Research Note

Comparative effects of in ovo versus subcutaneous administration of the Marek's disease vaccine and pre-placement holding time on the processing yield of Ross 708 broilers[1,2,3]

E. D. Peebles,[*,4] T. M. Barbosa,[†,5] T. S. Cummings,[†] J. Dickson,[†] S. K. Womack,[*] and P. D. Gerard[‡]

[*]*Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762;* [†]*Zoetis, 1040 Swabia Court, Research Triangle Park, NC 27709; and* [‡]*Department of Mathematical Sciences, Clemson University, Clemson, SC 29634*

ABSTRACT Effects of the in ovo (i.o.) or subcutaneous (s.c.) method of administration (moa) of the Marek's disease (MD) vaccine and 4 or 18 h pre-placement holding time (pht) on the processing yield of male broilers through 49 d of age (doa) were investigated. Ross 708 broiler hatching eggs (3,900) were either i.o.-vaccinated at 18 d of incubation or chicks from eggs that were not i.o.-vaccinated were s.c.-vaccinated at hatch. The i.o. injections (50 μL) were delivered by a commercial multi-egg injector and s.c. injections (200 μL) were delivered by an automatic pneumatic s.c. injector. The pht was imposed on chicks after vaccination. Sixteen birds were initially assigned to each of 15 replicate floor pens belonging to each of the moa and pht combination groups and were grown out through 48 doa. At 48 doa, 6 birds were randomly selected from each replicate pen and were weighed and fasted for 16 h before being processed. At 49 doa, whole carcass, fat pad, breast muscle, and tenders muscles weights were recorded. Whole carcass weight as a percentage of live BW, and fat pad, breast muscle, and tenders muscles weights as percentages of both live and whole carcass weights were calculated. Upon subjection of the data to a 2 × 2 factorial analysis, only a main effect due to moa was observed for tenders muscles weight as a percentage of live and whole carcass weights. Tenders muscles weight as a percentage of both live ($P \leq 0.010$) and whole carcass ($P \leq 0.004$) weight was higher in birds hatched from eggs that received i.o. rather than s.c. vaccinations. In conclusion, in comparison to s.c. vaccination, i.o. vaccination increased relative tenders weight yield, whether or not broilers were held for 4 or 18 h prior to placement. Therefore, with regard to broiler processing yield, i.o. and s.c. vaccinations were safe for the administration of the MD vaccine, with i.o. vaccination displaying a slight potential advantage.

Key words: broiler, holding time, in ovo injection, Marek's disease vaccination, processing yield

2017 Poultry Science 96:3944–3948
http://dx.doi.org/10.3382/ps/pex201

INTRODUCTION

The traditional method of administration (**moa**) of the Marek's disease (**MD**) vaccine to broilers has been by subcutaneous (**s.c.**) injection at d of hatch (Johnston et al., 1997; Ricks et al., 1998). However, upon determining the effects of in ovo (**i.o.**) and s.c. injections on hatchability, mortality, feed consumption and conversion, processing live BW, and condemnations, Gildersleeve et al. (1993) surmised that administration of the MD vaccine by i.o. injection results in reduced settlement costs and is safe and efficacious. Accordingly, 98 to 99% of broilers in the US poultry industry are currently vaccinated for MD by i.o. injection (Johnston et al., 1997; Jochemsen and Jeurissen, 2002).

Peebles et al. (2016) also examined the effects of the moa of the MD vaccine in a study which served as a counterpart to the present one. In that report, it was likewise shown that in comparison to non-injected controls, i.o. injection did not have a differential effect on fertile egg hatchability or total and yolk-free BW at hatch. Also, in comparison to s.c. injection, i.o. injection did not have a significantly different effect on

---

Received April 3, 2017.
Accepted June 14, 2017.

[1] This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station.

[2] This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 329260.

[3] Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.

[4] Corresponding author: d.peebles@msstate.edu

[5] Current address: Merck & Co., Inc., 2000 Galloping Hill Rd., Kenilworth, NJ 07033.

total and yolk-free BW, body length, or body mass index at placement, or on total and yolk-free BW at 7 d post-hatch. However, in comparison to non-injected controls, higher body lengths and lower body mass indices resulted in the hatchlings that had received i.o. injections. In a later companion report, Peebles et al. (2017) additionally noted that the 14 to 28 d of age (**doa**) feed consumption and 14 to 21 doa BW gain of birds belonging to the i.o. treatment group was greater in comparison to those belonging to the s.c. treatment group.

