Impact of an alternate feeding program on broiler breeder pullet behavior, performance, and plasma corticosterone

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ABSTRACT Broiler breeders are commonly feed restricted using some variation of skip-a-day feeding to prevent excessive body weight (BW) gain and poor flock uniformity that results in lower production levels. However, the level of feed restriction has increased leading to negative effects on broiler breeder welfare. Research needs to be conducted to evaluate alternative feeding programs to diminish the negative impact of restricted feeding on bird welfare. This research examined pullets that were fed soybean hulls (alternate day feeding, ATD) on the off day of a traditional skip-a-day feeding program in comparison to the standard skip-a-day program (SAD). The 2 dietary feeding treatments each had 3 replicate pens of 210 pullets each and were fed from wk 5 to 21 of age. Nitrogen-corrected true metabolizable energy and digestible amino acid coefficients of soybean hulls were determined. Body weight in the ATD feed program was significantly higher ($P < 0.001$) than the birds on the SAD feed program until 16 wk of age. Feed allocations for the SAD feeding program was increased at 11 wk of age to achieve similar BW prior to photo stimulation. The ATD feed program significantly improved BW uniformity of the birds for weeks 8, 12, 16, and 20. Hens fed on the SAD feed program had a lower mean egg production than the hens fed on the ATD program. There were significant differences on plasma corticosterone concentrations between the feeding days (24 or 48 h after feeding) in both feed programs. There was a shift in the behavior of the birds with significant differences in the feeding, foraging, and comfort behaviors between the feeding programs on the same feed day. Overall, feeding the ATD females soybean hulls on the off feed day improved the BW uniformity and egg production, but further research will be needed to determine potential differences in nutrient utilization or behavior of the pullets that positively impacted this flock performance.

Key words: feeding program, body weight, behavior, corticosterone, pullet, hen

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INTRODUCTION

Selection for increased growth rate has led to an increase in adult body weight (**BW**) and appetite for broilers and their parent stocks (Zuidhof et al., 2014). Broiler parent stock fed ad libitum commonly have a decline in production and increase in mortality with age (Renema and Robinson, 2004). Feed restriction is necessary to prevent excessive BW, maintain health and reproductive competence in broiler parent stocks. The continuing genetic progress for growth rate and increasing feed requirement of modern day broilers has resulted in the need for more severe feed restriction in their parent breeder stocks. Thus, feed intake is currently restricted to about 25 to 33% of the intake of ad libitum fed birds in rearing (de Jong et al., 2002).

There are performance and welfare implications for both ad libitum feeding and current industry feed restriction programs. The detrimental health effects of overeating make feed restriction programs the more welfare-friendly alternative. While feed restriction has the associated positive effect of limiting body size and improving overall health of the birds, the negative effects are behaviors indicative of frustration, boredom, and hunger, such as stereotypic object pecking, over drinking, and high activity (de Jong and Jones, 2006). Increasing feed restriction can also elevate plasma corticosterone concentrations (Mormède et al., 2007). Increased corticosterone levels may be indicative of signs of behavioral stress or be related to adaptive metabolic adjustments in the bird to cope with a decreased supply of energy. In addition, based on gene expression, the degree of fasting and stress may influence immune and metabolic processes of the birds (Sherlock et al., 2012).

Research has been conducted to decrease the prevalence of negative behaviors associated with the stress of feed restricted broiler breeders. Among some of the new

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management strategies are environmental enrichment, scattering feed on the litter, and restricted every-day feeding (**ED**). These strategies aim to provide the birds with either object stimuli or feed every day to satisfy the bird's natural instinct to peck and forage (de Jong et al., 2005; Leone and Estevez, 2008). Diluting the nutrient density of the feed is another strategy to decrease the welfare impact of feed restriction (Zuidhof et al., 1995). The optimal level of dilution and best diluent ingredients that have a positive effect on welfare are yet to be determined. Ingredients with low energy and crude protein (**CP**) levels such as soybean hulls, wheat midds, ground oats, and pea hulls are commonly used as diluents.

The current study investigates if an alternative feed program can improve broiler breeder pullet welfare without causing a negative influence on overall performance. Feeding soybean hulls on the OFF feed day as an alternative feed program may have the potential to satisfy the need for the pullets to peck or consume something on the day feed is not provided in a skip-a-day feed program. We hypothesize that feeding soybean hulls will decrease the prevalence of behaviors indicative of hunger or feeding frustration and thus improve the welfare of birds undergoing a feed restriction program.

