Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2021.19082.1694

Effect of Nutritional Variance of Energy and Crude Protein on Sex Ratio and Development of W-36 Parent Offspring

McCaide T. Wooten Zachary S. Lowman Christopher M. Ashwell

Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA

Poultry Science Journal 2021, 9(2): 255-262

Keywords
Hen
Layer
Sex ratio
Nutrition
Reproduction

Corresponding author
McCaide T. Wooten
caide.wooten.dvm@gmail.com

Article history
Received: April 29, 2021
Revised: November 10, 2021
Accepted: November 15, 2021

Abstract

Past studies have shown a link between maternal condition or resource availability, and the resultant sex ratio of offspring in avian species, both wild and domesticated. This study utilized 200 male and 400 female chicks from a W-36 white leghorn parent stock and assessed the effects of three diets with different caloric and protein concentrations on the sex ratios of the Hy-Line W-36 laying hens. As expected, the development of both parent and filial generations was significantly affected by diet or egg composition, respectively. Sex ratio was not significantly different from an assumed population proportion of 0.50 across all experimental groups, but did approach significance among offspring of parents switched from "High" to "Control" diets at 17 weeks of age. Results suggest that continual availability of either high- or low-nutrient density feed resources does not predispose hens to bias the sex ratio of their offspring; however, a sudden change in diet, particularly to one of lower protein concentration, may influence such an effect. Further trials are needed to assess the efficacy of diet alteration prior to reproductive maturity as a means of sex allocation manipulation.

Introduction

There is evidence that environmental conditions, specifically the availability and the quality of food resources, can bias the sex ratio of hatchlings in sexually dimorphic avian species (Bowers *et al.*, 2015; Howe, 1977; Kalmbach *et al.*, 2005; Love *et al.*, 2005; Nager *et al.*, 1999; Parker, 2002; Pike & Petrie, 2005; Pryke & Rollins, 2012; Trivers & Willard, 1973). Presumably, the sex requiring fewer resources to reach maturity will be produced in greater numbers when resources are scarce because its rearing is less costly (Trivers & Willard, 1973). The effect appears to be maternally derived; based on her condition and the resources to which she has access, the female will skew the sex ratio of her offspring towards the sex that is more likely to survive on lower quality egg contents during incubation and in a poorer rearing environment (Nager *et al.*, 1999; Pryke & Rollins, 2012; Trivers & Willard, 1973). Typically, this is the sex capable of surviving with a lower total intake and quality of feedstuffs and, on average, has a lower mature body weight (Nager *et al.*, 1999).

The mechanism by which this change occurs has not been definitively identified, but it has been proposed that increased maternal corticosterone production in response to stressful environmental conditions may play a major role in sex-biased maternal investment in offspring (Correa *et al.*, 2005; Goerlich-Jansson *et al.*, 2013; Love *et al.*, 2005; Merkling *et al.*, 2018; Navara, 2013; Pike & Petrie, 2003, 2005, 2006; Pinson *et al.*, 2011a; Pinson *et al.*, 2011b; Wrobel *et al.*, 2020). Wrobel *et al.* (2020) illustrated a significant influence of pre-ovulatory application of corticosterone in W-36 laying hens on the expression of multiple genes affecting oocyte maturation, the ionic gradient of the germinal disc, and epigenetic chromosomal modifications, all of which may be involved in sex ratio adjustment. Increased corticosterone levels in the bloodstream of the mother result in the reciprocal rise in egg yolk deposition (Love *et al.*, 2005; Pike & Petrie, 2003). In a study of European starlings (*Sturnus vulgaris*), high corticosterone eggs had male chicks with lower hatch rate (higher embryonic mortality), decreased weight at hatch, and slower cell-mediated immune responses.

Please cite this article as McCaide T. Wooten, Zachary S. Lowman & Christopher M. Ashwell. 2021. Effect of Nutritional Variance of Energy and Crude Protein on Sex Ratio and Development of W-36 Parent Offspring. Poult. Sci. J. 9(2): 255-262.