Studies have shown that providing broiler hatchlings with immediate access to feed and water is most beneficial, and that increasing their pre-placement holding time (**pht**) without access to food and water results in increased mortality and decreased post-hatch performance (Fanguy et al., 1980; Nir and Levanon, 1993; Stamps and Andrews, 1995; Willemsen et al., 2010). Delayed pht has also been associated with dehydration and an inadequate supply of energy (Vieira and Moran, 1999b). Franco et al. (2006) showed that as the pht of broiler chicks was increased in 12 h intervals up to 48 h, that there was a linear reduction in their live BW and feed intake. Vieira and Moran (1999a) have noted that a reduction in the BW gain of broilers subjected to a 24 h pht was sustained through market age (49 doa), and it has been advocated that the inability of rats to attain normal BW is associated with a depression in their muscle development (Elliott et al., 1943). In the study by Peebles et al. (2016), it was also reported that prolonging pht by 14 h was unfavorable to the early chick quality of Ross 708 broilers. A decrease in BW at 7 doa also resulted by extending pht an additional 14 h. Furthermore, in the later companion report by Peebles et al. (2017), it was shown that in response to an increase in pht from 4 to 18 h, a decrease in post-hatch BW gain through 35 doa was associated with a reduction in feed consumption through 28 doa.

In an earlier study, Peebles et al. (2014) reported a significant negative correlation between mean daily percentage incubational weight loss of embryonated Ross 708 broiler hatching eggs through 18.5 d of incubation with relative tenders muscle weights at processing on d 49 post-hatch, suggesting that various physiological variables in the early stages of a broiler's life can impact those in the very late stages of its life. Peebles et al. (2016) also noted that although there were no interactive effects of moa and pht on any of the early post-hatch quality variables of the Ross 708 chicks, in comparison to the 4 h pht and s.c. moa treatments, the 18 h pht and i.o. moa treatments, respectively, exerted similar effects on chick body length and body mass index. Peebles et al. (2017) similarly observed that although there were no main effects of pht or moa on Ross 708 broiler BW at 42 and 48 doa, or interactive effects between moa and pht on their performance through 48 doa, BW gain through 21 d post-hatch was affected by moa, and BW through 35 d post-hatch was affected by pht.

These previous studies suggest that moa and pht have the potential to have subsequent effects on various broiler processing yield characteristics through 49 d post-hatch despite inconsistent effects on prior performance. However, possible main and interactive effects of pht and MD vaccine moa on the subsequent processing yield of Ross 708 broilers have not been previously examined. Therefore, the objective of this study was to determine the main and interactive effects of the moa (s.c. or i.o.) of the MD vaccine, using current automated commercial application methods, and pht (4 or 18 h) on various processing yield variables of male Ross 708 broilers at 49 doa.

MATERIALS AND METHODS

Research Design and Treatments

The protocols for the current study were approved by the Institutional Animal Care and Use Committee of Mississippi State University. A total of 3900 Ross 708 (Aviagen, Inc., Huntsville, AL) broiler hatching eggs, that were obtained from a commercial source, were randomly set in each of 15 replicate setter trays belonging to each of 4 pre-assigned moa and pht treatment combinations. Vaccinations were administered by i.o. injection at 18 d of incubation to eggs in 30 of the 60 trays, and all eggs were subsequently transferred to hatching baskets. Information concerning incubation of the eggs in the setter and hatcher units and the candling and selection criteria of the experimental eggs were as described by Peebles et al. (2016).

At 21 d of incubation, 480 male hatchlings from eggs that received i.o. injections were not given s.c. injections, whereas 480 male hatchlings from eggs that were not i.o.-injected were vaccinated by s.c. injection at hatch. The i.o. injections (50 μL) were delivered by a commercial multi-egg injector (Embrex Inovoject M, Zoetis, Inc., Durham, NC) and the s.c. injections (200 μL) were delivered by an automatic pneumatic s.c. injector (Accuvac, Merial Co., Inc., Duluth, GA). The i.o. and s.c. injection solutions consisted of a MD vaccine (Poulvac Marek CVI + HVT; Zoetis, Florham Park, NJ) taken from the same lot that was diluted using Zoetis MD vaccine diluent. According to manufacturer recommendations, one full commercial dose (8,960 pfu) of the vaccine was administered in each diluted s.c. and i.o. injection volume. Further information concerning the storage and handling of the vaccine is provided by Peebles et al. (2017).