MATERIALS AND METHODS

A total of 1,260 one-day-old (Cobb 700) pullets were raised in 6 floor pens with new pine shavings in an environmentally controlled solid side wall poultry house. At 5 wk of age, 3 replicate pens (7.3 × 4.6 m^2, 210 pullets) were allocated to each feeding program (630 birds per treatment). Two pens were housed in the same room separated by a chain link partition. Birds in the same room were on the same feeding program. During rearing, the pullets ate from a chain feeder (14.3 m) and water was provided by nipple drinker line (6.1 m with 40 nipples). All birds were wing banded at 8 wk of age to track growth rate. The photoperiod to 22 wk of age was 23 h of light:1 h of darkness (23L:1D) for the first 3 D, followed by a 8L:16D pattern until 22 wk of age. The photoperiod was increased to 14L:10D at 22 wk of age and remained constant until the end of the study at 40 wk of age. All birds were fed a standard starter ration (2,910 kcal/kg, 18% CP) for the first 3 wk of age, followed by a grower diet (2,820 kcal/kg, 15% CP) to 25 wk of age. At 22 wk of age hens were transferred into individual broiler breeder cages due to a shortage of floor pen space. The individual cages had a nipple drinker and a feeder trough. A standard breeder layer diet (2,910 kcal/kg, 15.8% CP) was fed when hens reached 5% egg production at 25 wk. The amount of feed allocated was based on Cobb Breeder Management Guide Recommendations (Cobb-Vantress, 2014) and the BW of the birds. The starter, grower, and laying diets were formulated and mixed by a poultry integrator (CP = 15%; ME = 2.82 kcal/g). All procedures were approved by the University of Georgia Animal Care and Use Committee.

Experimental Design

During the first week of age, all birds were fed ad libitum. For the next 3 wk, all birds were fed a restricted amount of feed daily to achieve primary breeder target BW guidelines. At 5 wk of age, birds were divided into 2 treatments: 1 treatment with birds reared under a standard skip-a-day (**SAD**) feed program while the birds on the other treatment were reared on an alternate feed program (**ATD**). At the start of the feeding treatments, 5 wk, the overall mean BW and coefficient of variation (**CV**) for BW were similar between the treatments with no grading of birds or elimination of birds. The SAD birds were fed the grower diet and received twice the daily feed amount every other day (ON day). The ATD birds were also fed the grower diet and received twice the daily feed amount every other day (ON day) and soybean hulls (11–19 g/bird/day, increasing from 5–20 wk of age) on the day they would otherwise not receive feed (OFF day). The increasing amount of ground soybean hulls was to ensure all birds' access to the filler. The soybean hulls were fed in the chain feeder. Four rooms were used to house the birds, with 1 or 2 pens per room. Two rooms housed the ATD (3 pens, n = 210 birds/pen) birds and 2 separate rooms housed the SAD (3 pens) birds. The treatments were in different rooms to prevent having an effect on behavior of the SAD pullets when the ATD pullets were fed on the OFF feed day.

At 22 wk of age, all birds were weighed and 288 pullets (144 per treatment) were moved into individual cages in an environmentally controlled house. Birds were selected to give similar mean BW between the feeding treatments and the CV per treatment reflected the CV of the treatment at 20 wk of age. Treatment and replicate groups were randomly arranged throughout the house with 12 cages per replicate group and 12 replicate groups per treatment. The broiler breeder cages (48.3 cm length × 50.8 cm width × 43.2 cm high) were arranged in 3 tiers high and 2 to 48 cage rows back to back with a hen in each back to back cage sharing 2 nipples with the adjoining cage. Once in cages, the feeding treatments were discontinued and all birds were fed the rearing diet daily with no added soybean hulls.

TMEn Determination (in Leghorn roosters)

The nutrient availability in soybean hulls were determined by standard TMEn (Nitrogen-corrected true metabolizable energy) methods described by Sibbald (1976) and modified by Dale and Fuller (1984). Eight Single Comb White Leghorn roosters (60 wk of age) were fasted for 30 h to empty the digestive tract. Roosters were transferred to individual wire cages measuring 30.5 cm wide, 45.7 cm deep and 50.8 cm high. Each cage was equipped with a nipple drinker to provide free access to water and a stainless-steel excreta collection pan. Roosters were each precision-fed 30 g of soybean hulls. Excreta were collected for 48 h post feeding. To estimate endogenous energy excretion 10 roosters remained unfed for 48 h collection period.

Excreta were collected from each individual pan, dried, and weighed. Crude protein and moisture of the feces and soybean hulls were determined (AOAC, 2006, by the University of Georgia Agricultural and Environmental Laboratories, Athens, GA), with gross energy of feed and feces determined with a bomb calorimeter (University of Georgia Agricultural and Environmental Laboratories, Athens, GA).

Digestible Amino Acid Determination

The determination of the digestible amino acid coefficients of the soybean hulls followed the same procedures utilized for the TMEn determination except that 60-wk-old cecectomized roosters were utilized. The amino acid content of the soybean hulls and feces were determined (AOAC, 2006, and University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, MD) for calculation of the digestible amino acid coefficients for each diet.

Growth and Productivity

A sample BW (25% of the birds from each pen) was taken weekly and all birds were individually weighed at 8, 12, 16, and 20 wk of age during rearing and biweekly during lay. Coefficient of variation for BW was calculated on a per pen basis during rearing as a measure of flock uniformity. The BW for the caged layers was calculated per group (n = 12 birds per groups, 144 birds per treatment and total of 288 hens). Egg production in cages was monitored daily on a per hen basis from 24 to 40 wk of age. Average egg production was calculated on a pen basis (number of eggs laid per week was divided by 7 D and by the number of birds alive during each week).

