ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 4 (5): 268-273, 2005
ISSN 1682-8356

Incubator Temperature and Oxygen Concentrations During the Plateau Stage in Oxygen Uptake Affect Turkey Embryo Plasma T_4 and T_3 Concentrations[1]

V.L. Christensen[2,3], M.J. Wineland[3], I. Yildirum[4], B.D. Fairchild[3], D.T. Ort[3] and K.M. Mann[3]
[3]Department of Poultry Science, College of Agriculture and Life Sciences,
North Carolina State University, Raleigh, North Carolina 27695-7608, USA
[4]Department of Animal Science, Agriculture Faculty, The University of Selcuk,
42031 Campus Konya, Turkey
E-mail: vern_christensen@ncsu.edu

Abstract: Avian embryo thyroid responses to incubator temperature and oxygen concentrations during the plateau stage in oxygen consumption were measured. It was hypothesized that turkey embryo thyroid responds in a limited way at this critical time to environmental conditions to modulate basal metabolism. Turkey embryos were exposed to one of four incubator temperatures (36, 37, 38 or 39°C) beginning on the 25th day of incubation at the onset of the plateau, a time when plasma thyroxine (T_4) and triiodothyronine (T_3) concentrations normally increase. Blood was collected and thyroid hormone concentrations were measured at pipping (27th day) and hatching (28th day). Elevated temperatures depressed T_3 and T_4 concentrations and increased the T_3 to T_4 ratios. In a second experiment four oxygen concentrations (17, 19, 21 or 23% oxygen) were provided to the embryos using identical procedures. The 21% treatment significantly reduced T_3 and T_4 at pipping compared to all other treatments, but 23% oxygen increased plasma T_3 and the T_3 to T_4 ratio compared to all other treatments. The 17% oxygen treatment elevated T_3 compared to all other treatments. At hatching, 23% oxygen elevated T_3 and T_3 to T_4 ratios compared to all other treatments. When temperature and oxygen treatments were applied together in a factorial arrangement, temperature and oxygen affected T_3 and T_4 hormone concentrations independently but did not interact. Therefore, we conclude that temperature and oxygen are independent stimuli of the avian embryonic thyroid gland during the plateau stage, and that incubator temperature and oxygen concentrations can modulate development of turkey embryos by changing plasma T_3 and T_4 concentrations.

Key words: Embryonic thyroid, turkeys, environmental conditions

Introduction

Turkey embryo plasma thyroid concentrations increase developmentally as the hypothalamus matures (Christensen and Biellier, 1982). The peak coincides with a time called the plateau stage in oxygen consumption identified by the hypoxic and hypercapnic condition created for embryos (Rahn, 1981). Embryo thyroids from different genetic backgrounds respond differently to maternal dietary iodide and to the length of the incubation period (Christensen *et al*., 2002). Carbon dioxide has also been proposed as a stimulus for chick embryo thyroid hormone release (Buys *et al*., 1998). However, incubator temperature and oxygen concentrations eliciting embryonic turkey thyroid responses are unknown.

Organ system immaturity associated with thyroid hormones is the principal cause of morbidity associated with extreme prematurity in humans, and therapies for premature infants include the infusion of T_3 and T_4 (Yeung and Smyth, 2002). Organ system immaturity (Lilja and Olsson, 1987) and the onset of thermoregulation (Nichelmann *et al*., 2001) both of which are mediated by thyroid hormones are important components of hatchling quality as well. Therefore, the hypothesis was proposed that incubator temperature and oxygen concentrations or their interaction at the plateau stage in oxygen consumption stimulate the embryonic thyroid to release thyroxine (T_4) and or triiodothyronine (T_3) into the embryonic blood.

Materials and Methods

Incubator temperature and oxygen concentrations were tested to determine their effectiveness in affecting plasma thyroid hormones in embryonic turkeys. Experimental cabinets simulating commercial incubators were manufactured[3] and used. Each cabinet contained one incubator tray capable of holding 100 eggs. Digitized thermostats, connected to microprocessors with temperature sensitivity of ± 0.1°C, controlled the wet and dry bulb temperatures. Digital thermometers[4] were used in each cabinet to verify set point temperatures, and ports were used to infuse desired gaseous concentrations.

