, RP diets fed breeders spent less time drinking compared to C diets fed breeders. However, for the F0-diet effect, the time spent drinking of breeders in the F1 generation was increased by maternal RP dietary treatment compared with maternal C dietary feeding. In week 22, only a F1-diet effect ($P = 0.039$) was observed and again RP diets fed breeders drank less that C diets fed breeders.

***Sitting*** The results can be found in Table 3 and Figure 2. No dietary effects on sitting behavior of breeders were found in weeks 6 and 22. In week 11, the sitting behavior of breeders was influenced by the dietary treatments in both generations. For the F1-diet effect, the breeders fed with RP diets spent significantly more time sitting than the breeders fed with C diets at 15h30 ($P = 0.006$), and tended to sit more at 12h00 ($P = 0.064$). Regarding the F0-diet effect, at 15h30, the time spent sitting of breeders in the F1 generation was increased by maternal RP dietary feeding in the F0 generation compared with maternal C dietary feeding ($P = 0.033$). This F0-diet effect was more pronounced by RP diets feeding in the F1 generation (interaction between F0-diet and F1-diet: $P = 0.039$).

***Feather Pecking*** Results are listed in Table 3 and Figure 2. In week 6, no difference between the treatments was observed in feather pecking behavior. In week 11 at 15h30, RP F1-dietary treatment significantly increased the time spent feather pecking compared to C F1-diet fed breeders ($P = 0.018$). It tended to be the opposite at 12h00 ($P = 0.084$). In week 22, both an F0-diet ($P = 0.045$) and an F1-diet ($P = 0.004$) effect were noticed at 15h30. Concerning the F1-diet effect, RP diets fed breeders performed more feather