Blood Sample Collection

Twenty pullets from each pen were randomly allocated into 2 groups and marked by with a different color on their backs (10 birds/group). The groups were divided based on sample collection time (24 or 48 h after feeding). Blood samples were collected at 7:30 am before the light period started at 8:00 am at 8, 11, 16, and 20 wk of age. Collection of the blood sample from the brachial vein was completed within 1 min of initial physical contact with each pullet. Blood samples were centrifuged at 1,000× g at 4°C for 10 min. Plasma was collected from each sample and frozen at −80°C.

Plasma Corticosterone Determination

Plasma corticosterone concentration was determined using a corticosterone specific enzyme immunoassay kit (Enzo Life Sciences, Plymouth Meeting, PA). A 25 μL aliquot of plasma from each sample was mixed with steroid displacement reagent following the manufacturer's protocol. Corticosterone content in each sample was then determined following the manufacturer's protocol. Duplicate corticosterone determinations were made for each of the samples and mean values were used.

Table 1. Recorded behaviors of broiler breeder pullets.[1]

Behavior	Description
Feeding	Pecking at the feeder
Drinking	Pecking at the nipple drinker
Foraging	Pecking and/or scratching the litter
Walking	Walking or running without performing other behaviors
Comfort	Preening, sitting, nibbling, stroking, dust bathing and wing flapping

[1]Behavior definitions modified from the ethogram of de Jong et al. (2005).

Behavioral Data

Video cameras (IR Network Camera, Hikvision Digital Technology, City of Industry, CA) were mounted over each pen at 8 wk of age. Videos were recorded onto a digital recording unit and transferred to external hard drives daily. Scan sampling was used to observe the frequency of feeding, foraging, comfort, walking, or drinking (Table 1). An instantaneous observation was made by 1 individual every 5 min the first hour lights were on and every 10 min thereafter until lights went off and the observer was blind to the treatments. Behaviors were observed during 2 consecutive days (ON and OFF feed day) for each week at 8, 13, and 16 wk of age. Each week was treated as the average of behaviors performed on the ON feed and OFF feed days. Uninterrupted days with no weighing, blood collection, pen maintenance or unplanned events were chosen for behavioral evaluation. The behaviors were analyzed for the entire light period that day, with time 0 being the time lights came on (8 am) until the lights were turned off (4 pm). The observation areas were as follows: an area with a 6.1 m length of chain feeder (total length of feeder, 14.3 m, 43% of total feeder observed), the drinker line (6.1 m with 40 nipples), and open or unobstructed area (1.5 × 3.5 m) in each pen.

Body Composition

Ten birds per treatment (3–4 birds per pen) on the OFF feed day, before soybean hulls were provided, were randomly selected and killed at 7, 14, and 21 wk of age for dual-energy x-ray absorptiometry (DEXA, GE Healthcare, Chicago, IL), whole body composition and gene expression analyses. Parameters for body composition were bone mineral density (**BMD**), bone mineral content (**BMC**), bone area, total tissue weight, fat weight, lean muscle weight, and percent fat. A scan of the whole body was made and analyzed using small

Table 2. Oligonucleotide primer pairs for real time PCR.[1]

Gene Name	Primer	Oligonucleotide primer and probe sequence (5′-3′)	Product length (bp)	Annealing temperature (°C)
GAPDH	Forward	GCTAAGGCTGTGGGGAAAGT	116	56
	Reverse	TCAGCAGCAGCCTTCACTAC		
IFN-gamma	Forward	CTGAAGAACTGGACAGAGAG	264	57
	Reverse	CACCAGCTTCTGTAAGATGC		
IL-6	Forward	CAGGACGAGATGTGCAAGAA	233	57
	Reverse	TAGCACAGAGACTCGACGTT		
PEPCK	Forward	GGGGTGTCTATTGGGAAGGC	100	57
	Reverse	CACGGCTCCCCATTATCTGG		
GLUT2	Forward	GATCTGTGCGGACCGTATGT	150	57
	Reverse	GGATTTGGCTGGGAGCTTCT		
ACAT2	Forward	GCTGGGGTGAAAATGGGAGA	132	57
	Reverse	CCCTCTGCTAACTTGCCACT		

[1]GAPDH = glyceraldehyde 3-phosphate dehydrogenase; IFN-gamma = interferon gamma; IL-6 = interleukin 6; PEPCK = phophenolpyruvate carboxykinase; GLUT2 = glucose transporter 2; ACAT2 = acetyl-CoA acetyltransferase 2.

animal scan software (GE Healthcare, Little Chalfont, UK).

Gene Expression Using Quantitative Real-Time PCR

To evaluate the impact of the alternate feeding program on the immune response and changes in metabolism of the birds, the mRNA expression of the following target genes were measured: IFN-gamma and IL-6 (immune genes), phophoenolpyruvate carboxykinase (PEPCK), glucose transporter 2 (GLUT2) (glucose metabolism), and acetyl-CoA acetyltransferase 2 (ACAT2) (lipid metabolism). Total RNA was extracted from 50 mg liver samples using TRIzol reagent (Invitrogen, Waltham, Massachusetts, USA) according to the manufacturer's instructions. The isolated total RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, MA). Pairs of primers for each gene were designed (Table 2) and checked for target identity using the National Centre for Biotechnology Information (NCBI). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed in duplicate reactions including nuclease free water, the forward and reverse primers of each gene, cDNA and SYBR Green as a detector using CFX Connect^{TM} Real-Time PCR Detection System (Life Science Research, Bio-Rad, Hercules, CA). Glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as housekeeping gene. Samples were analyzed by using the $\Delta\Delta$CT method (Livak and Schmittgen, 2001). The values were reported as fold changes of the expression of the target genes in ATD pullets relative to SAD pullets.