Before placement, male chicks from each i.o. and s.c. vaccination treatment were subjected to 1 of the 2 pht periods (4 or 18 h), with 240 chicks now belonging to each of 4 moa and pht treatment combinations. Sixteen male chicks were randomly selected from each treatment combination replicate group and were assigned to each of 15 replicate floor pens belonging to each of the 4 moa and pht treatment combination groups (60 total pens). The chicks were held in baskets without access to feed and water inside their respective floor pens until their respective assigned pht was completed. After pht completion, the chicks were released into each floor pen of an isolated grow-out facility and were grown out through 48 doa. Chicks in all treatment groups were housed in the same facility. Commercial bird density in the pens was maintained by reducing bird numbers when necessary. A full description of the housing conditions and diets is provided by Peebles et al. (2017).

Data Collection

At 48 doa, individual live BW (**LBW**) was determined for 6 birds that were randomly selected from each replicate pen. Those birds were then fasted for 16 h before being processed. At 49 doa, whole carcass (**CW**), fat pad (**FPW**), breast muscle (major pectoral muscle; **BMW**), and tenders muscles (minor pectoral muscles; **TW**) weights were recorded. Whole carcass weight as a percentage of LBW (**CLBW**), and FPW, BMW, and TW as percentages of both LBW and CW (**FLBW** and **FCW**, **BMLBW** and **BMCW**, and **TLBW** and **TCW**, respectively) were calculated.

Statistical Analysis

A completely randomized experimental design was employed with pen as the experimental unit. There were 15 replicate units (pens) that belonged to each moa-pht treatment combination. All data were analyzed by two-way ANOVA in a 2 moa (i.o.-injected and s.c.-injected) × 2 pht (4 and 18 h) factorial arrangement of treatments to test for the main and interactive effects of moa and pht, with moa and pht serving as fixed factors and with replicate pen serving as a random factor. Individual sample data within each of the replicate units were averaged before analysis. All percentage data were subjected to an arcsine square root of the percentage data transformation before analysis (Snedecor and Cochran, 1974). All data were analyzed using the MIXED procedure of SAS software 9.4 (SAS Institute, 2012). Least squares means were compared using Fisher's Protected LSD in the event of significant global effects. F tests were performed to evaluate treatment effects, and comparison of least squares means was achieved using Student *t* tests (Littell et al., 2006). Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

No significant pht main effects or interactive effects between moa and pht were observed for any of the processing yield variables examined. Nevertheless, the means for each of the processing yield variables within the 4 h and 18 h pht treatment groups are provided in Table 1 for observation. There were also no significant main effects due to moa for LBW ($P = 0.897$), CW ($P = 0.729$), CLBW ($P = 0.139$), FPW ($P = 0.949$), FLBW ($P = 0.839$), FCW ($P = 0.757$), BMW ($P = 0.425$), BMLBW ($P = 0.140$), BCW ($P = 0.222$), or TW ($P = 0.074$) at 49 doa. However, there were significant main effects due to moa for TLBW ($P = 0.009$) and TCW ($P = 0.004$). The mean 49 doa values of TLBW and TCW were observed to be higher in the birds that received i.o. injections in comparison to those that received s.c. injections (Table 2).

Table 1. Mean male live BW (LBW) at 48 d post-hatch, and whole carcass weight (CW), CW relative to LBW (CLBW), fat pad weight (FPW), FPW relative to BW (FLBW), FPW relative to CW (FCW), breast muscle weight (BMW), BMW relative to BW (BMLBW), BMW relative to CW (BMCW), tenders muscles weight (TW), TW relative to BW (TLBW), and TW relative to CW (TCW) at 49 d post-hatch within the 4 and 18 h pre-placement holding time (pht) treatment groups, across the in ovo and subcutaneous injection treatment groups.[1]

Treatment	4 h pht	18 h pht
Variable		
LBW (kg)	3.64 ± 0.054	3.66 ± 0.051
CW (kg)	2.62 ± 0.038	2.63 ± 0.036
CLBW (%)	71.9 ± 0.14	71.9 ± 0.13
FPW (kg)	0.053 ± 0.0013	0.052 ± 0.0013
FLBW (%)	1.46 ± 0.037	1.42 ± 0.035
FCW (%)	2.04 ± 0.053	1.97 ± 0.051
BMW (kg)	0.65 ± 0.014	0.65 ± 0.013
BMLBW (%)	17.7 ± 0.16	17.8 ± 0.16
BMCW (%)	24.6 ± 0.22	24.7 ± 0.21
TW (kg)	0.13 ± 0.002	0.14 ± 0.002
TLBW (%)	3.68 ± 0.049	3.79 ± 0.046
TCW (%)	5.11 ± 0.065	5.28 ± 0.061

[1] Data from 6 birds within each of 30 replicate pens (N = 30) were used to calculate treatment means.