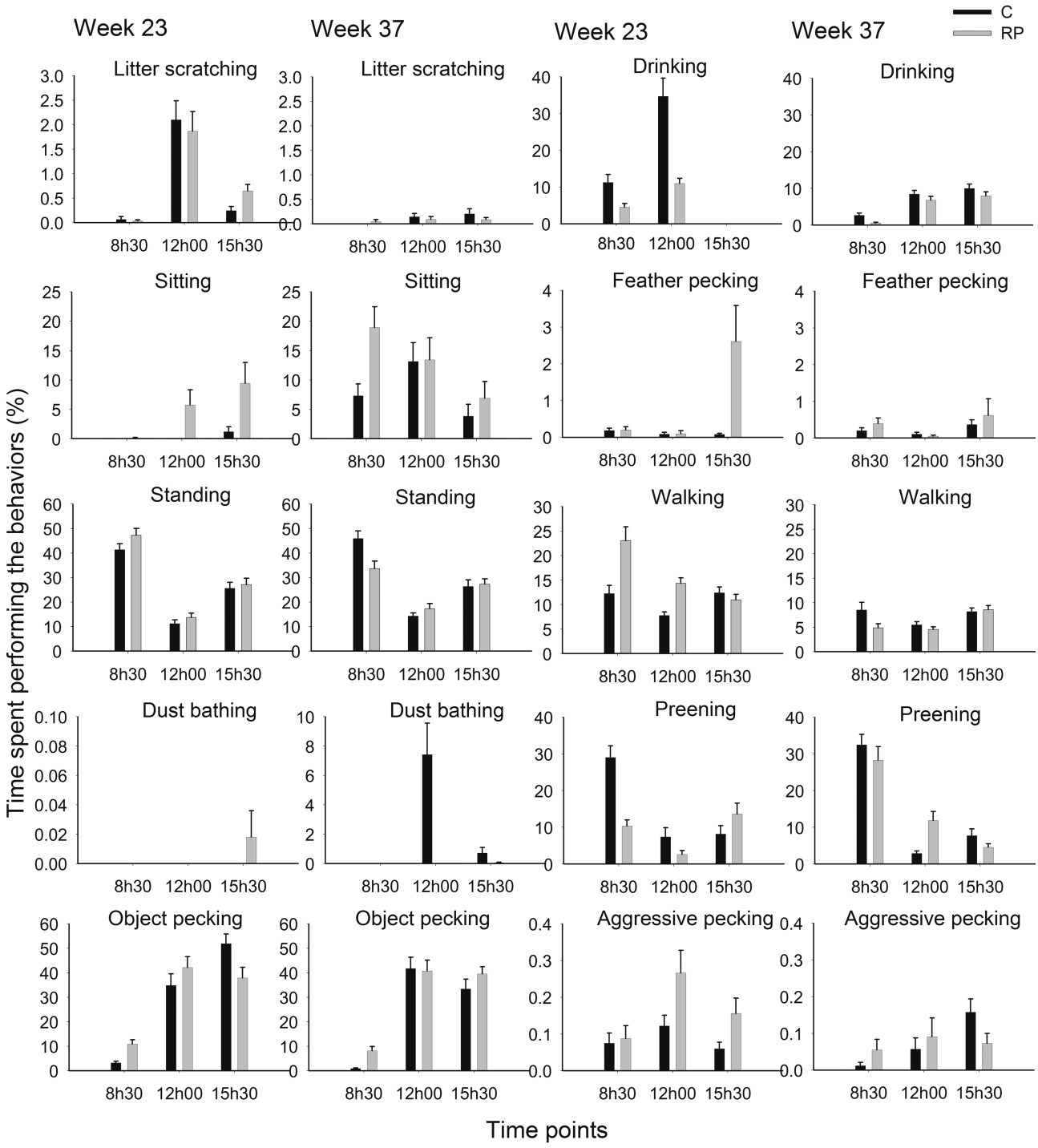


**Figure 1.** Percentage of time spent on different behaviors for breeders in the F0 generation under the control (C) and reduced balanced protein (RP) dietary treatment in weeks 23 and 37.

pecking behavior than C diets fed breeders. In terms of the F0-diet effect, RP diets feeding in the F0 generation significantly reduced feather pecking behavior in the F1 generation at 15h30. This F0-diet effect was again more pronounced by feeding the breeders RP diets in the F1 generation (interaction between F0-diet and F1-diet: $P = 0.036$).

Table 3 and Figure 3 contain the results of the following paragraphs.

***Standing*** At 12h00 in week 6, C diets fed breeders spent significantly more time standing compared to RP diets fed breeders ($P = 0.022$). However, at 12h00 of week 11, the difference between the 2 dietary treatments turned out to be opposite ($P = 0.001$) to that in week 6. In week 22, RP diets fed breeders tended to stand more than C diets fed breeders ($P = 0.078$).

***Walking*** In week 6 ($P = 0.075$) and week 11 ($P = 0.056$), C diets fed breeders tended to spend

**Table 3.** *P*-values of the effects of reduced balanced protein diet on the behavior of broiler breeders in the F1 generation in weeks 6, 11, and 22.

