RESEARCH NOTES

The Effect of Pyrazine Odor on Body Weight and the Weight of Various Organs in Chicks (*Gallus gallus domesticus*)

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ABSTRACT Embryonic and posthatch long-term exposure to the odor of 2-methoxy-3-isobutyl-pyrazine (2M3IP) was examined for its potential physiological consequences as reflected in changes in BW and organ weights in domestic chicks (*Gallus gallus domesticus*). Experiments were run from Day 1 of incubation to the age of 3 wk with a total of 360 fertile chicken eggs. The experimental design consisted of four treatment groups: PP chicks were exposed to 2M3IP during both incubation and posthatch rearing; PC chicks were exposed to 2M3IP during incubation only; CP chicks were exposed to 2M3IP during rearing period only; CC control chicks were not exposed to 2M3IP. Chicks were weighed immediately after hatch and at 3 wk of age, when they were necropsied. Various organs (thyroid, adrenal, testes, comb, liver, spleen, abdominal fat, and the bursa of Fabricius) were removed and weighed. Body weights of both sexes in the PP group were reduced. This reduction was significant in males relative to both CP and CC groups and in females only relative to the CP group. Effects of 2M3IP exposure on the examined organs were as follows: in males, adrenal gland weight significantly increased in the PP group *vs* all other groups. No weight differences were found between the other inspected organs among the four treatments. In females, comb weight significantly decreased compared with the rest of the groups when 2M3IP exposure occurred during incubation (PC). Further investigation is needed to study the mechanisms that underlie the differential effects of pyrazine odor on male and female chicks.

(*Key words*: 2-methoxy-3-isobutylpyrazine, chicks, long-term exposure, odor)

1999 Poultry Science 78:1786–1789

INTRODUCTION

Anatomical, neurophysiological, and behavioral evidence has established that some bird species, including domestic fowl (*Gallus gallus domesticus*), have a sense of smell (Jones and Roper, 1997). Various odors have been used in these studies, including the aromatic pyrazines (P), which are exceptionally odoriferous substances that are widely distributed in plant and animal species (Woolfson and Rothschild, 1990). The P nucleus comprises a six-numbered aromatic ring containing two para-oriented tertiary nitrogen atoms. Over 100 P compounds have been identified; some of them are odorless, whereas the methoxyalkylpyrazines produce one of the most powerful and persistent odors known to man (Moore and Brown, 1981; Moore *et al.*, 1990).

Few studies were directed to investigate the effect of 2-methoxy-3-isobutyl-pyrazine (2M3IP) odor on the behavior of chicks (Guilford *et al.*, 1987; Kaye *et al.*, 1989; Rowe and Guilford, 1996; Barnea *et al.*, 1999). None of the previously mentioned studies, however, examined the physiological effect. Therefore, this study examined the long-term exposure effect of 2M3IP on BW and weights of various organs of embryos and chicks until they reached the age of 3 wk.

MATERIALS AND METHODS

Incubation

Fertile white Shafrir eggs (Kvutzat Yavne, Israel) were incubated in two force-draft turning incubators,[2] 90 eggs in each. Eggs were maintained at 37.5 to 38.2 C and a relative humidity of 70 to 80%. The incubators were placed in separate rooms. The first room served for treatments with 2M3IP odor and the second for controls.

Management

On Day 20 of incubation, the incubators were checked every few hours. Chicks that hatched during that period

Received for publication March 10, 1999.
Accepted for publication July 26, 1999.
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Abbreviation Key: C = control; 2M3IP = 2-methoxy-3-isobutylpyrazine; OBP = olfactory binding protein; P = pyrazine.

were marked, weighed, and transferred to assigned pens in random order to form groups of seven to nine chicks (approximately half male, half female). The pens were white, wooden-walled cages, 100 × 100 × 60 cm (length by width by height), with a wood shavings litter-covered floor. In each of the two rooms there were 10 such pens. A continuous lighting program was provided. Heat was provided by infrared lamps (Philips, 250W) during the rearing period. Water and feed (commercial ration, 21% protein, 3,000 kcal ME/kg) were supplied to every pen for *ad libitum* intake. In the center of each feeder, standard perforated white film boxes were attached to serve as "odor generators" for the odor being tested (see subsequently).

