Effect of Gibberellic Acid on Production Characteristics of Aged and Force Molted Chickens in Cages

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ABSTRACT Production characteristics of individually caged 72-week-old brown egg type hens or 86-week-old hens of a similar strain that had previously been subjected to different watering systems (continuous flow cups *vs.* intermittent flow troughs) and the stress and recovery of a forced molting program were evaluated in two trials over a 4 to 5-week interval following 6 alternate day subcutaneous injections (2.4 mg/bird) of gibberellic acid (GA_3).

Egg production and feed consumption of the 72-week-old hens maintained at constant temperature (20.0 ± 1.0 C), humidity (55%), and light intensity (10.76 lx) were significantly greater (GA_3 × time, $P<.05$) in the GA_3 treated birds and were accompanied by significantly larger eggs (GA_3 × time, $P<.05$) with generally thinner shells. In the second trial, the 86-week-old hens responded with similar trends but the differences were less and related in part to prior watering system. No statistically significant differences were found for egg production, feed consumption, and egg weight as previously observed, but there was significantly greater shell thickness of eggs from the GA_3-treated birds previously adapted to cups as compared to trough waterers (GA_3 × time × waterer, $P<.025$). No statistically significant effects of GA_3 on body weight were observed in either trial.

The data suggest that the differences in response between the two trials may be related to the initial differences in egg production and, thus, the levels of circulating gonadal hormones, since GA_3 has been shown to have androgenic activity in chicks, and estrogenic as well as other hormonal activity in other species.

(Key words: gibberellic acid, hens, age, force molting, production)

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INTRODUCTION

Gibberellic acid (GA_3) is one of at least 52 known gibberellins. It is the most common of this group of endogenous plant growth promoting metabolites derived from cultures of the fungus *Gibberella fujikuroi.* The effects of GA_3 on various aspects of plant growth and development has been extensively researched (Moore, 1979); however, a relative dearth of information is available on its potential metabolic effects in mammalian and avian species. The early studies of Peck *et al.* (1957) and Kimura *et al.* (1959) on the toxicological and pharmacological effects of GA_3 clearly demonstrated that it was essentially nontoxic when tested by various routes of injection or by oral, ocular, or aerosol application in rats, mice, guinea pigs, rabbits, cats, or dogs. Because of the possible use of gibberellins in spray applications for promoting plant growth in field crops and the presence of potentially high residual levels on plant materials used in poultry feeds, Warden and Schaible (1958) fed GA_3 to broiler chicks to 4 weeks of age. They found no significant effects on body weight, feed efficiency, or livability at levels 10 times that anticipated as potential residues. A subsequent study was conducted by Champion and Schnabel (1962) to determine the effects of gibberellins injected into fertile eggs prior to incubation and at 3.5, 4.5, and 8 days after incubation. The authors suggested that gibberellin injected prior to incubation does not produce any gross cell abnormalities or specific embryonic mortality pattern, does not exhibit an important nutritional role for embryo development or for the growing chick to 2 weeks posthatching, nor does it apparently mimic, supplement, or alter the endogenous growth-promoting hormones of the chick embryo or progeny to 2 weeks of age. However, based on gross observations, they also reported that the chicken embryo is apparently more sensitive to injections of gibberellins following

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differentiation and specialization rather than in the initial stages of development.

In contrast to the absence of, or negative effects of, GA_3 on mammalian and avian species, more recent evidence indicates that it might be biologically active in animal organisms. Studies by Gawienowski and coworkers (1977a,b) have clearly demonstrated that GA_3 not only exhibits synergistic uterotrophic effects with estrodiol in the immature mouse, but that it also possesses androgenic properties in male chicks based on the chick comb bioassay. In the latter case, estradiol (E_2) prevented the gibberellin-induced stimulation. Moreover, the mode of estrogenic actions of GA_3 and E_2 based on the mouse uterine bioassay has recently been suggested (Gawienowski and Chatterjee, 1980). These authors postulated that because GA_3 has demonstrated in mammals a number of hormone-like actions, the activity of GA_3 may be via the prostaglandins. Their study revealed that a significant increase in mouse uterus weight produced by either GA_3 or E_2 was almost completely blocked by the prostaglandin inhibitor indomethacin, indicating an apparent relationship between biosynthesis of prostaglandins and the action of GA_3 and E_2. Also, GA_3 has been patented as a medicine (Lab. Laroche Navarron, 1967) for protein deficiency, states of stress, and a number of other metabolic conditions.

The studies reported herein were conducted to investigate whether the hormonal actions of GA_3 reported for mammals might have an influence on the production potential of laying hens approaching the end of their first laying year or in similar physiologically fatigued hens maintained under the stress of different watering systems and partially rejuvenated by a forced-molting program.