Table 2. Mean male live BW (LBW) at 48 d post-hatch, and whole carcass weight (CW), CW relative to LBW (CLBW), fat pad weight (FPW), FPW relative to BW (FLBW), FPW relative to CW (FCW), breast muscle weight (BMW), BMW relative to BW (BMLBW), BMW relative to CW (BMCW), tenders muscles weight (TW), TW relative to BW (TLBW), and TW relative to CW (TCW) at 49 d post-hatch within the in ovo and subcutaneous injection treatment groups, across the 4 and 18 h pre-placement holding time treatment groups.[1]

Treatment	In ovo	Subcutaneous
Variable		
LBW (kg)	3.65 ± 0.053	3.66 ± 0.052
CW (kg)	2.62 ± 0.038	2.63 ± 0.037
CLBW (%)	71.7 ± 0.14	72.0 ± 0.14
FPW (kg)	0.052 ± 0.0013	0.052 ± 0.0013
FLBW (%)	1.44 ± 0.037	1.44 ± 0.036
FCW (%)	2.01 ± 0.053	2.00 ± 0.052
BMW (kg)	0.64 ± 0.013	0.66 ± 0.013
BMLBW (%)	17.6 ± 0.16	17.9 ± 0.16
BMCW (%)	24.5 ± 0.21	24.8 ± 0.21
TW (kg)	0.14 ± 0.002	0.13 ± 0.002
TLBW (%)	3.82^{a} ± 0.048	3.64^{b} ± 0.047
TCW (%)	5.33^{a} ± 0.064	5.06^{b} ± 0.062

[a,b] Means within a row with no common superscript differ significantly ($P \leq 0.05$).

[1] Data from 6 birds within each of 30 replicate pens (N = 30) were used to calculate treatment means.

Upon comparing the effects of 100 μL of the MD vaccine administered by hand i.o. injection to embryos at 17 or 18 d of incubation with its s.c. administration to chicks at hatch, Sharma et al. (1984) observed that embryo vaccination did not lead to reductions in subsequent BW gain, and concluded that it could be safely used as an embryonal vaccine in chickens. Gildersleeve et al. (1993) showed that in comparison to the conventional s.c. method in which a 200 μL volume of the MD vaccine was given, i.o. administration of 50 μL of the MD vaccine improved live production results more safely and efficiently in integrated commercial broiler operations. In addition, Peebles et al. (2017) reported in a subsequent companion article, that the effects of administering 50 μL of the MD vaccine by i.o. injection, or 200 μL of the vaccine by s.c. injection, on the mean BW of the birds at each of the weekly age periods through 48 d post-hatch did not differ significantly.

In spite of the fact that moa was shown by Peebles et al. (2017) to have no significant effect on mean BW at each of the weekly age periods, there were significant integrated responses of feed consumption and BW gain to moa in the various age intervals examined. The 14 to 28 d feed consumption and 14 to 21 d BW gain of s.c.-vaccinated birds was lower than that of i.o.-vaccinated birds. Previous reports directly comparing effects of i.o. and s.c. administration of the MD vaccine on broiler processing yield are lacking in the literature. In the current study, relative tenders muscles weight was greater at 49 doa in the birds that received i.o injections in comparison to those that received s.c. injections. This may be related to the increased feed consumption and BW gain noted in those birds between 14 and 28 doa by Peebles et al. (2017). The noted isolated effect on the tenders muscle group, exclusive of the other processing variables examined, is difficult to explain. However, it is noteworthy that Peebles et al. (2014) also showed that the incubational characteristics of Ross 708 broiler hatching eggs was associated with relative tenders muscle yield at 49 d post-hatch. These results nevertheless suggest that although i.o. vaccination displays some potential advantage over s.c. injection of the MD vaccine, these moa basically have no differential effects on the processing yield of Ross 708 broilers.

Peebles et al. (2016) also reported that although chick body length, relative yolk-free BW, and yolk moisture content at placement were increased by an increase in pht from 4 to 18 h, the delay in pht caused total absolute BW, body mass index, absolute and relative yolk sac weight, and yolk-free body moisture content at placement, and BW at 7 doa, to be decreased. Oliveira et al. (2015) reported that a 24 h pht including feed and water restriction caused Ross 708 broilers to experience a lower feed intake and BW gain, and a higher feed conversion ratio through 21 doa post-hatch in comparison to those provided immediate access to water and feed. Peebles et al. (2017) further showed that an increase in pht from 4 to 18 h significantly reduced the BW of the Ross 708 broilers at placement (d 0) as well as on d 7, 14, 21, and 35, and further noted that there were likewise significant integrated responses of feed consumption and BW gain to pht in the various post-hatch age intervals examined. The increase in pht from 4 to 18 h decreased feed consumption through 28 d post-hatch and BW gain through 35 d post-hatch. Peebles et al. (2017) concluded that a pht without available food and water for 18 h or more can result in significant reductions in broiler post-hatch BW, and suggested that the basis for the relationship between reduced feed intake and growth suppression is a reduction in nutrient availability.