Odor Application

A stock solution of 2M3IP[3] was prepared by adding 10 μL 2M3IP to 101 μL distilled water. This concentration was chosen because of a successful effect in a previous behavioral and physiological works (Barnea *et al.*, 1999; Barnea, unpublished data). A 5-mL aliquot of solution was placed in the treatment incubator every 12 h from Day 1 of incubation until hatch. In the control incubator a 5-mL aliquot of distilled water was added at the same times. During the rearing period, 1 mL solution was injected into deodorizers in the treatment pens every morning after feeding. The same amount of distilled water was injected to similar "odor generators" into the control group pens at the same times.

Experimental Procedure

At the age of 1 d, chicks were divided into four groups according to hatching and rearing conditions (P and C = control). Chicks of the first group were incubated and reared in the presence of P (PP). Chicks of the second group were incubated in the presence of P, but were reared in an odor-free environment (PC). The third group of chicks was incubated in the control incubator, but reared in the presence of P (CP). The last group consisted of chicks that were incubated and reared with no P, which served as controls (CC). On Day 21, all chicks were weighed. Ten chicks from each group (males and females) were killed by cervical dislocation, and necropsy was performed. Comb, thyroid gland, adrenal gland, bursa of Fabricius, gonads, spleen, liver, and abdominal fat were removed, cleaned of adhering tissues, and weighed. Glands and organ weights were computed as a percentage of BW. The entire experimental procedure was performed twice.

Statistical Analysis

The effect of P odor on the inspected parameters was tested by three-way ANOVA (repetition, sex, and treatment). The results showed an interaction between repetition and treatment in BW ($F = 3.24$; df = 2; $P < 0.02$) and a marginally significant interaction between sex and treatment in BW ($F = 2.34$; $P < 0.07$). Therefore, we separated females from males and carried out two different analyses using two-way ANOVA (repetition, treatment). Comparisons were made between least squares means by T-test with simultaneous (Holm's modified Bonferroni) correction (Aickin and Gensler, 1996).

RESULTS AND DISCUSSION

Table 1 summarizes the effects of P exposure during both incubation and rearing (PP), during incubation only (PC), during rearing only (CP), and with no P at all (control; CC) on body weight and relative organ weights (percentage of BW) of male chicks.

In males, BW was affected by treatment ($F = 13.73$; df = 2; $P < 0.0001$); exposure to 2M3IP during incubation and rearing decreased BW. A comparison of the BW differences between treatments revealed a decrease in the PP group compared with the CP group ($P < 0.01$) and the control group ($P < 0.0006$) and a marginally significant decrease ($P < 0.06$) relative to the PC group. Adrenal glands were also affected by treatment ($F = 6.96$; df = 2; $P < 0.0008$). Relative adrenal gland weights in the PP group were significantly increased in response to pyrazine exposure during incubation and rearing compared with the rest of the groups. The thyroid of the PP group was heavier than that of the other groups, but only marginally heavier ($P < 0.06$) than for the other groups. No effect of treatment was found on testes, comb, liver, abdominal fat, spleen, and bursa of Fabricius.

Table 2 summarizes the effects of P exposure during incubation and rearing (PP), during incubation only (PC), during rearing only (CP), and no P at all (control; CC) on BW and relative organ weights (percentage of BW) of female chicks. In females, BW was affected by treatment ($F = 3.4$; df = 2; $P < 0.03$). However, comparison of BW differences among treatments revealed a decrease of the PP group only in comparison with the CP group ($P < 0.004$). Comb was affected by treatment ($F = 3.03$; df = 2; $P < 0.046$). Comb weight decreased in response to P exposure when this exposure was limited to incubation time only (PC; $P < 0.05$). No significant effects of treatments were found on any of the other organs tested (ovaries, thyroid, adrenal glands, liver, abdominal fat, spleen, and bursa).

Long-term exposure to 2M3IP decreased BW. The effect was observed in both sexes, although the effect was greater in males. In contrast to our results, Jemiolo and Novotny (1994) showed significantly greater somatic growth in male mice exposed to another P compound (2,5-dimethylpyrazine) compared with mice exposed to water; female growth was not affected by this treatment. The difference in type of response between mice and chicks might result from differences in species as well as from the kind of P used.

[3]99%, Pyrazine Specialties Inc., Athens, GA.