However, females matured from eggs with elevated corticosterone levels appeared to be unaffected (Love *et al.*, 2005). A similar study in which Japanese quail (*Coturnix coturnix japonica*) were subjected to chronic exogenous corticosterone administration via Silastic implants showed a significant negative linear relationship between fecal corticosterone concentrations and male sex ratio per clutch of eggs (Pike & Petrie, 2006). Conversely, Pinson *et al.* (2011a) found that acute administration of corticosterone to laying hens (*Gallus gallus domesticus*) five hours before ovulation resulted in significantly more male (82.6%) than female embryos.

It is still unknown whether a causal mechanism for biases in the offspring sex ratio of birds can be confidently applied across species (Pike & Petrie, 2003). In a study with the blue-faced parrot finch (*Erythrura trichroa*), a species with no discernible sexual dimorphism that is insensitive to changes in nutritional quality (in terms of body condition), yielded puzzling results. Birds on low-quality diets produced male-biased offspring (72.9%), but those on high-quality diets had an unbiased offspring sex ratio. In the juvenile offspring, females were much more susceptible to the effects of a low-quality diet than male birds, thus providing a possible explanation for the skewed sex ratio from parents fed low-quality diet. This implies that maternal condition may not play as much of a role in determining the sex of offspring as the expected rearing environment (Pryke & Rollins, 2012). Similar results were seen in both the great skua (*Stercorarius skua*) and the common grackle (*Quiscalus quiscula*, Icteridae), in which wild populations would progressively produce higher numbers of the less "metabolically expensive" sex with the deterioration of resource availability (Howe, 1977; Kalmbach *et al.*, 2005).

This study aims to identify whether environmental adjustments similar to those referred to in the included literature can create complementary bias in the sex ratio of the domestic chicken, which may have been subject to the loss of such a trait due to many years of dependence on humans for survival. If present, positive results could be of major benefit to both the commercial egg- and meat-production industries.

Materials and Methods

In this study, 200 male and 400 female day-old W-36 parent stock chicks were obtained from Hy-Line International (Dallas Center, Iowa). The chicks were vaccinated with HVT/IBD, Rispens, and SB1. All males were dubbed at hatch. Birds were neck-tagged and evenly separated into three groups, each corresponding to a diet of specific total crude protein (CP) and metabolizable energy (ME): "Control" (C)- 18% CP, 2998 kcal/kg ME, "Low" (L)- 12% CP, 2205 kcal/kg ME, and "High" (H)- 24% CP, 3219 kcal/kg ME (Table 1). Chicks were brooded in a Petersime Brood-Unit (Model 25D 24) for 4 weeks; then, birds were divided by treatment and moved to grow-out cages. At 17 weeks of age, 4 males and 24 females from each treatment group were randomly selected for breeding. An additional 12 females from both the H and L groups were switched to the control diet at this time and were labeled as HC and LC, respectively. Body and testicle weights were recorded from unsaved males, histological slides of testicles were prepared, and semen production was monitored for a separate project, details presented in Lowman *et al.*, (2014). At 19 weeks, females were artificially inseminated twice weekly with 50 µL of pooled semen from all 4 males of the corresponding group. For HC and LC females, H and L semen was used, respectively. Semen was collected from the males using the abdominal massage method described by Burrows and Quinn (1937). Birds were stimulated 2 times at each collection; semen was immediately collected from the ejaculatory groves of the phallus.

Eggs were collected for two weeks after the second week of artificial insemination (birds were inseminated twice a week) and stored at 60°F and 65% RH until incubation. Eggs were incubated in a NatureForm NMC 1000 setter (Jacksonville, FL) at 99.5°F and 55 % RH for 18 days. For the last 3 days of incubation, eggs were placed in hatchers (G.Q.F. Model 1520 Circulating Air Incubators) at 99.5°F and 65 % RH. At hatch, chicks were weighed, tagged, and evenly mixed in each level of Petersime Brood-Units. All chicks were fed a standard layer starter (19.5% CP, 2992 kcal/kg) and water ad libitum. The birds were weighed weekly to track the rate of growth. Weights were recorded for three weeks before termination.. All unhatched eggs were analyzed for fertility and, if applicable, the stage of embryonic death (i.e., early, middle, or late). After 3 weeks, birds were euthanized via cervical dislocation, and sexes were determined visually by secondary sex characteristics. Birds called into question were necropsied to confirm sex.