Statistical Analysis

Body weight and egg production results were analyzed using SLICE analysis (SAS, 2013, Cary, NC). Slice analysis specifies effects to test for differences between interactions LS-mean, to produce tests of simple effects (Winer, 1971). The analysis allows one to evaluate the effect of the treatments without having an impact by the differences over time (week). Plasma corticosterone, uniformity, behavior, body composition, and gene expression results were compared using GLM (SAS, 2013, Cary, NC). Individual bird was used the experimental unit for all parameters. Behavior data were analyzed for each individual week (8, 13, and 16), and with no differences in a specific behavior over time the data were pooled and analyzed by ON or OFF with respect to the treatment. Behavior data were arc sine transformed and original means reported. Differences were deemed to be significant when the P-value was less than or equal to 0.05.

Table 3. Body weight of pullets reared with an SAD or ATD feeding program from 5 to 21 wk of age.[1,2]

Age (week)	SAD (g)	ATD (g)	STD[3] (g)
8	793 ± 8[b]	828 ± 8[a]	860
12	1207 ± 8[b]	1236 ± 8[a]	1205
16	1527 ± 8[b]	1562 ± 8[a]	1570
20	1838 ± 8[a]	1852 ± 8[a]	2100

[1]Values are means ± SEM of 3 replicate pens.

[2]SAD = Skip-a-day feeding program; ATD = alternate feeding program, STD = Cobb 700 target body weight.

[3]Pullets were held under target body weight during rearing at Cobb Technical staff suggestions.

[a,b]Superscripts between treatment within age indicate significant differences $P < 0.05$.

RESULTS

Body Weight and Uniformity

Body weight was not significantly different at 5 wk (547 and 557 g for the SAD and ATD, respectively) when feeding treatment was initiated at 20 wk age (Table 3). In contrast, BW at 8, 12, and 16 wk of age was significantly different ($P < 0.001$) between feed programs. Body weight was not significantly different

Table 4. Coefficient of variation (CV) of BW of pullets reared with SAD or ATD feeding program from 5 to 21 wk of age.[1,2]

Age (week)	SAD[1] (CV)	ATD (CV)
8	14.00 ± 0.34[b]	12.74 ± 0.34[a]
12	14.54 ± 0.34[b]	13.25 ± 0.34[a]
16	14.52 ± 0.34[b]	13.23 ± 0.34[a]
20	14.85 ± 0.34[b]	13.31 ± 0.34[a]

[1]Values are means ± SEM of 3 replicate pens.
[2]SAD = Skip-a-day feeding program; ATD = alternate feeding program.
[a,b]Superscripts within age indicate significant differences $P < 0.05$.

Table 5. Protein, energy and intake of feed and soybean hulls of pullets reared with an SAD or ATD feeding program from 5 to 21 wk of age.[1,2]

Source	SAD	ATD
Cumulative Feed (g/bird)	3,677.2	3,558.0
Cumulative Soybean hulls (g/bird)	–	983.0
Cumulative TME soybean hulls (kcal/kg)	–	643.8
Cumulative CP intake (g/bird)	573.3	664.8
Cumulative AME (kcal/kg)	10,376.7	10,687.2

[1]SAD = Skip-a-day feeding program; ATD = alternate feeding program.
[2]TME = Nitrogen corrected true metabolizable energy, CP = crude protein, AME = apparent metabolizable energy.

during the laying period from 26 to 42 wk with a maximum difference of 49 g at 38 wk (data not shown). The ATD feeding program significantly improved BW uniformity for each week (Table 4). Body weight uniformity during lay did not differ for the hens in the 2 treatments (data not shown).

Feed Intake and Digestibility

Feed intake was similar through 10 wk of age for both treatments and was adjusted weekly to meet recommended breeder target BW during rearing. Not including the soybean hulls that were fed, pullets under the ATD feed program were fed 3.2% less feed compared to the pullets under the SAD program (Table 5). However, pullets under the ATD feed program consumed more crude protein and energy with the added opportunity to eat soybean hulls on off feed days (+76.4 g more CP and +310.5 kcal more energy per bird). The TMEn value of the soybean hulls was approximately 658 kcal/kg. The CP level of the hulls was 9.7% with individual amino acid digestibility coefficients less than 10% (Table 6) with a mean digestible coefficient of 47.5%. During the egg production phase of the experiment (22–40 wk) feed intake did not differ between treatments.

Egg Production

Overall egg production was significantly different between treatments ($P = 0.04$). Hens fed on the standard SAD program during rearing had a lower mean egg production than hens fed on the ATD program (77.7 vs.