| | 8h30 | | 12h00 | | 15h30 | | By each week (pool of 3 time points) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | F0-diet | F1-diet | F0-diet | F1-diet | F0-diet | F1-diet | F0-diet | Time * F0-diet | F1-diet | Time * F1-diet | Time |
| Week 6 | | | | | | | | | | | |
| Litter scratching | – | ns | – | 0.003 | – | ns | 0.031 | ns | 0.037 | 0.002 | 0.040 |
| Drinking | ns | ns | ns | ns | ns | ns | (0.051) | ns | (0.072) | ns | 0.002 |
| Sitting | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Feather pecking | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Standing | – | ns | – | 0.022 | – | ns | ns | ns | ns | (0.054) | 0.006 |
| Walking | – | – | – | – | – | – | ns | ns | (0.075) | ns | 0.002 |
| Dust bathing | – | – | – | – | – | – | – | – | – | – | – |
| Object pecking | – | – | – | – | – | – | ns | ns | ns | ns | 0.032 |
| Week 11 | | | | | | | | | | | |
| Litter scratching | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Drinking | – | (0.055) | – | <.0001 | – | 0.015 | 0.041 | ns | <.001 | (0.081) | 0.001 |
| Sitting | ns | ns | ns | (0.064) | 0.033 | 0.006 | ns | (0.069) | 0.005 | 0.040 | (0.059) |
| Feather pecking | – | ns | – | (0.084) | – | 0.018 | ns | ns | ns | 0.043 | ns |
| Standing | – | ns | – | 0.001 | – | ns | ns | ns | 0.027 | 0.041 | <0.0001 |
| Walking | – | – | – | – | – | – | ns | ns | (0.056) | ns | 0.005 |
| Dust bathing | – | – | – | 0.005 | – | (0.095) | ns | ns | 0.022 | 0.029 | 0.029 |
| Object pecking | – | – | – | – | – | – | ns | ns | ns | ns | 0.004 |
| Week 22 | | | | | | | | | | | |
| Litter scratching | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Drinking | – | – | – | – | – | – | ns | ns | 0.039 | ns | 0.009 |
| Sitting | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Feather pecking | ns | ns | ns | ns | 0.045 | 0.004 | ns | 0.037 | ns | 0.028 | ns |
| Standing | – | – | – | – | – | – | ns | ns | (0.078) | ns | 0.026 |
| Walking | – | – | – | – | – | – | ns | ns | (0.085) | ns | ns |
| Dust bathing | – | – | – | – | – | – | ns | ns | ns | ns | ns |
| Object pecking | – | ns | – | ns | – | (0.084) | ns | ns | (0.084) | ns | 0.007 |

"–" means that the analyses were not run. ns = no significant difference.

more time walking compared to RP diets fed breeders. However, in week 22, it tended to be the opposite to walking behavior observed in weeks 6 and 11 ($P = 0.085$).

***Dust Bathing*** No dust bathing was found in week 6, and no difference was observed in week 22. In week 11, RP diets fed breeders spent significantly more time dust bathing compared to C diets fed breeders at 12h00 ($P = 0.005$) and a same tendency of the F1-diet effect was found at 15h30 ($P = 0.095$).

***Object Pecking*** No differences were observed in weeks 6 and 11. In week 22, C diets fed breeders were found to have a tendency of more object pecking than RP diets fed breeders at 15h00 ($P = 0.084$).

No dietary effects were observed between the treatments in terms of preening and aggressive behavior (not shown in the figures).

## *Age and Time Point Effects of the Behavior in the F0 and F1 Generation*

Age effects on the behavior in the 2 generations are briefly described in this section (Tables 2 and 3). In the F1 generation, the highest time spent on litter pecking was found in week 6 compared to the other ages ($P < 0.05$). Sitting and preening behavior increased with age during the rearing period of F1 generation and the laying period of F0 generation with an exception of less sitting behavior in week 22 of the F1 generation ($P < 0.05$). The time spent on standing and dust bathing was higher with increasing age during the rearing period of F1 generation and the laying period of F0 generation, respectively ($P < 0.05$). Walking and aggressive behavior decreased with age during the laying period of F1 generation ($P < 0.05$). No significant age effect was found for drinking, object pecking, and feather pecking behavior.

Overall, time spent on drinking, litter scratching, and dust bathing behavior was the highest after feeding at 12h00 ($P < 0.05$). Standing, walking, preening, and feather pecking behaviors were noticed most frequently before feeding at 8h30 and after feeding at 15h30, while the least at 12h00 ($P < 0.05$). Time spent on sitting, object pecking, and aggressive pecking behavior was the highest after feeding (at 12h00 and 15h30) compared with before feeding (at 8h30) ($P < 0.05$).

## DISCUSSION

The results of the current study showed that the RP dietary treatment in the current generation could induce a considerable effect on the behavior of breeders, as it affected the duration of many behaviors. Moreover, maternal RP dietary treatment of breeders in the F0 generation was observed to express effects on the behavior of breeders in the next (F1) generation. For example, a significant F0-diet effect was found on litter scratching, drinking, sitting, and feather pecking behavior of breeders in the F1 generation.