Tissue samples were collected from all late dead embryos for sexing via PCR amplification. DNA was extracted using the Qiagen DNA extraction kit protocol. In 600 µL of cell lysis solution and 6 µL of proteinase K, ~ 10 ng samples were incubated in a rotor oven at 55°C overnight. 200 µL of protein precipitation solution was added to each sample, then vortexed, and centrifuged. Supernatant was added to 600 µL of isopropanol, gently inverted and then centrifuged a second time to form a DNA pellet. The supernatant was then discarded and samples were rinsed with 500 µL of 70% ethanol and centrifuged a final time. The supernatant was discarded and DNA pellets were allowed to dry before being suspended in 50 µL of DNA rehydration solution. PCR reactions

were composed of GoTAQ 10x buffer, TAQ polymerase, magnesium chloride, DNTPs, and Ribo (256 bp) and XhoI (415 bp) sexing primers, as described by Clinton *et al.* (2001) PCR was run for approximately 1.5 hours. Samples were then run on an agarose gel containing ethydium bromide for approximately 30 minutes.

Table 1. Ingredient composition of diets

Ingredients (g/kg)	Control	Low	High
Yellow Corn (8.5% CP^)	521.7	372.0	415.5
Oats (9.0% CP^)	0	250.1	0
Poultry Fat	64.1	0	90.6
Poultry By-Product Meal (58.4% CP^)	105.5	0	125.1
Soybean Meal (48% CP^)	196.5	72.2	264.7
Soybean Hulls (4.6% CP^)	5.4	186.9	0.30
Calcium Carbonate	86.5	88.4	88.0
Dicalcium Phosphate	12.6	20.5	8.30
Salt	2.5	2.5	2.5
DL-Methionine	1.2	2.4	1.0
Choline	0	1.0	0
Trace Mineral Mix*	2.0	2.0	2.0
Vitamin Pre-Mix†	1.0	1.0	1.0
Selenium	1.0	1.0	1.0
Formulated Percentages			
Crude Protein	18	12	24
Crude Fat	10	2.99	12.3
Crude Fiber	4.4	10.46	1.69
Calcium	4	4	4
Metabolizable Energy (kcal/kg)	2998	2205	3219
Analysis (actual percentages)			
Crude Protein	18.6	12.4	24.8

^CP values for ingredients were sourced from NRC (1994).
*Mineral Content per g of Mix: 60mg Manganese, 60mg Zinc, 40mg Iron, 5mg Copper, 1.25mg Iodine, 0.5mg Cobalt.
†Vitamin Content per g of Mix: 13,200 IU Vitamin A, 400 IU Cholecalciferol, 66 IU Vitamin E, 34.6µg, Cobalamin, 13.2mg Riboflavin, 110mg Niacin, 22mg Pantothenic Acid, 4mg Vitamin K, 2.2mg Folic Acid, 4mg Thiamine, 8mg Pyridoxine, 252µg Biotin.

Two more replicates were conducted using the aforementioned methodology. However, blood was taken at the time of hatch from a random selection of C, H, and L chicks of the second hatch to measure corticosterone levels and all chicks were only tracked for growth for three weeks before termination. For the third hatch, blood samples were taken for PCR sexing at hatch, but chicks were not kept for tracking growth due to space limitations. Egg production records were kept for parental groups during the trial.

Weight and blood corticosterone experimental data collected during the trial were analyzed by ANOVA (JMP 16, SAS, Cary, NC) using Tukey-Kramer comparison of means. Sex ratio data were analyzed by two-tailed one-sample proportion *Z* hypothesis tests (Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au) utilizing an assumed population proportion of p=0.50 (50% male, 50% female). Dietary treatments were used as the experimental units, and an alpha of 0.05 was used to establish the statistical significance.

Results

There were no significant differences between the observed sex ratios and the assumed ratio of p= 0.50 across all treatments. This finding was consistent for hatched, late dead, and total offspring produced for all hatches (Tables 2-4).

Table 2. Percent late dead offspring by treatments

Treatments	% Male (#/n)	% Female (#/n)	*P*-Value
Low	56.76 (21/37)	43.24 (16/37)	0.4109
High	56.67 (17/30)	43.34(13/30)	0.4650
Control	45.28 (24/53)	54.72 (29/53)	0.4519
Low-Control, LC	38.46 (5/13)	61.54 (8/13)	0.4053
High-Control, HC	46.67 ((7/15)	53.33 (8/15)	0.7965

**P*-values <0.05 represent sex ratios that differed significantly from an assumed population ratio of 0.50 (50% Male, 50% Female).