Experiment 1: Four temperature treatments, 36, 37, 38 and 39°C, were selected. All temperatures were below

the actual shell temperature of an incubating egg during the latter half of development. Eggs from a commercial strain of turkeys[5] were incubated together until the beginning of the 25th day of development. Days 25 and 26 are the plateau stage in oxygen consumption for turkeys (Rahn, 1981). At the completion of 24 d of incubation, the eggs were candled to determine embryo viability. Following candling and removal of nonviable embryos, randomly selected viable embryos were transferred to one of the four experimental cabinets. Each cabinet operated at one of the treatment temperatures listed above.

Embryo blood samples were obtained beginning at external piping (approximately 26 to 27 d of incubation) and at hatching (approximately 28 d) by decapitation and the collection of trunk blood into a collection vial containing 10 mg of EDTA. The collected blood was placed quickly on ice then centrifuged under refrigeration (4°C) at 700 g for 20 minutes. Following centrifugation, plasma was decanted, frozen (-22°C) and stored for later analysis. Ten embryos were sampled per treatment at each stage of development.

Plasma concentrations of T_4 and T_3 were determined using radioimmunoassays as reported previously (Christensen and Davis, 2001). The T_3 to T_4 ratios were computed by dividing the T_3 concentration by that of T_4. The ratio indicates the deiodination from the prohormone to the active form by the monodeiodinase system of the embryo (McNabb, 1988). Intra assay variation was 1.2%. All samples were analyzed in one assay so no interassay variation existed.

Experiment 2: In Experiment 2, four oxygen concentrations were the treatments. The oxygen concentrations tested were 17, 19, 21 and 23% fractional concentrations of the air within the cabinet at sea level. Concentrations lower than ambient oxygen concentration (20.9%) were maintained by infusing nitrogen gas into the cabinet at a rate that resulted in the desired concentration of 17 or 19%. Concentrations were measured using an oxygen meter[6], and the flow rates from adjacent oxygen or nitrogen storage tanks were adjusted at hourly intervals to maintain the desired oxygen level.

Eggs were again incubated in a commercial machine until the completion of the 24th day of development. Eggs were candled to determine viability of the embryos then randomly distributed viable embryos were transferred to the four experimental incubator cabinets with the desired oxygen concentration. Embryo blood plasma was collected and analyzed identically as described above for Experiment 1. The intra assay coefficients of variation were 1.1% for Experiment 2.

Experiment 3: The most extreme treatment levels altering thyroid hormone concentrations in the previous experiments were combined in a factorial arrangement for Experiment 3. The highest (39°C) and lowest (36°C) temperatures as well as the greatest (23%) and least oxygen (17%) concentrations were examined simultaneously in Experiment 3. The incubator cabinet temperatures and oxygen concentrations were arranged as a 2 x 2 factorial. Temperatures and oxygen concentrations were maintained identically as described in the previous two experiments. Fertilized eggs were incubated for 25 days in an incubator then at the time of transfer to the machines used for hatching, they were randomly assigned to one of the four cabinets operating at 36 C with 17 or 23% oxygen or 39 C with 17 or 23% oxygen. Ten embryos or hatchlings per treatment combination were sampled at external pipping and at hatching to obtain blood plasma. All sampling techniques were described above, and the intra assay coefficient of variation for Experiment 3 was 1.1%.

Statistical analysis: Data for all experiments were analyzed using the general linear models procedure (SAS Inst., 1998). Experiments 1 and 2 were analyzed with four levels of treatment. In Experiment 3, the data were analyzed as two temperatures by two oxygen concentrations in a factorial arrangement. Means determined to differ significantly ($P < 0.05$) were separated using the least square mean procedure. All means given in tables are least square means. All possible main and interaction effects were tested for significance. All probabilities were based on $P < 0.05$ unless otherwise noted.

Results

Experiment 1: Blood plasma T_3 and T_4 concentrations of embryos exposed to different temperatures during hatching are given in Table 1. At 27 days, T_4 concentrations were not affected by any temperature treatment, but T_3 concentrations were significantly depressed by 38 and 39 C treatments compared to 36 C. Because of the depressed T_3 concentrations, T_3 to T_4 ratios were elevated in the 39 C treatment compared to 38°C and 37°C but not 36°C.