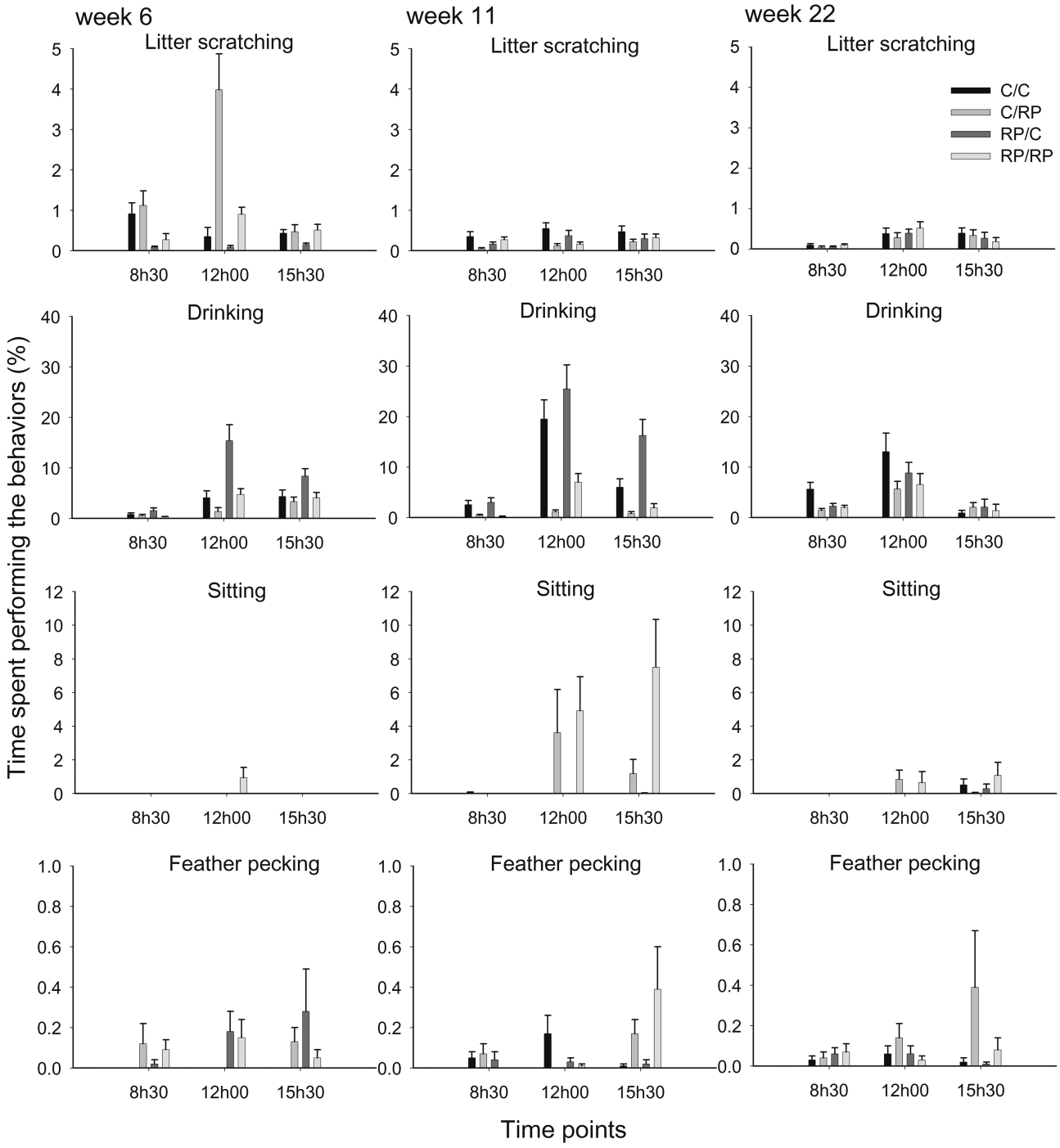


**Figure 2.** Percentage of time spent on different behaviors (had both F0-diet and F1-diet effect) for broiler breeders in the F1 generation in weeks 6, 11, and 22. C/C = breeders fed with control diet in the F1 generation and their maternal breeders fed with control diet; C/RP = breeders fed with RP diet in the F1 generation and their maternal breeders fed with control diet; RP/C = breeders fed with control diet in the F1 generation and their maternal breeders fed with RP diet; RP/RP = breeders in the F1 generation and their maternal breeders both fed with RP diet.