TABLE 1. Effect of pyrazine on BW (grams) and inspected glands and organs (percentage of BW) in male chicks

Tested parameters	PP (n = 16)	PC (n = 12)	CP (n = 8)	CC (n = 11)
BW, g	591.5 ± 27.9[b]	671.2 ± 32.2[ab,*]	749 ± 32.2[a]	781.4 ± 33.6[a]
Testes, mg % of BW	20 ± 2[a]	17 ± 2[a]	17 ± 3[a]	19 ± 2[a]
Adrenal, mg % of BW	14 ± 0.6[a]	11 ± 0.7[b]	11 ± 0.8[b]	10 ± 0.8[b]
Thyroid, mg % of BW	12 ± 0.9[a]	15 ± 1[a,*]	12 ± 1[a]	11 ± 1[a,*]
Comb, mg % of BW	28 ± 5[a]	36 ± 6[a]	40 ± 7[a]	44 ± 6[a]
Liver, % of BW	3.5 ± 0.2[a]	3.0 ± 0.2[a]	2.8 ± 0.3[a]	3.4 ± 0.2[a]
Abdominal fat, % of BW	1.2 ± 0.1[a]	1.3 ± 0.1[a]	1.1 ± 0.2[a]	1.3 ± 0.1[a]
Spleen, mg % of BW	110 ± 8[a]	120 ± 9[a]	90 ± 11[a]	110 ± 10[a]
Bursa, mg % of BW	250 ± 20[a]	270 ± 24[a]	260 ± 28[a]	290 ± 24[a]

[a,b]Values (mean ± SE) within rows with different letters are different from each other ($P < 0.05$).

[1]Male chicks were exposed to pyrazine during both incubation and rearing (PP), during incubation (PC), during rearing (CP), and not at all (CC).

*Marginal significance ($P < 0.06$).

Adrenal glands were heavier in PP than in CC males. In females, although no significant effect of treatment on adrenal gland weight was observed, those of the PP birds were heaviest. Stress enhances the activity of the avian adrenal gland. When stress is chronic, hypertrophy of the gland may occur (Harvey *et al.*, 1986). Thus, the reduction in growth rate observed in chicks exposed to P during incubation and rearing time may reflect long-term stress. Jemiolo and Novotny (1994) found significant growth inhibition of testes and uterus of juvenile mice exposed to 2,5-dimethylpyrazine. In another work (A. Barnea, personal communication), 2M3IP periodically inhibited egg laying in chickens and geese. The present experiment ended long before the chicks reached sexual puberty, making it difficult to pinpoint a similar effect. However, the weight of the female chicks' comb in the control group (CC) was significantly greater than that of the group that was exposed to P during incubation (PC). Because comb size is dependent on sex hormones (Johnson, 1986), it is possible that the diminished comb growth is an early manifestation of gonadal inhibition. One may suggest that the lack of response in the PP female is due to habituation or down regulation of receptors and that the gonadal suppressions only produced by exposure to P at incubation time.

Because 2M3IP is an odorant, one may suggest that its physiological effects are the sole results of olfactory stimulation. Pyrazines cannot cross freely through the aqueous barrier that exists in the olfactory epithelium to reach the chemosensory cells. It was found that there is a special protein, an olfactory binding protein (OBP), that transfers P across the barrier to the chemosensory cells (Pelosi, 1996; Lobel *et al.*, 1998). Whereas it was originally believed that OBP are only present in the olfactory mucous, P OBP were found in some organs such as the saliva glands (Marchese *et al.*, 1998). Similarly, receptors for OBP are not restricted to olfactory tissues, as OBP were detected in a variety of other tissues (Boudjelal *et al.*, 1996), including brain and testes (Schoentgen and Jolles, 1995). This suggests that OBP may play a role not only in olfactory signal transduction but perhaps may have a much broader role within the body, including detoxification or signaling.

Our study on the effect of long exposure to 2M3IP yielded two novel findings in avian species. First, exposure to an odorant may affect certain physiological parameters. Second, as in the mice, some effects may be sex-dependent. The possible routes through which odors affect avian physiology are of interest for further research. If certain physiological parameters can be manipulated

TABLE 2. Effect of pyrazine on BW (grams) and inspected glands and organs (percentage of BW) in female chicks