At 28 days a more dramatic effect of incubator temperature on thyroid hormone concentrations was noted as 36 C elevated embryonic plasma T_4 compared to 37, 38 and 39°C. No differences were noted at 28 days in plasma concentrations of T_3, but the T_3 to T_4 ratio was elevated at 38°C and 39°C compared to 36°C.

Experiment 2: The effects of oxygen on embryonic plasma T_4 and T_3 are shown in Table 2. At 27 days oxygen concentrations of 21% reduced T_4 significantly compared to all other treatment levels. The 17% oxygen treatment elevated T_3 at pipping and 21% oxygen resulted in the lowest T_3 concentration compared to all other treatments.

Table 1: Plasma thyroid hormone concentrations (ng/ml) in turkey embryos exposed to different incubation temperatures during hatching

Temperature (C)[1]	Day of incubation	
	27[2]	28[3]
	Thyroxine	
36	79.1	20.5[a]
37	84.6	11.8[b]
38	50.4	12.8[b]
39	37.3	8.8[b]
Mean ± SEM	62.9±10.1	13.5±1.5
Probability	NS	0.05
	Triiodothyronine	
36	10.3[a]	8.5
37	8.9[ab]	9.0
38	6.5[b]	8.7
39	6.4[b]	8.1
Mean ± SEM	8.0±0.6	8.6±0.3
Probability	0.05	NS
	Ratio	
36	0.18[ab]	0.49[b]
37	0.15[b]	1.11[ab]
38	0.16[b]	1.24[a]
39	0.25[a]	1.26[a]
Mean ± SEM	0.19±0.01	1.02±0.08
Probability	0.05	0.05

[a,b]Columnar means with different superscripts differ significantly (P< 0.05).

[1]Incubator set point temperature during days 25 to 28 of incubation was at 36, 37, 38 or 39°C.

[2]Day 27 embryos were pipping the shell externally preparatory to hatching.

[3]Day 28 poults were free from the shell and the down had dried except on the neck.

At 28 days the opposite was noted as the highest oxygen concentration (23%) elevated plasma T_3 concentrations compared to all other treatments with no significant differences in plasma T_4 concentrations. At 27 days the T_3 to T_4 ratio was not affected by the oxygen concentration in the cabinet, but at hatching the 23% oxygen concentration elevated the ratio compared to all other levels.

Experiment 3: When examined as a factorial arrangement, both temperature and oxygen again affected plasma T_4 and T_3 concentrations, but they did not interact (Table 3). Neither temperature nor oxygen concentration affected embryonic plasma T_4 at 27 days in Experiment 3. Incubator temperature of 39°C depressed plasma T_4 at 28 days compared to 36°C, and 17% oxygen elevated T_4 compared to 23%, but again the main factors did not interact significantly. Exposure to 36°C elevated T_3 concentrations compared to 39°C in 27 d turkey embryos, resulting in significantly elevated T_3 to T_4 ratios.

Table 2: Plasma thyroid hormone concentrations (ng/mL) in turkey embryos exposed to different incubator oxygen concentrations during hatching

	Day of incubation	
Oxygen (%)[1]	27[2]	28[2]
	Thyroxine	
17	93.7[a]	13.9
19	94.8[a]	21.1
21	29.9[b]	16.3
23	83.3[a]	15.2
Mean ± SEM	75.4±7.6	16.6±1.3
Probability	0.006	NS
	Triiodothyronine	
17	16.3[a]	8.8[b]
19	11.8[b]	8.3[b]
21	6.8[c]	8.7[b]
23	11.3[b]	10.0[a]
Mean ± SEM	11.5±0.6	8.9±0.2
Probability	0.0001	0.05
	Ratio	
17	0.19	0.66[b]
19	0.18	0.46[b]
21	0.38	0.71[b]
23	0.21	1.06[a]
Mean ± SEM	0.24±0.04	0.72±0.08
Probability	NS	0.05

[a,b]Columnar means with different superscripts differ significantly (P<0.05).

[1]Incubator oxygen fractional concentration during days 25 to 28 of incubation was at 17, 19, 21, or 23%.

[2]Day 27 embryos were pipping the shell externally preparatory to hatching.

[3]Day 28 poults were free from the shell and the down had dried except on the neck.