### *The RP Dietary Effect From the Previous and the Current Generation on Litter Scratching, Drinking, Feather Pecking, and Sitting Behavior of Breeders*

For young breeders, in week 6, RP diet fed breeders (C/RP and RP/RP breeders) conducted more litter scratching behavior compared to C diet fed breeders (C/C and RP/C breeders) at 12h00 which was approximately 2 and a half hours after feeding. Litter scratching is an indicator of foraging behavior which could reflect the feeding motivation of breeders (Mason and Mendl, 1997). In addition, Hughes and Wood-gush (1973) reported that domestic fowls under nutritional deficiency (e.g., Ca) turned out to be more active. The increased time spent litter scratching in the

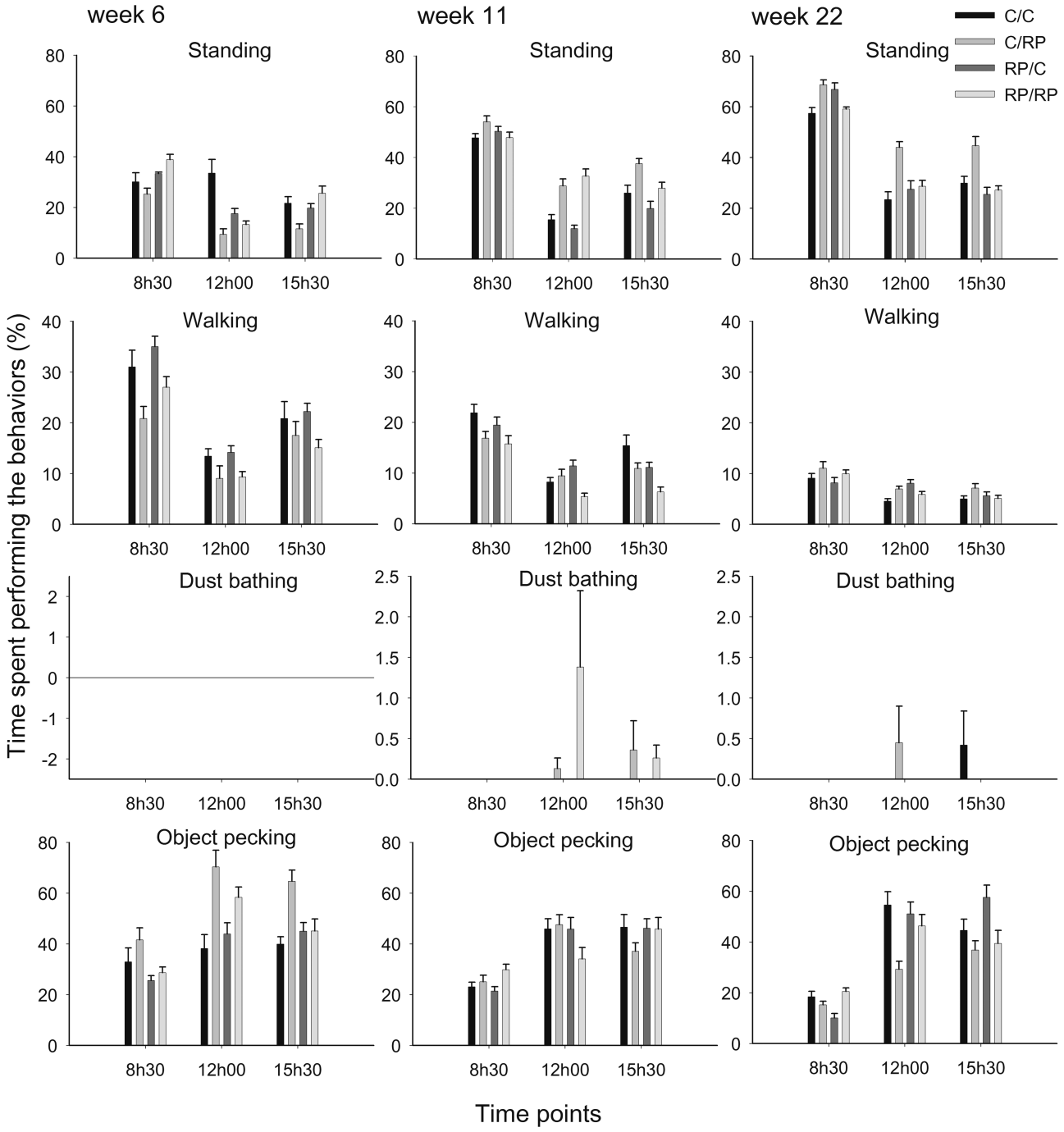


**Figure 3.** Percentage of time spent on different behaviors (had only F1-diet effect) for broiler breeders in the F1 generation in weeks 6, 11, and 22. C/C = breeders fed with control diet in the F1 generation and their maternal breeders fed with control diet; C/RP = breeders fed with RP diet in the F1 generation and their maternal breeders fed with control diet; RP/C = breeders fed with control diet in the F1 generation and their maternal breeders fed with RP diet; RP/RP = breeders in the F1 generation and their maternal breeders both fed with RP diet.

RP breeders as compared to the C breeders may be an expression of feeding frustration. Hence, it is speculated that RP diet might produce a nutritional deficiency for the breeders and that the breeders are more motivated to continue feeding following the feeding period in week 6. The difference in litter scratching was only observed in week 6 of the F1 generation and not at later ages and also not during the laying period of the F0 generation. Young breeders have relatively high metabolic requirements since they are in a period of rapid growth (Mench 2002). The effect of RP diet on the litter scratching behavior of breeders may therefore be more pronounced in this earlier age (week 6).