Table 6. Crude protein, amino acid content, and digestibility coefficient of soybean hulls.[1]

Amino Acid	Feed Content (%)	Digestibility Coefficient (%)
Crude protein	11.19	
Alanine	0.45	39
Arginine	0.48	68
Aspartic Acid	0.95	54
Cysteine	0.16	41
Glutamic Acid	1.14	51
Histidine	0.26	47
Isoleucine	0.40	44
Leucine	0.66	52
Lysine	0.73	53
Methionine	0.13	58
Phenylalanine	0.39	54
Proline	0.55	56
Serine	0.53	49
Threonine	0.36	51
Tryptophan	0.06	56
Tyrosine	0.36	47
Valine	0.46	34

[1]Values are reported on an as fed basis. The dry matter content of soybean hulls was 88%.

79.2 eggs/hen) in the 15-wk laying cycle with most of the difference in egg production occurring between 27 and 31 wk of age (Figure 1).

Plasma Corticosterone

The plasma corticosterone concentration in ATD reared pullets was greater than in SAD reared pullets, 48 h after feeding at 21 wk of age (Table 8). At 12, 16, and 21 wk of age plasma corticosterone values were greater in both SAD and ATD pullets 48 h after consuming the ON day feed amount than values measured at 24 h after feeding (Table 7).

Behavior Observations

The birds on the SAD and ATD feed program had a significantly higher percentage of birds feeding and drinking on the ON feed day than the OFF feed day (Figure 2). The birds on the SAD feed program had a significantly higher percentage of birds around the feeder on the ON feed day but lower percentage on the OFF feed day. There were no significant differences between the feed programs on drinking or walking behaviors. There were significant differences for foraging behaviors in the birds with a higher percentage of birds foraging on the OFF feed day than the ON feed day in both feed programs. The birds on the ATD feed program spent less time foraging than the birds on the SAD feed program on the OFF feed day; the inverse was observed on the ON feed day. The birds on the SAD feed program exhibited more comfort behaviors on the OFF feed day than did those on the ATD feed program. There were no significant differences between the treatments on the ON feed day.

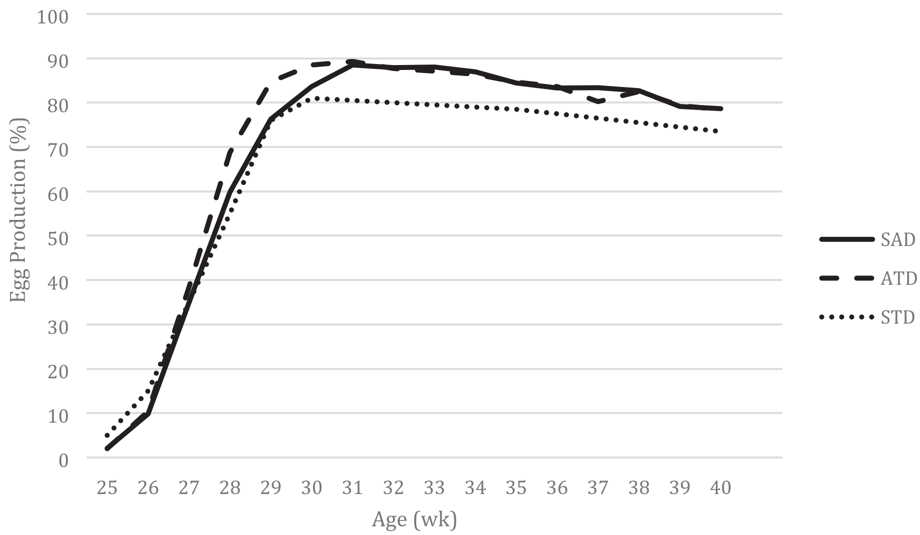

Figure 1. Egg production ($P = 0.04$) of hens that had been reared with an SAD or ATD feeding program from 5 to 21 wk of age. Values are means for 12 replicate groups of 12 individually caged hens per groups. Breeder egg production guidelines (STD) are also included in the graph.

Table 7. Plasma corticosterone concentrations of pullet reared with an SAD or ATD feeding program from 5 to 21 wk of age.[1,2]

Age (week)	Hours after feeding	SAD (ng/mL)	ATD (ng/mL)
8	24	0.215 ± 0.036	0.127 ± 0.037
8	48	0.191 ± 0.036	0.174 ± 0.037
12	24	0.204 ± 0.035	0.222 ± 0.038
12	48	0.624 ± 0.036[3]	0.705 ± 0.037[3]
16	24	0.233 ± 0.035	0.264 ± 0.037
16	48	0.366 ± 0.035[3]	0.529 ± 0.037[3]
21	24	0.109 ± 0.036	0.167 ± 0.037
21	48	0.229 ± 0.036 [b,3]	0.348 ± 0.037 [a,3]

[1]Values are the mean ± SEM of 20 replicates samples.
[2]SAD = skip a day. ATD = alternative feed program (soybean hulls).
[3]Values at 48 h are significantly different from the corresponding 24 h value.
[a,b]Superscripts within column and week indicate significant differences $P < 0.05$.