Table 3. Percent hatched offspring by treatments

Treatments	% Male (#/n)	% Female (#/n)	*P*-Value
Low	48.11 (178/370)	51.89 (192/370)	0.4672
High	50.66 (193/381)	49.34 (188/381)	0.7967
Control	47.40 (246/519)	52.60 (273/519)	0.2362
Low-Control, LC	55.06 (98/178)	44.94 (80/178)	0.1770
High-Control, HC	43.48 (80/184)	56.52 (104/184)	0.0769

*P-values <0.05 represent sex ratios that differed significantly from an assumed population ratio of 0.50 (50% Male, 50% Female).

Table 4. Percent total offspring by treatments

Treatments	% Male (#/n)	% Female (#/n)	*P*-Value
Low	48.89 (199/407)	51.11 (208)	0.6542
High	51.09 (210/411)	48.91 (201/411)	0.6585
Control	47.20 (270/572)	52.80 (302/572)	0.1805
Low-Control, LC	53.93 (103/191)	46.07 (88/191)	0.2774
High-Control, HC	43.72 (87/199)	56.28 (112/199)	0.0764

*P-values <0.05 represent sex ratios that differed significantly from an assumed population ratio of 0.50 (50% Male, 50% Female).

Birds that had been switched from the H diet to the C diet produced more female offspring at hatch at a level that approached significance for both hatched (P=0.0769) and total (P=0.0764) offspring (Tables 3 and 4).

Blood corticosterone concentrations at hatch were significantly higher in offspring from parents on the L diet (P < 0.01). Offspring from birds on the H diet had slightly higher blood corticosterone concentrations at hatch than did the C offspring, but not at a level of statistical significance (Figure 1).

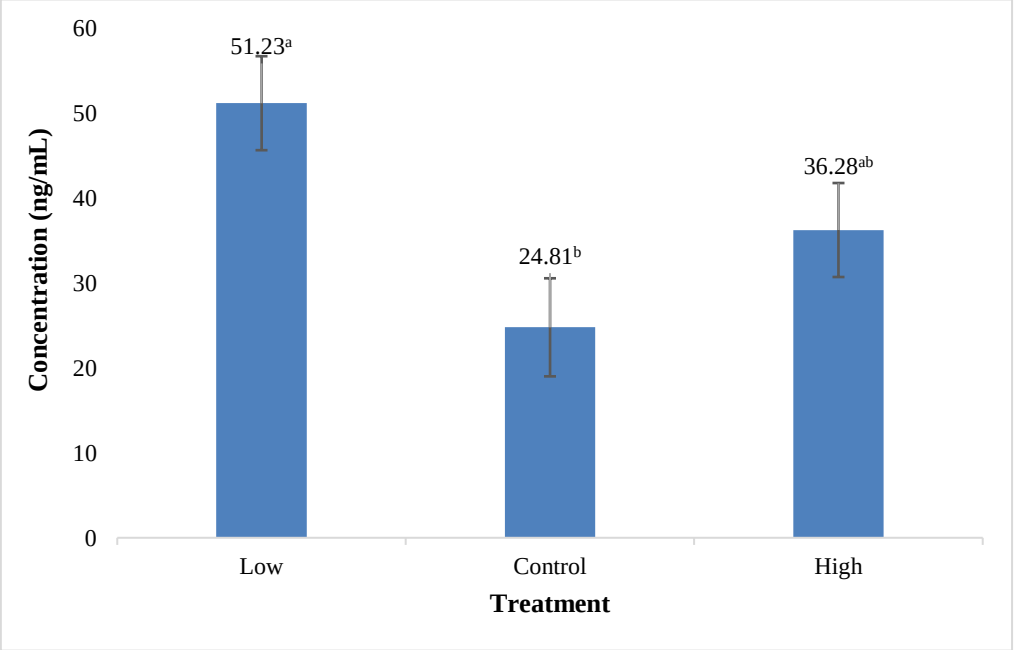

Figure 1. Mean offspring blood corticosterone concentration at hatch
[a,b]Concentrations within bars with different superscripts differ significantly (P <0.01)

Between 21 and 26 weeks, overall lay percentage was reduced in both the H and L groups compared to the control and was significantly lower (P < 0.01) in the L group. The L group came into peak lay, approximately 80%, about 4 weeks later than the H and C groups. Birds that transitioned diets showed rapid changes in egg production between 21 and 26 weeks of age; although not statistically significant, production for the HC group was comparable to that of the C group at week 21, but had decreased by week 26. Conversely, production of the LC group was comparable to the L group at 21 weeks, but was the same as the C group by 26 weeks (Figure 2).