During both rearing and onset of laying period, C diet fed breeders spent more time drinking compared to RP diet fed breeders. There is sufficient evidence to show that there is a positive relationship between dietary protein content level and water intake

of chickens (Wheeler and James, 1950; Marks and Pesti, 1984; Francesch and Brufau, 2004). Similar observations were made for feed controlled broiler breeders by Hocking et al. (2001) who reported that breeders fed a high protein diet consumed more water than breeders fed a low protein diet. Francesch and Brufau (2004) reported that excessive dietary protein in bird feed needs to be catabolized and excreted through the kidneys in the form of uric acid. The process of forming and draining uric acids requires certain amount of water consumption. Li et al. (2018a) also reported that high soybean content in the diet increased the water intake and excreta moisture of broiler breeders, which resulted in the wetting of litter. Besides the diet effect, polydipsia might also be involved due to the controlled feeding program. Polydipsia was observed in controlled fed broiler breeders (Savory et al., 1992). There was a possibility that the higher water requirement of the C fed breeders induced a more severe polydipsia compared to the RP fed breeders which could greatly contribute to the higher water consumption.

With respect to feather pecking, RP diet fed breeders spent significantly more time feather pecking compared to the C diet fed breeders at 15h30 in weeks 11 and 22 of the F1 generation. This is in agreement with the feather condition scores reported in Li et al. (2018a) which is that RP diet fed breeders had worse feather coverage than C diet fed breeders. Sulfur containing AA such as methionine and cysteine are essential for feather keratin formation. It is speculated that the craving for protein and certain AAs (feather as a protein source) triggered feather pecking behavior as feather picking and ingestion was consistently observed during the breeder management. The fluff feather on the thigh and low abdominal part was found the most damaged during the feather condition evaluation.