Body Composition

At 7 wk of age, ATD pullets had significantly higher BMD, BMC, bone area, total tissue weight, and lean muscle weight compared to SAD pullets (Table 8). At weeks 14 and 21 of age, there were no significant differences in body composition parameters between the ATD and SAD pullets.

Gene Expression for Immunity, Glucose Metabolism, and Lipid Metabolism

There were no significant differences in expression of key immune genes (IFN-gamma and IL-6), PEPCK, and Glut 2 between birds fed on SAD or ATD treatments (data not shown). The ATD feed program significantly depressed ACAT2 mRNA expression (1.09 vs. 0.33) compared to SAD feed program at 21 wk of age ($P < 0.05$).

DISCUSSION

The results from the current research indicate the benefit of feeding broiler breeder pullets a small amount of soybean hulls on the off day of an SAD feed restriction program. Providing the soybean hulls improved BW uniformity and resulted in greater cumulative egg production. However, the reasons for these improvements are not understood. Feeding the soybean hulls slightly increased the caloric intake of the birds on the ATD feed program. This increase in calories was likely not enough to alleviate the metabolic stress associated with the fasting period. Since corticosterone levels significantly increased in both the SAD and ATD birds when measured at 48 h after feeding the diet at 12, 16, and 21 wk of age. It is possible, that feeding the soybean hulls did alleviate some of the metabolic stress and elevation of corticosterone in the ATD birds during the last 24 h leading up to feeding. In addition, there was a shift in the behavior of the pullets on the ATD feeding program. The ATD birds spent less time feeding compared to the SAD birds on the ON feed day, the pattern is reversed for the OFF feed day. The SAD birds spend more time foraging in the on the OFF feed day than on the ON feed day. The ATD birds do not exhibit significant differences in foraging behavior between feed days. It is also interesting to note that on the OFF feed day the ATD birds exhibited significantly less comfort behavior than the SAD birds on the OFF day.

Body Weight and Uniformity

There is very little information on the actual nutritional value of soybean hulls. Soybean hulls are reported to contain about 10 to 12% CP, 43% crude fiber and digestible energy content of 2,070 kcal/kg (Chee et al.,

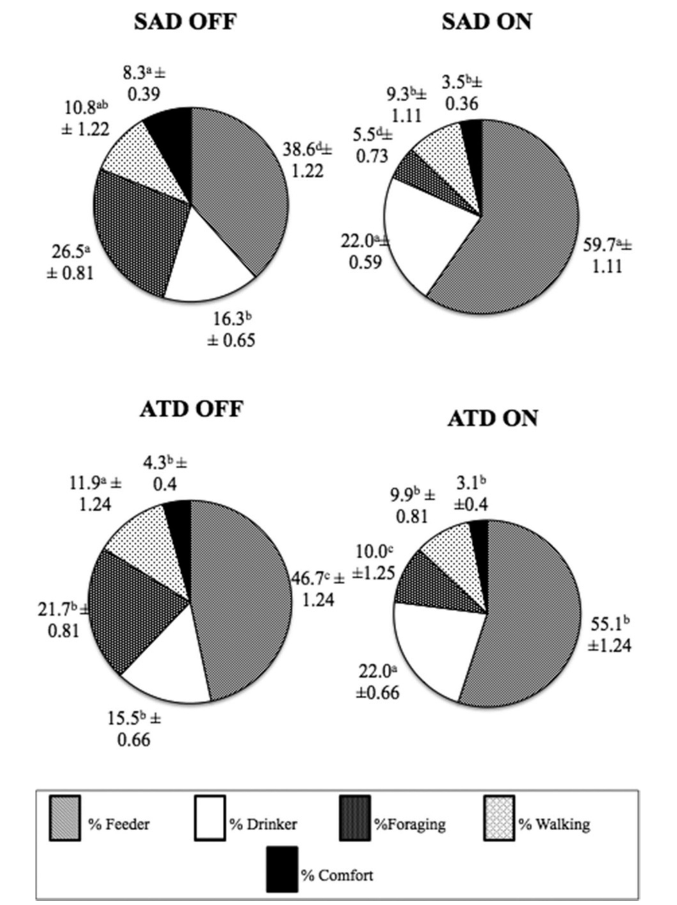

Figure 2. Effect of feeding program on the percentage of birds located at the feeders or drinker and observed behaviors (foraging, walking, or comfort) over total number of birds in view (%). Feeding programs were as follows: SAD = skip-a-day feeding program, ATD = alternate feeding program (soyhulls). Data were collected from 3 observational areas (feeder, drinker and open area) in each of the pens, every 10 min during the entire photoperiod (8:00 to 16:00). Preening, sitting, nibbling, stroking, dust bathing and wing flapping behavior are all categorized as comfort behavior. Observations occurred at 8, 13, and 16 wk for 2 consecutive days. Pullets were fed every day between 8:00 AM to 8:30 AM. [a-d]Superscripts within behavior and day indicate significant differences $P > 0.05$.