Discussion

The hypothesis was proposed that temperature and oxygen modulate avian embryonic thyroid function during the plateau stage in oxygen consumption. In mammals modulation is performed maternally primarily by the placenta (Yeung and Smyth, 2002). In the absence of maternal modulators, the control mechanisms for avian embryo thyroid are probably intrinsic but are unknown. The current study is the first to show clearly that the embryonic turkey thyroid at the plateau stage in oxygen consumption responds to both temperature and oxygen stimuli. Data from the current study indicated also that both factors affect monodeiodination as well. Overall, temperature was a more effective stimulus, but oxygen effects were seen as well as those of temperature. Both T_3 and T_4 increase exponentially during the plateau stage in oxygen consumption attaining their greatest concentrations at any time in the life of the bird (Decuypere *et al.*, 1991). The increased embryonic T_3

Table 3: Plasma thyroid hormone concentrations (ng/mL) in turkey embryos exposed to different incubator temperatures and oxygen concentrations during hatching

Temperature (C)[1]		Day of incubation		
	Oxygen (%)[2]	27[3]	28[4]	
	Thyroxine			
36	17	25.6	12.9	
	23	24.0	9.1	
	Mean		11.0[a]	
39	17	22.8	9.2	
	23	25.8	8.0	
	Mean		8.6[b]	
	Mean ± SEM	24.5±2.1	9.8±0.8	
	Temperature (T)	NS	0.01	
	Oxygen (O)	NS	0.01	17 = 11.1[a]
				23 = 8.5[b]
	T x O	NS	NS	
		Triiodothyronine		
36	17	11.3	9.6	
	23	10.3	8.5	
	Mean		10.8[a]	
39	17	6.9	9.1	
	23	9.3	8.4	
	Mean		8.1[b]	
	Mean ± SEM	9.5±0.6	8.9±0.2	
	Temperature (T)	0.02	NS	
	Oxygen (O)	NS	0.02	17 = 9.3[a]
				23 = 8.4[b]
	T x O	NS	NS	
		Ratio		
36	17	0.54	0.90	
	23	0.57	1.08	
	Mean		0.56[a]	
39	17	0.29	1.65	
	23	0.46	1.19	
	Mean		0.38[b]	
	Mean ± SEM	0.46±0.06	1.21±0.1	
	Temperature (T)	0.05	NS	
	Oxygen (O)	NS	NS	
	T x O	NS	NS	

[a,b]Columnar means with different superscripts differ significantly ($P < 0.05$).

[1]Incubator set point temperature during days 25 to 28 of incubation was at 36, 37, 38 or 39°C.

[2]Incubator oxygen fractional concentration during days 25 to 28 of incubation was at 17, 19, 21, or 23%.

[3]Day 27 embryos were pipping the shell externally preparatory to hatching.

[4]Day 28 poults were free from the shell and the down had dried except on the neck.

and T_4 concentrations in precocial species distinguish these species from altricial (McNabb, *et al.*, 1984; McNabb, 1988; and Vyboh *et al.*, 2001). If T_3 and T_4 are depressed, then hatchling maturity may be delayed (Christensen *et al.*, 2003). If maturity and function are delayed in a vital tissue, then hatchling health and survival may be jeopardized as in humans (Christensen and Biellier, 1982; Christensen *et al.*, 2003).

Immature thyroid gland and thyroid function in premature human fetuses is characterized by several conditions. These include, high circulating levels of thyrotropin releasing hormone (TRH), high circulating levels of thyroid stimulating hormone (TSH); immature thyroid hormone biogenesis; limited thyroglobulin stores; low thyroxine-binding globulin concentrations. Additional considerations are the predominant conversion of T_4 to reverse T_3 and sulfated analogues; minimal T_4 to T_3 conversion, and the progressive maturation of T_4 production and T_4 (via T_3) negative feedback control of TSH. Extra-thyroidal tissue characterizations include

developmentally programmed maturation of thyroid hormone nuclear receptors in individual fetal tissues and developmentally programmed maturation of thyroid hormone gene transcription in individual fetal tissues (Yeung and Smyth, 2002). The functions listed above are "developmentally programmed" by the mammalian fetus in conjunction with production of TRH from placental and fetal gut tissues, particularly the pancreas, and to low or absent levels of TRH-degrading activity in fetal blood (Fisher, 1999). Organ system immaturity appears to be the principal cause of morbidity associated with extreme prematurity in humans, and therapies for premature infants include the infusion of T_4, T_3, dexamethasone, and hydrocortisone (Yeung and Smyth, 2002).