Interestingly, passing over F0-diet effects was observed at different ages for the 3 behaviors discussed above. The RP dietary treatment in the F0 generation significantly decreased litter scratching (week 6) and feather pecking (week 22, at 15h30) behaviors whereas it increased drinking behavior (a tendency in week 6 and a significant difference in week 11) of the breeders in the F1 generation. Animals could adapt to the nutrient deficiency by increasing absorption rates and utilization efficiency (Ashwell 2010). Initial environmental or nutritional alterations in parental animals were reported to pass over effects to subsequent generations (Ho and Burggren, 2010). A nitrogen retention test was conducted in digestibility cages with the male offspring of the current F1 generation breeders (Lesuisse et al., 2018b). A tendency toward a better nitrogen retention was observed for the offspring of breeders receiving RP diets in F0 and/or F1 generation compared to C/C progeny (Lesuisse et al., 2018b). Therefore, it is hypothesized that RP dietary treatment of breeders in the F0 generation probably enhanced the protein and AA (including methionine and cysteine) utilization to cope with nutritional deficiencies which might subsequently pass over this effect to offspring. The potential better protein utilization ability of RP/C and RP/RP breeders might contribute to a better coping ability toward the controlled feeding program as indicated by the less litter scratching (foraging motivation) and feather pecking (protein/AA (methionine and cysteine) craving) after feeding. The result of feather pecking behavior is highly corresponding to the feather condition of the breeders (Li et al., 2018a). Conversely, the drinking behavior of the breeders descending from RP diet fed breeders was increased compared to the breeders descending from C diet fed breeders (weeks 6 and 11). We did not find appropriate explanations regarding this RP F0-diet effect on drinking behavior of breeders. Further investigation is needed to verify and explain this finding.

The time spent sitting of the breeders fed with RP diets was longer compared to C diets fed breeders. This was in accordance with the observations of Van Emous et al. (2014) and Hocking et al. (2001). To maintain the target BWs throughout the trial, breeders on RP diet received on average 10% more feed than C diet breeders. The 10% higher amount of RP diet might give the breeders higher gut fulfillment feeling which might induce a temporary satiety as compared to C breeders. With a satiety feeling, breeders tend to perform more resting behavior such as sitting. Moreover, the RP dietary treatment in the F0 generation increased sitting behavior of offspring breeders (F1 generation). The RP F0-diet effect was mainly pronounced by also feeding breeders RP diet in the F1 generation (C/RP vs RP/RP). It was probably the 10% more feed amount together with the potential better protein utilization contributed to satiety status and thus more resting/sitting behavior of breeders.

### *The RP Dietary Effects from the Current Generation on Walking, Object Pecking, Dust Bathing, and Preening Behavior of Breeders*

For walking behavior, in weeks 6 and 11 of the F1 generation, C diet fed breeders tended to walk more than RP diet fed breeders. Before feeding time, walking behavior is assumed to reflect anticipation of daily meal (Savory and Maros, 1993) and expression of an appetitive element of foraging behavior (Kostal et al., 1992). During the process of management, most of the breeders were noticed to stand beside the pen door and wait for the feed arrival as well as walking back and forth to the pen door. Hence, C diet might be more attractive to C diet fed breeders than RP diet's appeal to RP diet fed breeders before feeding time during the rearing period. Before feeding, RP breeders had a stronger motivation in obtaining meal worms instead of the RP diet which indicated that the RP diet was not an ideal diet for the breeders (Li et al., 2018b).

After feeding finished, walking behavior can reflect the activity level of the breeders which could indirectly indicate their satiation level. Corresponding to the above discussed more sitting behavior of RP breeders, it was not a surprise that RP breeders tended to performed less walking behavior compared with C breeders. Conversely, during onset of the laying period in week 22 of the F1 generation and week 23 of the F0 generation, RP diet fed breeders spent more time walking compared to C diet fed breeders. Photostimulation started in week 21 for both generations. Light stimulation to the brain of chickens was reported to trigger the hypothalamus to release the gonadotrophin-releasing hormone which is associated with the increase of plasma luteinizing and follicle-stimulating hormone (Dunn and Sharp 1999; Nicol 2015). Behavioral changes such as increased activity and restlessness are stimulated by these hormone changes (Nicol 2015). The alteration in walking behavior between the 2 dietary treatments during the onset of lay might be induced by the photostimulation and the subsequent hormonal changes. How exactly the phtostimulation possibly influenced walking behavior still needs to be investigated further.