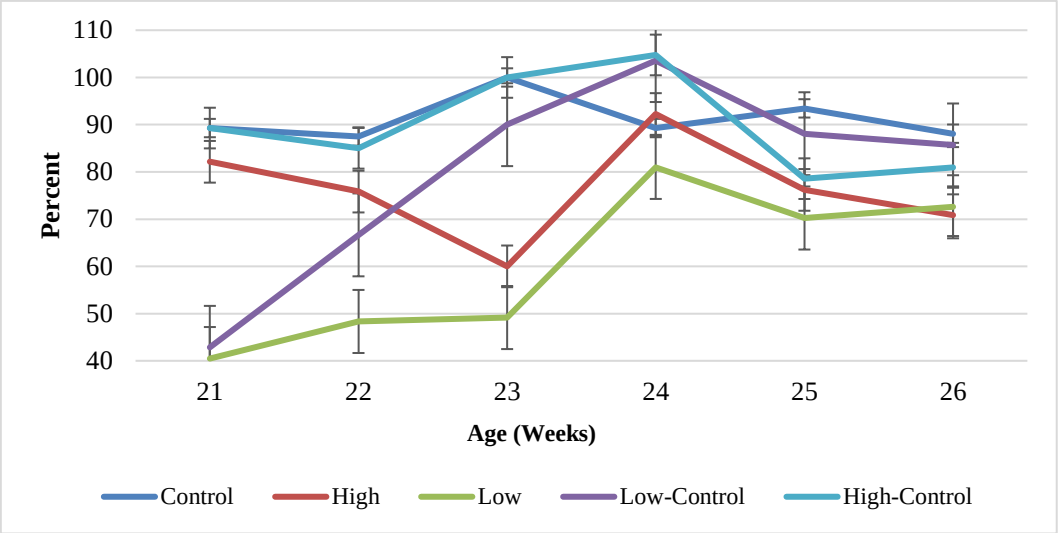

Figure 2. Parent stock percent egg production 21-26 weeks.
The control, high, and low groups were composed of 24 females each, the low-control and high-control groups were composed of 12 females each.

At hatch, there were significant differences in offspring weights between groups. The H offspring were significantly larger ($P < 0.0059$) than the L offspring, when analyzed only by treatment (Table 5). When further broken down by treatment and sex, there were no significant differences between the males of each treatment, but the H females were significantly larger than the L females, with the C females falling in the middle (Table 6). At the day 7 of weighing, both the C and the H groups were significantly larger ($P < 0.0001$) than the L birds (Table 5). The C and H males were both significantly larger than the L males, with the females following the same pattern (Table 6). 14- and 21-day weights revealed similar results; the H group weights were significantly larger than the L group with the C group being in the middle (Table 5). At day 21, there were no significant differences between the male weights of each group. However, the C females were significantly heavier than the L females ($P < 0.001$) (Table 6).

Table 5. Total mean body weights for offspring by treatments

	Day 0 wt (g)	± SE	Day 7 wt (g)	± SE	Day 14 wt (g)	± SE	Day 21 wt (g)	± SE
Low	40.04 ^{b}	0.22	63.97 ^{b}	0.43	122.58 ^{b}	1.80	176.20 ^{b}	2.27
Control	40.54 ^{ab}	0.20	68.39 ^{a}	0.42	128.55 ^{ab}	1.77	187.84 ^{a}	2.22
High	40.98 ^{a}	0.20	67.78 ^{a}	0.42	129.71 ^{a}	1.76	183.13 ^{ab}	2.21

^{a,b}Means within each column with different superscripts differ significantly ($P < 0.01$