2005). However, the protein content of soybean hulls varies depending on processors, with research indicating a range of 9.2 to 18% CP (Cole et al., 1999). In addition, due to their high fiber content, soybean hulls are known to be poorly digestible for nonruminant animals. The TMEn analysis of the soybean hulls used in our trial indicated an energy value on an as fed basis of 658 kcal/kg. The overall average digestibility coefficient for the amino acids in soybean hulls was about 50%, thus providing 4.5% digestible protein. In addition, soybean hulls provided some energy and amino acids which contributed to the birds on the ATD feeding program having greater BW at 8, 12, and 16 wk of age than the birds fed the feed only on the SAD feeding program. In order to achieve target BW prior to lay, the birds on the SAD feed program were fed more feed compared to the ATD birds (starting at 11 wk of age). The increase in feed (0.5 to 3 g more per bird) eliminated differences in mean BW between feed programs by 20 wk.

Body weight uniformity provides an estimate of the variability in a flock and is a crucial measurement for broiler breeder management. Highly uniform flocks have better performance than more variable flocks, making it easier to meet the nutritional requirements of the uniform flock (Hudson et al., 2001). In a 1989 paper, Bennett and Lesson found BW to be consistently heavier in the ED vs. the SAD fed pullets and no difference in BW uniformity; however, they did note that ED fed pullets were more aggressive toward the feed and ate in 1 h vs. 2.5 h for the SAD fed pullets. They speculated that in a commercial facility with more limited feeder space that BW uniformity might decline. Interestingly, we found an improvement in uniformity for birds on the ATD feed program compared to SAD feed program throughout the rearing period including at 20 wk of age when the pullets fed on the SAD feeding program were being fed more to align their average BW with the ATD pullets. Previous researchers suggested that qualitative restriction improves the CV of BW due to efficiency of nutrient utilization on the OFF day (Pinchasov et al., 1993). Morrissey et al. (2014) reported the greatest improvement in BW uniformity in pullets fed on an ED feed program when soybean hulls were added as filler in the diet compared to different fillers or on an SAD feed program. While this study is not a comparison of ED to SAD as in the Morrissey et al. (2014) study, these data agree that feeding soybean hulls to the pullets with the ATD treatment resulted in increased weight gain over the SAD treatment but an improvement in nutrient utilization would have to be confirmed in a future study.

Behavior Observations

The difference in behaviors between the ATD and SAD pullets can be attributed to providing soybean hulls in the ATD feed program. Van Emous et al. (2015b) indicated that an increase in focus on the feeder lead to more tranquil birds that exhibit more comfort behaviors. The increase in feeding behavior by birds on the ATD feed program on the OFF feed day compared to those on the SAD program, potentially improving the welfare of birds by decreasing distracting them, and perhaps resulting in more consistent daily behaviors in the ATD. Drinking behavior was similar for both feed programs. The birds spent more time at the drinker on the ON day than the OFF feed day. The increase in drinking behavior on the ON day is likely to be related to increased feeding behavior. Bennett and Lesson (1989) reported a feed to water consumption ratio of 2:1. Therefore, higher feed consumption leads to higher water consumption on the ON feed day. The lack of difference between the feeding programs on the OFF feed day suggested that average everyday water consumption was enough to satisfy the increased feeding behaviors associated with soybean hull consumption.

Foraging is a normal behavior that has its own positive reinforcement. However, it can also show a lack

Table 8. Body composition of pullets reared with an SAD or ATD feeding program from 5 to 21 wk of age.[1,2]

Age and Treatment	BMD (g/cm^2)	BMC (g)	Bone Area (cm^2)	Fat (%)	Tissue (Fat + Lean) (g)	Fat (g)	Lean (g)
7 wk							
SAD[1]	0.12[a]	11.87[a]	97.06[a]	15.34	684.92[a]	108.86	585.13[a]
ATD	0.13[b]	13.65[b]	107.10[b]	16.24	752.96[b]	127.01	630.49[b]
Pooled SEM	0.002	0.488	3.324	0.975	0.041	0.019	0.031
P value	0.015	0.015	0.042	0.517	0.013	0.148	0.031
14 wk							
SAD	0.19	26.73	157.20	5.75	1,288.20	77.11	1,211.09
ATD	0.16	24.23	153.90	5.31	1,279.13	68.04	1,211.09
Pooled SEM	0.013	1.030	3.471	0.673	0.075	0.022	0.067
P value	0.288	0.097	0.513	0.647	0.836	0.574	0.956
21 wk							
SAD	0.18	33.31	185.40	6.76	1,891.48	122.47	1764.47
ATD	0.19	36.07	191.70	6.63	1,864.27	122.47	1741.80
Pooled SEM	0.016	1.058	2.994	1.117	0.115	0.043	0.133
P value	0.108	0.073	0.152	0.933	0.688	0.964	0.783

[1]Values are the mean of 10 replicate birds per treatment.
[2]SAD = skip a day. ATD = Alternative feed program (soybean hulls). BMD = Bone mineral density. BMC = Bone mineral content.
[a,b]Superscripts within column and week indicate significant differences $P < 0.05$.

of satiety and feed seeking. The number of birds foraging also varied based on the feed program and feed day. Previous research has shown that increasing feeding time by diet dilution leads to decreased foraging behaviors away from the feeder (Hocking et al., 2001; Van Emous et al., 2015a). The results of our study showed that the birds on both feed programs had a significantly higher number of birds foraging on the OFF feed day than during the ON day. The increase in foraging in the OFF days suggests a lack of satiety. However, by providing the birds with soybean hulls we saw a significant decrease in foraging of the birds on the ATD feed program compared to those on the SAD feed program (OFF feed day). The results suggest soybean hulls fed to the ATD pullets provided some satiety on the OFF feed day.