Object pecking, a so-called post-feeding appetitive behavior by the lack of satiety (Mason and Mendl 1997), tended to be conducted more by C diet fed breeders at 15h30 in week 22 and week 23 of the F1 and F0 generations, respectively. Under controlled feeding program, the C diet fed breeders had a stronger post-feeding appetite compared with the RP diet fed breeders. This result is reasonable as the RP diet fed breeders received 10% more feed amount which provide more gut full feeling (6 h after feeding) and hence cause less object pecking. Similarly, Van Emous et al. (2014) reported that feeding breeders with low protein diet but 4.6% or 10% higher feed amount reduced stereotypic pecking significantly. However, at 8h30, so before feeding in week 23 of the F0 generation, RP diets fed breeders tended to perform more object pecking than C diets fed breeders. Therefore, we can conclude that the hunger releasing effect of 10% more RP diet could not last for a long time and at least not until the morning of the next day. Before feeding, RP diet fed breeders might be more frustrated because the RP diet might not be able to fulfill their nutritional requirements.

By looking at the results of drinking behavior and the correlated litter condition (Li et al., 2018a), we can explain the difference in dust bathing behavior of the broiler breeders in week 11 of the F1 generation and week 37 of the F0 generation. Fine-grained substrates such as peat, sand, and lignocellulose are preferred by chickens to perform dust bathing behavior since fine particles are better in penetrating and cleaning their plumage (Van Liere et al., 1990; Scholz et al., 2010; Nicol, 2015). Wet litter is not suitable for birds to perform dust bathing behavior (Blokhuis and De Wit, 1992). Dust bathing behavior was also observed more on dry litter by turkeys (Wu and Hocking, 2011). The litter of RP diet fed breeders was significantly drier than C diet fed breeders in week 10 which is corresponding to the drinking behavior results (Li et al., 2018a). Unsurprisingly, RP diet fed breeders were observed to conduct more dust bathing behavior with the dry litter compared to C diet fed breeders. However, in week 37 of the F0 generation, C breeders tended to dust bath more compared to RP breeders while the difference between the 2 treatments in drinking behavior had disappeared. It seems likely that the litter wetness of the C group decreased with aging and was not a limiting factor for dust bathing anymore. This might be contributed by the elevation of feed amount in accordance to the increasing of egg production which reduced the chance of polydipsia.

In week 23 of the F0 generation, C breeders spent more time preening compared to RP breeders but no differences were found in the other observation weeks of the 2 generations. Preening and dust bathing were considered as comfort behaviors by some studies (Van Rooijen, 2005; Morrissey et al., 2014) but the time spent preening and dust bathing behavior does not necessarily indicate the comfort status of birds. Unsettled short bouts of preening and dust bathing may also indicate a degree of frustration (Merrill et al., 2006; Nicol et al., 2011; Alvino et al., 2013). The preening behavior can also act as a displacement behavior under mild and short-term frustration such as motivational conflicts, frustration of consummatory acts, and physical thwarting of performance (Delius, 1967; Duncan and Wood-Gush, 1972; Webster, 1995; Hocking et al., 2007). Hence, we cannot draw a conclusion on the meaning of the difference in time spent on comfort behavior between the treatments.

## CONCLUSION

The behavioral observations in the current study indicate that the RP dietary treatment may increase feeding frustration of the breeders. This might be induced by the craving for "improved" feed (protein requirement). However, drinking behavior decreased dramatically by the RP dietary treatment which resulted in better litter quality in turn provided more suitable substrate for dust bathing behavior. Moreover, feeding the breeders RP diet had positive effects on the next generation such as decreased litter scratching and feather pecking behavior. This is probably caused by the potentially increased protein utilization inherited from the maternal breeders. On the other hand, the sitting behavior of the breeders was increased by the RP dietary treatment in both current and previous generations. This is probably a positive indicator of satiety. More detailed information in terms of preening and dust bathing behavior needs to be collected to investigate the comfort status of the breeders. In general, positive effects were found by reducing protein level of breeder diets. However, negative side effects were also observed, which merit further study.

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