Little is known of morbidity resulting from embryonic thyroid immaturity in turkeys. Depressed T_3 and T_4 concentrations have been noted in weak neonatal poults classified as "flip-overs" (Christensen *et al.*, 2003). Prior to the current study, it was known that TRH and TSH were effective secretagogues for both T_4 and T_3 in the turkey embryo at the plateau (Christensen and Phelps, 2001). Also, providing the turkey breeder hen with adequate dietary iodine (Christensen and Davis, 2001) or exposing the incubating eggs to longer incubation periods (Christensen *et al.*, 2000) fostered embryonic survival and elevated T_4 and T_3 concentrations. Prolongation of the incubation period following long term storage of eggs prior to placement in incubators also delays the increase of T_3 and T_4 (Christensen *et al.*, 2003). Genetic selection for economically desirable traits such as rapid growth changes embryonic organ form (Lilja and Olsson 1983), and function (Christensen *et al.*, 2000) and embryonic livability declines (Christensen *et al.*, 1993). The changes in growth may also be accompanied by different times of thyroid hormone surges (Dunnington *et al.*, 1992; McNabb *et al.*, 1993; Buys *et al.*, 1998; Christensen *et al.*, 2000). The actual hatching process of turkey embryos, the time when the hormonal surge occurs under normal conditions, can cause exposure to hypoxia for as short as 2 to 3 hours or as long as 72 hours (Christensen, unpublished data). The importance of the length of the plateau and hatching period for hatchling quality needs more study.

Thyroid hormones in avian embryos are intimately involved in both growth and functional maturation of a number of organs. The organs include but are not limited to intestine (Black, 1978), adrenal and kidney (Doneen and Smith, 1982), lung (Hylka and Doneen, 1984), heart and liver (Nobikuni *et al.*, 1989) and skeletal muscle (Nobikuni *et al.*, 1989; Decuypere *et al.*, 1991). Does thyroid maturation necessarily match the final maturation of all organ systems for which it is responsible? The answer is probably not (Christensen and Biellier, 1982). Thus, one possible solution to synchronizing maturation may be to control temperature and oxygen concentration during the plateau.

In conclusion, data from the current study suggest that both temperature and oxygen at the plateau in oxygen consumption alter embryonic plasma thyroid hormone concentrations. Thus, incubation environment during the plateau of turkey embryos may be used as a management tool to optimize survival (Christensen and Biellier, 1982). Increasing embryonic thyroid hormone plasma concentrations may also assist in improving the survival of turkey hatchlings posthatching (Christensen *et al.*, 2003).

References

Black, B.L., 1978. Morphological development of the epithelium of the embryonic chick intestine in culture: Influence of thyroxine and hydrocortisone. Am. J. Anat., 153: 573-600.

Buys, N., E. Dewil, E. Gonzales and E. Decuypere, 1998. Different CO_2 levels during incubation interact with hatching time and ascites susceptibility in two broiler lines selected for different growth rate. Avian Path., 27: 605-612.

Christensen, V.L. and H.V. Biellier, 1982. Physiology of turkey embryos during pipping and hatching. IV. Thyroid function in embryos from selected hens. Poult. Sci., 61: 2482-2488.

Christensen, V.L. and G.S. Davis, 2001. Maternal dietary iodide influences embryonic thyroid function and survival. Poult. Sci., 80: 1286-1292.

Christensen, V.L., G.S. Davis and K.E. Nestor, 2002. Environmental incubation factors influence embryonic thyroid hormones. Poult. Sci., 81: 442-450.

Christensen, V.L., W.E. Donaldson and K.E. Nestor, 1993. Embryonic viability and metabolism in turkey lines selected for egg production or growth. Poult. Sci., 72: 829-838.

Christensen, V.L., J.L. Grimes, W.E. Donaldson and S. Lerner, 2000. Correlation of body weight with hatchling blood glucose concentration and its relationship to embryonic survival. Poult. Sci., 79: 1817-1822.

Christensen, V.L. and P. Phelps, 2001. Injection of thyrotrophin-releasing hormone in turkey embryos elevates plasma thyroxine concentrations. Poult. Sci., 80: 643-646.