Tested parameters	PP (n = 6)	PC (n = 10)	CP (n = 7)	CC (n = 10)
BW, g	613.3 ± 27.5[b]	668.2 ± 21.3[ab]	773.8 ± 27.5[a]	684.1 ± 21.3[ab]
Ovaries, mg % of BW	24 ± 3[a]	27 ± 2[a]	20 ± 2[a]	22 ± 2[a]
Adrenal, % of BW	15 ± 1[a]	13 ± 1[a]	12 ± 1[a]	11 ± 1[a]
Thyroid, % of BW	16 ± 1[a]	12 ± 1[a]	12 ± 1[a]	13 ± 1[a]
Comb, % of BW	18 ± 2[ab]	13 ± 1[b]	19 ± 2[ab]	20 ± 1[a]
Liver, % of BW	3.2 ± 0.2[a]	3.2 ± 0.15[a]	3.0 ± 0.17[a]	3.2 ± 0.15[a]
Abdominal fat, % of BW	1.4 ± 0.14[a]	1.3 ± 0.11[a]	1.4 ± 0.12[a]	1.3 ± 0.11[a]
Spleen, % of BW	120 ± 16[a]	120 ± 13[a]	110 ± 14[a]	120 ± 13[a]
Bursa, % of BW	270 ± 28[a]	230 ± 21[a]	280 ± 24[a]	250 ± 21[a]

[a,b]Values (mean ± SE) within rows with different letters are different from each other ($P < 0.05$).

[1]Female chicks were exposed to pyrazine during both incubation and rearing (PP), during incubation (PC), during rearing (CP), and not at all (CC).

or controlled by odorants during different phases of development, odorants may have value in poultry breeding.

ACKNOWLEDGMENTS

This research was financially supported by the Israeli Ministry of Science. The authors wish to thank E. Arnon for her skillful technical assistance.

REFERENCES

Aickin, E., and H. Gensler, 1996. Adjusting for multiple testing when reporting research results. Am. J. Publ. Health. 86:726–728.

Barnea, A., G. Gvaryahu, and M. Rothschilds, 1999. The effect of the odor of pyrazine and colors on recall of post event and learning in the domestic chick (*Gallus gallus domesticus*). *in*: Insects and Birds. H. Van Enden, ed. Chapman and Hall,

Boudjelal, M., A. Sivaprsadarao, and J. B. Findlay, 1996. Membrane receptor for odour-binding proteins. Biochem. J. 317:23–27.

Guilford, T., C. Nicol, M. Rothschild, and B. P. Moore, 1987. The biological roles of pyrazines: Evidence for warning odor function. Biol. J. Linn. Soc. 31:113–128.

Harvey, S., C. G. Scanes, and K. I. Brown, 1986. Adrenals. Pages 479–493 *in*: Avian Physiology. P. Sturkie, ed. Springer-Verlag, Berlin, Germany.

Jemiolo, B., and M. Novotny, 1994. Inhibition of sexual maturation in juvenile female and male mice by chemosignal of female origin. Physiol. Behav. 55:519–522.

Johnson, A. L., 1986. Reproduction in the female. Pages 101–131. *in*: Avian Physiology. P. Sturkie, ed. Springer-Verlag, Berlin, Germany.

Jones, R. B. and T. J. Roper, 1997. Olfaction in the domestic fowl: A critical review. Physiol. Behav. 62:1009–1018.

Kaye, H., N. J. Mackintosh, M. Rothschild, and B. P. Moore, 1989. Odor of pyrazine potentiates an association between environmental cues and unpalatable taste. Anim. Behav. 37:563–568.

Lobel, D., S. Marchese, J. Krieger, P. Pelosi, and H. Breer, 1998. Subtypes of odorant-binding proteins—Heterologous expression and ligand binding. Eur. J. Biochem. 254:318–324.

Marchese, S., D. Pes, A. Scaloni, V. Carbone, and P. Pelosi, 1998. Lipocalins of boar salivary glands binding odours and pheromones. Eur. J. Biochem. 252:563–568.

Moore, B. P., and W. V. Brown, 1981. Identification of warning odor components, bitter principles and antifeedants in an aposematic beetle: *Metriorrhynchus rhiphidius* (Coleoptera: Lycidae). Insect Biochem. 11:493–499.

Moore, B. P., W. V. Brown, and M. Rothschild, 1990. Methylalkylpyrazines in aposematic insects, their host plants and mimics. Chemoecology 1:43–51.

Pelosi, P. 1996. Perireceptor events in olfaction. J. Neurobiol. 30:3–19.

Rowe, C., and T. Guilford, 1996. Hidden color aversions in domestic chicks triggered by odors of insects warning display. Nature, 383:520–522.

Schoentgen, F., and P. Jolles, 1995. From structure to function: Possible biological roles of a new widespread protein family binding hydrophobic ligands and displaying a nucleotide binding site. FEBS Lett. 369:22–26.

Woolfson, A., and M. Rothschild, 1990. Speculating about pyrazines. Proc. R. Soc. Lond. B 242:113–119.