MATERIALS AND METHODS

Experiment 1. Thirty hens of a commercial brown egg type strain housed 3 birds per 30.5 × 45.7 cm cage in a modified stair step system with continuous flow trough watering system for the previous 72 weeks (Grover and Anderson, 1980) were selected for this study. One-half of these hens was injected subcutaneously on 6 alternate days with .4 ml of solution containing 400 μg of GA_3 or a total of 2.4 mg. The compound was dissolved in a 1:11 ethanol-sesame oil mixture with the addition of 4.0 mg $NaHCO_3$/.4 ml of injection solution. The remaining 15 hens were treated in a like manner except that the injection solution contained the ethanol-sesame oil-$NaHCO_3$ mixture only. Following the injection interval, the hens were transferred at random to individual triple deck battery cages in an environmental chamber maintained at a constant 20 ± .5 C, 55% relative humidity, 10.76 lx, and a 16 hr light: 8 hr dark cycle. A commercial breeder mash was fed in individual feeders to all birds, and water was supplied by a continuous flow cup (CFC) watering system. Data were recorded for body weight, egg production, egg weight (Mettler E200 balance), and egg shell thickness determined by an Ames micrometer with a convex anvil head on an individual bird basis for the following 4 consecutive weekly intervals. Feed consumption and conversion data were obtained only during the postinjection intervals.

Experiment 2. To test further the efficacy of GA_3 in altering the production potential of physiologically fatigued hens, a second study was conducted with a different commercial strain of brown egg-type layers, which had previously been maintained on different watering systems and which had recovered from a moderately severe molting program. The birds were molted according to a program in which the lights were turned off and feed removed for one week resulting in an average body weight loss of approximately .5 kg. At this time the birds received their first meal and thereafter we followed the program outlined by Savage (1976) with the exceptions that a breeder mash was fed, and once feeding was resumed, no further fast days were used. At no time during this process was the water turned off. Production peaked in the high seventies to low eighties at 86 weeks of age when they were selected for this experiment.

Thirty hens from the CFC waterer group and 30 from the intermittent flow trough (IFT) system group (i.e., 4 daily watering of 15 min each) from this study (Grover and Anderson, unpublished data) were used. Birds were transferred at random from either 3 or 7 bird cages of the same modified stair-step cage system used in Experiment 1 to individual bird, triple deck laying cages with CFC waterers in a controlled environment room maintained at 25.6 ± 1 C; 10 to 30 lx, and a 16 hr light: 8 hr dark cycle. Three replicates of 5 birds from each prior watering system were injected subcutaneously with a GA_3 solution of the same composition at the same dosage level and schedule as in

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TABLE 1. *Effect of subcutaneous injections of gibberellic acid (GA_3) on mean production parameters of 72-week-old brown egg type chickens (Experiment 1)*

Parameters	GA_3	Time postinjection, weeks 0	1	2	3	4	Mean, weeks 1-4
Body weight, kg	0	2.38[1]	...	2.34	...	2.29	2.32
	+	2.40	...	2.34	...	2.37	2.36
Feed consumed, g/bird/day	0	...	...	106.6	...	117.9	112.2
	+	...	...	109.9	...	139.8	124.8
Eggs/bird/week	0	4.00	4.13	4.07	3.80	4.13	4.03
	+	4.67	4.27	4.54	5.40	5.13	4.84
Feed consumed/egg, g	0	...	...	221.4	...	225.5	238.5
	+	...	...	178.3	...	188.1	183.2
Egg weight, g	0	68.8	67.3	66.5	65.7	67.6	66.8
	+	68.7	67.4	67.6	69.2	68.4	68.2
Shell thickness, mm × 100	0	33.0	32.5	33.0	34.8	34.3	33.6
	+	34.8	32.7	32.7	35.0	33.8	33.6

[1] Mean of 15 females per group.

Experiment 1. Commercial layer mash from the same source as in Experiment 1 containing 17% protein by analysis was fed to all birds. Data on feed consumption, body weight, egg production, feed conversion, egg weight, and shell thickness were collected during the 11-day injection interval and for 5 consecutive weekly intervals thereafter.

Least squares analyses on the variances associated with repeated observations on the same individuals (Snedecor and Cochran, 1956) were conducted for all parameters in both experiments and were based on the postinjection intervals only unless otherwise stated.