Table 6. Total mean body weights for offspring by treatment x sex

	Day 0 wt (g)	± SE	Day 7 wt (g)	± SE	Day 14 wt (g)	± SE	Day 21 wt (g)	± SE
Low Female	39.84 ^{c}	0.28	62.48 ^{c}	0.51	117.56 ^{b}	2.27	165.55 ^{c}	2.54
Low Male	40.36 ^{abc}	0.34	66.16 ^{b}	0.62	130.16 ^{a}	2.79	192.28 ^{a}	3.12
Control Female	40.01 ^{bc}	0.26	66.33 ^{b}	0.52	124.13 ^{ab}	2.35	176.82 ^{b}	2.63
Control Male	41.17 ^{a}	0.29	71.02 ^{a}	0.59	133.89 ^{a}	2.59	201.19 ^{a}	2.90
High Female	41.03 ^{ab}	0.28	65.78 ^{b}	0.55	125.48 ^{ab}	2.52	169.47 ^{bc}	2.80
High Male	40.93 ^{abc}	0.27	69.69 ^{a}	0.54	133.39 ^{a}	2.35	195.2 ^{a}	2.63

^{a,b,c} Means within columns with different superscripts differ significantly ($P < 0.01$)

Discussion

Several studies have demonstrated a relationship between a female's physical condition and the sex ratio of her offspring in multiple avian species (Bowers *et al.*, 2015; Howe, 1977; Kalmbach *et al.*, 2005; Love *et al.*, 2005; Nager *et al.*, 1999; Parker, 2002; Pike & Petrie, 2005; Pryke & Rollins, 2012; Trivers & Willard, 1973). However, our results were not indicative of such a phenomenon. Hens raised from hatch on diets with either high or low energy and crude protein levels did not skew the sex ratio of their offspring to any appreciable degree. Although, there were changes that approached significance in the sex ratio of offspring from birds that had experienced a change in diet at the onset of lay. A repeat study utilizing larger sample sizes with comparable results between experimental groups would result in statistical significance. Our

experimental design was novel in that we raised our parent stock on experimental diets differing in nutritional content; all of the previously cited reports studied birds subsisting in wild populations or raised under standardized dietary protocols . As such, it may be that the hen's physiological response to an abrupt change in resources, or her perception of a change, is the causative trigger that leads to a bias of the sex ratio of her offspring.

As expected, the offspring from parents raised on the low nutrient diet had the highest blood corticosterone levels at hatch, indicative of the higher deposition in the yolks of their eggs. This is in concordance with the findings of Schoech *et al.*, (1997) and Pike & Petrie, (2006) who reported that birds would respond to stressful events, like changes in diet, via the production of hormones, particularly the stress hormone corticosterone (Pike & Petrie, 2003). There is evidence that corticosterone inhibits testosterone production (Wingfield *et al.*, 1994). It was also shown that acute application of testosterone several hours prior to ovulation in domestic chickens can skew the resulting sex ratio of offspring toward males (Pinson *et al.*, 2011b). This finding was also reflected with chronic application of testosterone via Silastic tube implantation in the Homing Pigeon (*Columba livia palumbus*) (Goerlich-Jansson *et al.*, 2013). As such, the inhibition of testosterone production via the action of chronically increased corticosterone levels in stressed hens may be a possible explanation for the skewing of the sex ratio toward females in poultry (Pike & Petrie, 2006). However, Pinson *et al.* (2011a) showed that acute application of corticosterone in domestic chickens, though far exceeding physiological capabilities, stimulates the production of more male offspring. Our results indicate that high corticosterone levels, as seen in the offspring from the parents raised on the low-nutrient diet, may not necessarily cause any change in sex ratio. Again, this suggests that it is more likely a relatively acute *change* in hens' circulating hormone concentrations, perhaps in response to changing environmental conditions, that stimulates changes in offspring sex ratio, rather than the concentrations themselves.

The delay in egg production in the hens on the low nutrient diet appears to be similar to research from broiler breeders (Joseph *et al.*, 2000). Walsh and Brake (1997) reported that if pullets do not meet the recommended threshold of energy and protein intake prior to photo stimulation, they will have a delayed onset of lay and can have decreased rates of lay once in production. Conversely, the hens that were over-conditioned did not lay as well as the control birds. However, these birds did come into lay slightly earlier. The same trend has also been reported in the broiler breeder industry and is one of the reasons for the implementation of feed restriction programs in broiler breeders. More trials focused on examining how much of an increase in diet nutrient levels as well as how long those levels need to be sustained to stimulate a sex ratio bias, if one can be physiologically stimulated by this mechanism, will need to be conducted. It would also need to be determined if the sacrifice in egg production at the beginning of the laying period would justify the theoretical increase in female chicks produced.