Christensen, V.L., J.L. Grimes, M.J. Wineland and G.S. Davis, 2003. Accelerating embryonic growth during incubation following prolonged egg storage 2. Embryonic growth and metabolism. Poult. Sci., 82.

Christensen, V.L., D.T. Ort and J.L. Grimes, 2003. Physiological factors associated with weak neonatal poults (Meleagris gallopavo). Int. J. Poult. Sci., 2: 7-14.

Decuypere, E., E. Dewil and E.R. Kuehn, 1991. The hatching process and the role of hormones. Pages 239-256 *in:* Avian Incubation. S. G. Tullett, ed. Butterworth-Heineman, London, UK.

Doneen, B.A. and T.E. Smith, 1982. Ontogeny of endocrine control of osmoregulation in chick embryo. II. Actions of prolactin, arginine vasopressin, and aldosterone. Gen. and Comp. Endocrinol., 115: 2133-21.

Dunnington, E.A., F.M.A. McNabb, T.B. Freeman, N. O'Sullivan and P.B. Siegel, 1992. Genetic analyses of incubation time in weight-selected lines of chickens and their crosses. Arch. Geglugelkd., 56: 77-80.

Fisher, D.A., 1999. Hypothyroxinemia in premature infants: Is thyroxine treatment necessary? Thyroid 9: 715-720.

Hylka, V.W. and B.A. Doneen, 1984. Ontogeny of embryonic lung: Effect of pituitary gland, corticosterone, and other hormones upon pulmonary growth and synthesis of surfactant phospholipids. Gen. and Comp. Endocrinol, 51: 108-121.

Lilja, C. and U. Olsson, 1987. Changes in embryonic development associated with long-term selection for high growth rate in Japanese quail. Growth, 51: 301-308.

McNabb, F.M.A., 1988. Peripheral thyroid hormone dynamics in precocial and altricial avian development. Am. Zoologist, 28: 427-465.

McNabb, F.M.A., L.J. Lyons and T.E. Hughes, 1984. Free thyroid hormones in altricial (ring doves) versus precocial (Japanese quail) development. Endocrinol., 115: 2133-2136.

McNabb, F.M.A., E.A. Dunnington, P.B. Siegel and S. Suvarna, 1993. Perinatal thyroid hormones and hepatic 5'deiodinase in relation to hatching time in weight-selected lines of chickens. Poult. Sci., 72: 1764-1771.

Nichelmann, M., O. Janke and B. Tzschentke, 2001. Efficiency of thermoregulation in precocial avian species during the prenatal period. J. Thermal Biol., 26: 273-280.

Nobikuni, K., K.O. Koga and H. Nishiyama, 1989. The effects of thyroid hormones on liver glycogen, muscle glycogen and liver lipid in chicks. Jpn. J. Zootech. Sci., 60: 346-348.

Rahn, H., 1981. Gas exchange in avian eggs with special reference to turkey eggs. Poult. Sci., 60: 1971-1980

SAS Institute, 1998. SAS/STAT Guide for Personal Computers, Version 6 Edition. SAS Institute, Cary, NC.

Vyboh, P., M. Zeman, J. Buyse, M. Jurani and E. Decuypere, 2001. Developmental changes in thyrotropic and somatotropic effect of TRH in precocial Japanese quail and altricial European starlings. Gen. and Comp. Endocrinol., 124: 293-299.

Yeung, M.Y. and J.P. Smyth, 2002. Hormonal factors in the morbidities associated with extreme prematurity and the potential benefits of hormonal supplement. Biol Neonate, 8: 1-15.

Abbreviation Key: T_4 = thyroxine; T_3 = triiodothyronine; TRH = thyrotropin releasing hormone; TSH = thyroid stimulating hormone

[1]The mention of trade names in this publication does not imply endorsement of the products mentioned nor criticism of similar products not mentioned.

[2]To whom correspondence should be addressed: vern_christensen@ncsu.edu

[3]Cumberland Hatchery Systems, Mt. Olive, NC 28365.

[4]Cox Records, 69 McAdenville Rd., Belmont, NC 28012-2434.

[5]Nicholas Turkey Breeding Farms, 19449 Riverside Dr., Sonoma, CA 95476-1209.

[6]Dyna Tech, Compton CA 90220.