The birds on the SAD feeding program exhibit more comfort behaviors on the OFF rather than the ON feed day from 13 and 16 wk. The comfort behaviors are inverse at 8 wk with a greater number of birds displaying comfort behaviors even though they do not receive any feed on the OFF feed day. The birds engage in comfort behaviors even though they do not receive any feed on the OFF feed day. These results suggest the birds get more comfortable or acclimated with the feeding program over time. This agrees with previous research which shows that birds on an SAD feeding program used to the feed volume over time showing no differences in comfort on the late phase of production (Morrisey et al., 2014). In contrast, the birds on the ATD feeding program do not have any difference in comfort behaviors between the ON and OFF feed day. The lack of difference suggests that the birds have similar behaviors on the ON and OFF feed day. Preening is also added to the number of comfort behaviors; however, displacement preening might have been taking place. Displacement preening is considered a negative behavior and is different from normal preening (Duncan and Wood, 1972). Therefore, an over estimation of comfort behaviors might have been made since the distinction on the behaviors was not made during these observations.

Overall, the behavior of the birds on the ATD diet was more similar on both ON and OFF feed days; while the birds on the SAD diet have very different behaviors on the ON than the OFF feed day. The results of this study suggested that by feeding soybean hulls on the off feed day we can change the behavior of the birds leading to more similar behavioral time budget on both ON and OFF feed days.

Egg Production

There was a significant difference in overall egg production between the treatments. Since the CVs from rearing were maintained when the hens were transferred to cages, the initial differences in egg production might be attributed to the differences in uniformity. As noted in previous studies, better uniformity leads to higher egg production (Abbas et al., 2010). The difference in CP intake through rearing may have also impacted egg production. Joseph et al. (2000) reported a decrease in early and late stage egg production when birds were reared on 14% CP diet compared to a 16 and 18% CP diet. Providing the soybean hulls marginally increased digestible amino acid levels in the ATD pullets through much of the rearing period. Thus, the increased CP for the ATD pullets may have contributed to the increase in egg production, but the difference in flock uniformity was likely the major determinant in the increased egg production.

Plasma Corticosterone

Previous research indicated changes in plasma corticosterone levels were attributed to restriction in feed intake and are usually elevated during fasting (Mench, 1991; Hocking et al., 1996; De Beer et al., 2008). Mench (1991) determined birds on an everyday feeding program have lower levels of plasma corticosterone. However, there were no significant differences between our feeding programs until 21 wk. In a 2010 study, de Jong et al., suggested that differences in plasma corticosterone can be attributed to differences in metabolic rate as well as differences in level of stress. The question becomes whether increased plasma corticosterone concentrations were due to psychological/behavioral stress, metabolic stress or both. A recent publication suggested that high levels of feed restriction require corticosterone regulation for glucose homeostasis during OFF feed day regardless of the feed program (Arrazola et al., 2019). Similar results were observed in the current research. The difference between corticosterone levels at 24 and 48 h were observed as the degree of restriction increased (from 12 to 21 wk). The results suggested the nutrient levels in soybean hulls were not enough to decrease the metabolic stress of the birds. In fact, the birds on the SAD feed program at 21 wk of age have a lower plasma corticosterone level than the ATD birds. The difference was due to the decrease in restriction on the SAD birds associated with the increase in feed amount to achieve target BW. Taken together the current results suggest that the degree of feed restriction and the length of the fasting period between feedings had the most influence on plasma corticosterone. The results suggested that the amount of soybean hulls fed to the birds was not enough to decrease the level of metabolic stress in feed restricted pullets.

Body Composition and Gene Expression

The lack of differences between body composition at 14 and 21 wk of age and gene expression suggested the feed program did not have an impact on the bird's body composition. However, the difference in BW gain and egg production suggested that the addition of soybean hulls have an impact on the birds. The difference in the expression of ACAT2 gene at 21 wk of age might be an indicator of changes in birds' metabolism. Regassa et al. (2016) previously showed that the broilers exhibit lower levels of ACAT2 after feeding compared to birds in a fasted state. The current results contradicted the findings by Regassa et al. (2016), who suggested further research needs to be conducted. Differences in plasma glucose as observed by Arrazola et al. (2019) might provide an insight on the effect of the ATD and SAD feeding program on the metabolic stress and physiological/behavioral stress.

SUMMARY

From the results of the study, we concluded that the addition of soybean hulls on the off feed day of a standard skip-a-day program improved BW uniformity and subsequent egg production; however, a welfare benefit to the soybean hull feeding was unclear and additional research is warranted to evaluate the impact of the filler. Further research is also needed to determine the potential differences in nutrient utilization and behavior in pullets reared with ATD and SAD feeding programs and what changes contribute to improved BW uniformity and egg production.

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