RESULTS

Experiment 1. Results of alternate day subcutaneous administration of GA_3 on production parameters of 72-week-old brown egg type female chickens are presented in Table 1 with the mean squares from the variance analysis shown in Table 2. In those hens receiving GA_3 there was significantly greater feed intake with time, which was partially reflected in a slight (.08 kg) but nonsignificant increase in body weight 4 weeks postinjection ($P>.05<.10$). The significantly greater mean egg production ($P<.05$) in those birds administered GA_3 was accompanied by 23% less feed consumed per egg. The progressive increase in egg numbers with time reached a maximum at 3 weeks postinjection and declined slightly the following week. In contrast, those hens to which the carrier solution had been administered maintained production at an essentially constant level throughout the entire 4 week postinjection period. Following an initial decline, egg weight increased in a similar manner to 3 weeks postinjection in GA_3 -treated birds, whereas the carrier-injected birds showed a progressive decline to this point. Shell thickness dropped 6.0% from the injection period to the end of the 1st week postinjection in the GA_3 birds and fluctuated in concert with the controls thereafter. The controls were unchanged throughout the experimental period. No significant differences in shell thickness were observed except for the variable fluctuations with time (Table 2) when the analysis was based on the 4 postinjection periods. However, when another analysis was conducted to include the marked drop in shell thickness indicated, the GA_3 × time interaction attained statistical significance at the 5% level of probability.

Experiment 2. In view of the apparent stimulatory effects of GA_3 on production parameters in Experiment 1 and a possible relationship to the maintenance of anabolic

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TABLE 2. *Mean squares from analyses of variance of production parameters (Experiment 1)*

Source of variation		df	Body weight	Feed consumed	Feed consumed/egg	df	Eggs/bird/week	Egg weight	Shell thickness
GA_3	(G)	1	.0217	2392.7535	45838.1760†	1	19.200*	61.0614	2.2688
Error a		28	.1390	931.5679	12917.9871	28	4.295	59.1426	8.4115
Time	(T)	1	.0002	6408.7335***	7229.2327	3	1.200	3.4831	37.1339***
GT		1	.0224	1294.5615*	2225.2860	3	3.089†	17.6360††	.1743
Error b		28	.0216	240.2107	14177.4000	84	1.173	5.1237	2.4880

*P<.05.
***P<.005.
†P>.05<.10.
††P<.025.

processes during different stressful situations purported in the patent issued to Lab. Laroche Navarron (1967), a second experiment was conducted. Birds previously maintained on either the IFT or CFC watering systems and that had experienced the stress and recovery of the forced molting procedure described previously were used in this study. The results are presented in Tables 3 and 4.

Although no significant differences were observed as a result of GA_3 or previous watering system based on body weight *per se*, an analysis of the changes in body weight determined from the end of the injection period to the end of either postinjection period 4 or 5 (Table 3) revealed a significantly greater gain ($P<.05$) in those birds previously adapted to the IFT waterers. It would appear that the major portion of this response was due to the .09 kg mean increase in weight of those hens treated with GA_3. As observed in Experiment 1, the changes in body weight were accompanied by similar, although in this case nonsignificant, changes in feed consumption. The overall lower egg production of the IFT birds having received GA_3 throughout this trial accompanied by the higher apparent feed consumption is reflected in the significantly poorer ($P<.05$) feed conversion of these groups. Egg production followed similar, but statistically nonsignificant, trends as those observed in Experiment 1 in that eggs per bird per week in the CFC groups treated with GA_3 progressively increased to the 3rd week postinjection returning to a level below that at the start of the study. The controls remained essentially constant or declined slightly irrespective of prior watering system. No consistent trends were found in the GA_3-treated hens derived from the IFT water system.

Although there were no statistically significant effects of GA_3 on egg weight as observed in Experiment 1, there was a consistent but smaller time trend increase in egg size in the GA_3-treated hens from those groups that had been maintained on the CFC watering system throughout their entire laying cycle. A small but transient shift in mean egg weight at 2 and 3 weeks postinjection was also evident in the IFT waterer groups. Contrary to Experiment 1, there was a significant increase in shell thickness with time that occurred only in those groups having previously been exposed to the CFC watering system.

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TABLE 4. *Mean squares from analyses of variance of production parameters (Experiment 2)*

Source of variation	df	Body weight	Feed consumed	Feed consumed/egg	Eggs/bird week	Egg weight	Shell thickness
GA_3 (G)	1	.1739	601.6667	4161.6681†	.3530	10.5337	1.8868
Waterer (W)	1	.0756	40.3441	4432.4415†	2.1660†	30.0475	.1102
GW	1	.0244	119.8507	5512.3335*	1.6000	.6121	.0796
Error a	8	.1118	178.9500	856.9013	.5130	10.0108	1.4646
Time (T)	4	.0086***	286.4414†	1212.1404†	.3200	.7279	2.6199***
WT	4	.0014	38.6494	389.1561	.2460	.1294	.0390
GT	4	.0012	99.9754	277.6761	.1950	.8676	.0497
WGT	4	.0009	221.2044	788.9481	.1640	.6579	.2383††
Error b	32	.0013	125.6892	479.8097	.2075	.6821	.0654

*P<.05.
***P<.005.
†P>.05<.10.
††P<.025.