In offspring of parents on the low diet, initial hatch weight was the lowest of all birds and weight gain was decreased, suggesting a link in total nutritional deposition in the egg and the resultant size and growth pattern of offspring. Both male and female offspring of parents on the low diet hatched smaller than all other chicks, but did maintain a comparable rate of growth. There was not a negative effect on growth rate associated with smaller egg size or lower nutrient contents, indicating that the poor nutritional status of parents does not adversely affect offspring in any way other than initial size. Chicks of parents on the high diet were the largest of all treatment groups for both sexes at hatch. However, by three weeks of age, high diet offspring had been overtaken in size by control offspring and were more comparable in weight to low diet offspring. Based on these data, it appears that the larger egg size and increased nutrient deposition within the yolk of eggs from high diet parents was initially beneficial to their offspring; however, their slower rate of growth compared to control offspring on the same diet suggests a physiological disparity. There may be an epigenetic change occurring as a result of higher nutrient intake and obesity in the parental generation predisposing offspring to slower growth.

The presence of a nutritionally-linked phenomenon for the manipulation of offspring sex ratio in domestic fowl could result in the implementation of nutritional or managerial changes to attain results seen in the laboratory setting. Practical use of our findings depend heavily on the ease of integration of new practices into the current production system at the breeder level and economic feasibility. Further trials are needed to justify industrial integration. Based on our results, new studies could use similar diets to evaluate a potential link between acute diet changes, blood corticosterone concentrations, and biasing of sex ratios in parent-generation commercial breeders. Assuming similar results, larger sample sizes would be necessitated to establish statistical significance.

Conclusion

Continual access to either high-quality or low-quality diet from hatch to sexual maturity does not seem to affect the resultant sex ratio of offspring in W-36 layers. There may be a mechanism of sex ratio adjustment associated with the sudden change of diet

type at the onset of sexual maturity, perhaps hormone-linked; further study is required to confirm this hypothesis. Parents on low-protein and energy diets have offspring with significantly smaller hatch weights, but the differences between these offspring and those of parents on high-protein and energy diets become indiscernible within a few weeks of hatching.

References

Bowers EK, Thompson CF & Sakaluk SK. 2015. Persistent sex-by-environment effects on offspring fitness and sex-ratio adjustment in a wild bird population. Journal of Animal Ecology, 84: 473–486. DOI: 10.1111/1365-2656.12294

Burrows WH & Quinn JP. 1937. The Collection of Spermatozoa from the Domestic Fowl and Turkey. Poultry Science, 16: 19-24. DOI: 10.3382/ps.0160019

Clinton M, Haines L, Belloir B, & McBride D. 2001. Sexing chick embryos: A rapid and simple protocol. British Poultry Science, 42: 134-138. DOI:10.1080/713655025

Correa SM, Adkins-Regan E & Johnson PA. 2005. High progesterone during avian meiosis biases sex ratios toward females. Biology Letters, 1: 215-218. DOI: 10.1098/rsbl.2004.0283

Goerlich-Jansson VC, Muller MS & Groothuis TGG. 2013. Manipulation of Primary Sex Ratio in Birds: Lessons from the Homing Pigeon (*Columba liviadomestica*). Integrative and Comparative Biology, 53: 902–912. DOI: 10.1093/icb/ict056

Howe HF. 1977. Sex-ratio adjustment in the common grackle. Science, 198(4318): 744-746. DOI: 10.1126/science.198.4318.744

Joseph NS, Robinson FE, Korver DR & Renema RA. 2000. Effect of dietary protein intake during the pullet-to-breeder transition period on early egg weight and production in broiler breeders. Poultry Science, 79: 1790-1796. DOI: 10.1093/ps/79.12.1790

Kalmbach E, Furness RW & Griffiths R. 2005. Sex-biased environmental sensitivity: Natural and experimental evidence from a bird species with larger females. Behavioral Ecology, 16: 442-449. DOI: 10.1093/beheco/ari018

Love OP, Chin EH, Wynne-Edwards KE & Williams TD. 2005. Stress hormones: A link between maternal condition and sex-biased reproductive investment. The American Naturalist, 166: 751-766. DOI: 10.1086/497440

Lowman ZS, Wooten MT, Parkhurst CR & Ashwell CM. 2014. Nutritional Effects on Reproductive Performance of Hy-Line W-36 Parent Stock Males. Poster presented at the annual meeting of the Poultry Science Association, Corpus Christi, TX.

Merkling T, Nakagawa S, Lagisz M & Schwanz LE. 2018. Maternal Testosterone and Offspring Sex-Ratio in Birds and Mammals: A Meta-Analysis. Evolutionary Biology, 45: 96–104. DOI: 10.1007/s11692-017-9432-9

Nager RG, Monaghan P, Griffiths R, Houston DC & Dawson R. 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Sciences of the United States of America, 96: 570-573. DOI: 10.1073/pnas.96.2.570

Navara KJ. 2013. The Role of Steroid Hormones in the Adjustment of Primary Sex Ration in Birds: Compiling the Pieces of the Puzzle. Integrative and Comparative Biology, 53: 923–937. DOI: 10.1093/icb/ict083

NRC (National Research Council). 1994. Composition of Feedstuffs Used in Poultry Diets. In Nutrient Requirements of Poultry, 9th Rev. Ed.: 61–79. National Academies Press: Washington, DC.

Parker TH. 2002. Maternal condition, reproductive investment, and offspring sex ratio in captive red junglefowl (*gallus gallus*). The Auk, 19: 840-845. DOI: 10.1642/0004-8038(2002)119[0840:MCRIAO] 2.0.CO;2

Pike TW & Petrie M. 2003. Potential mechanisms of avian sex manipulation. Biological Reviews, 78: 553-574. DOI: 10.1017/S1464793103006146

Pike TW & Petrie M. 2005. Maternal body condition and plasma hormones affect offspring sex ratio in peafowl. Animal Behaviour, 70: 745-751. DOI: 10.1016/j.anbehav.2004.12.020

Pike TW & Petrie M. 2006. Experimental evidence that corticosterone affects offspring sex ratios in quail. Proceedings of the Royal Society, 273(1590): 1093-1098. DOI: 10.1098/rspb.2005.3422

Pinson SE, Parr CM, Wilson JL & Navara KJ. 2011a. Acute corticosterone administration during meiotic segregation stimulates females to produce more male offspring. Physiological and Biochemical Zoology, 84: 292-298. DOI: 10.1086/659373

Pinson SE, Wilson JL & Navara KJ. 2011b. Elevated testosterone during meiotic segregation stimulates laying hens to produce more sons than daughters. General and Comparative Endocrinology, 174: 195-201. DOI: 10.1016/j.ygcen. 2011.08.020

Pryke SR & Rollins LA. 2012. Mothers adjust offspring sex to match the quality of the rearing environment. Proceedings of the Royal Society B, 279(1744): 4051-4057. DOI: 10.1098/rspb.2012.1351

Schoech SJ, Mumme RL & Wingfield JC. 1997. Corticosterone, reproductive status and body mass in a cooperative breeder, the Florida scrub-jay (*Aphelocoma coerulescens*). Physiological and Biochemical Zoology, 70: 68–73. DOI: 10.1086/639545

Trivers RL & Willard DE. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science,179(4068): 90-92. DOI: 10.1126/science.179.4068.90

Walsh TJ & Brake J. 1997. The effect of nutrient intake during rearing of broiler breeder females on subsequent fertility. Poultry Science, 76: 297-305. DOI: 10.1093/ps/76.2.297

Wingfield JC, Suydam R & Hunt K. 1994. The adrenocortical responses to stress in snow buntings (Plectrophenax nivalis) and Lapland longspurs (*Calcarius lapponicus*) at Barrow, Alaska. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 108: 299–306. DOI: 10.1016/0742-8413(94)00030-E

Wrobel ER, Bentz AB, Lorenz WW, Gardner ST, Mendonça MT & Navara KJ. 2020. Corticosterone and testosterone treatment influence expression of gene pathways linked to meiotic segregation in preovulatory follicles of the domestic hen. PLOS ONE, 15(5). DOI: 10.1371/journal.pone.0232120