DISCUSSION

It is apparent that under the conditions of these studies, egg production, feed consumption and conversion, egg weight, and shell thickness were affected to varying degrees by GA_3 administered subcutaneously at 2.4 mg to hens nearing the end of their first laying year. In spite of the differences between strains, physical environment before and during the experimental periods, and the stresses that may have been associated with the forced molting program used, the overall results between Experiments 1 and 2 agree quite well particularly when compared on the basis of previous watering systems. The lower and more variable responses to GA_3 obtained in the second experiment in comparison to the first appeared to be related in part to the higher mean egg production for all groups during the injection interval (75.1 *vs.* 61.9%) and, therefore, presumably higher circulating estrogenic hormone levels. Thus, it would appear that if part of the metabolic activity of GA_3 in the bird is to enhance or induce an increase in estrogen levels and/or produce direct estrogen-like action, similar to that demonstrated by Gawienowski *et al* (1977a) with mouse uterine tissue, one might anticipate the lower production response of force molted hens maintained throughout on the CFC watering system. This does not, however, explain the essential absence of response in those birds adapted to the IFT following their transfer to the CFC watering system. If, however, the activity of GA_3 is more universal and mediated through a possible involvement of the prostaglandins and their biosynthesis, as indicated by Gawienowski and Chatterjee (1980) in mature ovariectomized mice, then other physiological systems may also be directly or indirectly involved with the end result representing a balancing between systems. If such a hormonal interplay or integrated feedback were operative, the action of GA_3 would be dependent on the response of the predominant system. This hypothesis may be helpful in explaining the more variable and weaker production responses found whan GA_3 was administered to birds stressed by the transfer from the IFT to the CFC watering system. Most of the increase in apparent feed consumption was associated with increased body weight and not with changes in egg production, in egg weight, or in shell thickness. It is also conceivable that the apparent dif-

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TABLE 3. *Effect of subcutaneous injections of gibberellic acid (GA_3) and previous watering system on mean production parameters of 86-week-old brown egg type females following a forced molt (Experiment 2)*

Parameter	Water[1] system	GA_3	Time postinjection, weeks 0	1	2	3	4	5	Mean, weeks 1-5
Body weight, kg	C	0	2.63[2]	2.62	2.63	2.61	2.67	2.66	2.64
		+	2.70	2.67	2.71	2.71	2.74	2.71	2.71
	I	0	2.52	2.48	2.49	2.54	2.57	2.54	2.52
		+	2.62	2.61	2.69	2.68	2.68	2.71	2.67
Feed consumed, g/bird/day	C	0	135.6	116.8	127.7	107.9	129.7	126.6	121.7
		+	127.7	116.9	134.6	131.0	128.8	114.9	125.2
	I	0	127.5	115.5	120.1	122.6	125.4	119.2	120.8
		+	138.0	123.5	138.3	124.5	128.6	133.7	129.7
Egg/bird/week	C	0	5.53	5.33	5.00	5.00	5.33	5.20	5.17
		+	5.40	5.47	5.47	5.60	5.33	4.87	5.35
	I	0	5.33	5.40	5.07	5.07	5.47	5.00	5.20
		+	4.77	4.27	4.23	5.03	5.07	4.60	4.64
Feed consumed/egg, g	C	0	172.0	153.9	180.7	152.3	171.4	170.4	165.7
		+	166.2	149.7	173.1	164.0	169.6	162.3	163.7
	I	0	170.8	150.5	167.4	169.4	160.0	168.7	163.2
		+	202.3	205.4	231.9	173.0	177.5	210.1	199.6
Egg weight, g	C	0	65.9	65.6	65.3	64.7	65.0	64.9	65.1
		+	65.9	66.0	66.2	66.1	66.3	65.8	66.1
	I	0	64.8	65.5	63.3	63.1	64.2	64.1	63.8
		+	64.7	64.3	65.2	64.2	64.1	64.4	64.4
Shell thickness, mm × 100	C	0	36.3	38.5	37.3	37.1	38.2	35.6	37.3
		+	37.9	38.9	38.8	39.4	40.7	36.4	38.8
	I	0	36.8	37.8	36.9	37.7	38.3	35.4	37.2
		+	36.9	38.6	37.2	37.0	38.3	35.7	37.4

[1] C, Continuous flow (cups); I, intermittent flow (trough).

[2] Mean of 3 replicates of 5 females.

ferences in response between experiments may be partly related to the 5.6 C difference in ambient temperature.

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