Vieira and Moran (1999b) have summarized that depressed growth as the result of a delayed pht and early nutrient inadequacies is associated with decreased skeletal muscle growth which extends to marketing age, thereby leading to reduced broiler meat yield. Joseph and Moran (2005) reported that prolonging the pht of male and female Ross 308 broilers in the hatcher from 3 to 15 h exerted no major effects on their subsequent grow-out performance or their carcass qualities and breast meat yields. However, a 24 h pht without access to feed and water has been reported to eventually reduce the slaughter carcass weight and percentage of grade A carcasses of male Ross 308 broilers (Vieira and Moran, 1999a). The results of this current study are similar to those of Joseph and Moran (2005). Despite noted adverse effects on broiler performance through 35 doa post-hatch, a delay of up to 18 h was observed to have no negative impact on processing yield. Conversely, increasing the delay up to 24 h pht may be deleterious to processing yield and carcass quality. It is suggested that such a delay would negatively impact the yield in Ross 708 broilers, as has been shown by Vieira and Moran (1999a) for Ross 308 broilers. Further research would be needed to confirm this suggestion.

In conclusion, i.o. and s.c. injections were equally safe for administration of the MD vaccine, and when compared to the s.c. injection of the MD vaccine, i.o. injection not only exerted no adverse effects on the processing yield of Ross 708 broilers, but displayed a slight advantage, in that tenders weight yield as a percentage of both live and whole carcass weight was greater in those that had received i.o rather than s.c. injections. The corresponding results of this study and that of Peebles et al. (2014), concerning tenders muscle yield at 49 d post-hatch, also confirm that the early subjection of broilers to various physiological challenges can ultimately affect processing yield. In addition, there were no interactive effects observed between moa of the MD vaccine and pht. Although prolonging pht by 14 h reduced BW gain through 35 d in association with the reduction in feed consumption through 28 d, because there was no interaction between moa and pht for any of the processing yield variables examined, it may also be concluded that an increase in pht from 4 to 18 h does not exacerbate the effect of administering the MD vaccine to Ross 708 broilers by either i.o. or s.c. injection. In light of the results of the current investigation, effects of the i.o. administration of vaccines for other viral diseases of major concern to poultry producers including avian influenza, Newcastle disease, and coronaviruses on subsequent processing yield deserve further investigation.

ACKNOWLEDGMENTS

This work was fully supported by funds provided by Zoetis Global Poultry.

REFERENCES

Elliott, T. S., R. C. Wiggington, and K. C. Corbin. 1943. The number and size of muscle fibres in the rat soleus in relation to age, sex, and exercise. Anat. Rec. 85:307–308.

Fanguy, R. C., L. K. Misra, K. V. Vo, C. C. Blohowiak, and W. F. Krueger. 1980. Effect of delayed placement on mortality and growth performance of commercial broilers. Poult. Sci. 59:1215–1220.

Franco, J. R. G., A. E. Murakami, M. R. M. Natali, E. R. M. Garcia, and A. C. Furlan. 2006. Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. Braz. J. Poult. Sci. 8:233–241.

Gildersleeve, R. P., C. M. Hoyle, A. M. Miles, D. L. Murray, C. A. Ricks, M. N. Secrest, C. J. Williams, and C. L. Womack. 1993. Developmental performance of an egg injection machine for administration of Marek's disease vaccine. J. Appl. Poul. Res. 2:337–346.

Jochemsen, P., and S. H. Jeurissen. 2002. The localization and uptake of *in ovo*-injected soluble and particulate substances in the chicken. Poult. Sci. 81:1811–1817.

Johnston, P. A., H. Liu, T. O'Connell, P. Phelps, M. Bland, J. Tyczkowski, A. Kemper, T. Harding, A. Avakian, E. Haddad, C. Whitfill, R. Gildersleeve, and C. A. Ricks. 1997. Applications in *in ovo* technology. Poult. Sci. 76:165–178.

Joseph, N. S., and E. T. Moran, Jr. 2005. Effect of flock age and postemergent holding in the hatcher on broiler live performance and further-processing yield. J. Appl. Poult. Res. 14:512–520.

Littell, R. C., G. A Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. SAS Institute, Inc., Cary, NC.

Nir, I., and M. Levanon. 1993. Effect of posthatch holding time on performance and on residual yolk and liver composition. Poult. Sci. 72:1994–1997.

Oliveira, T. F. B., A. G. Bertichini, R. M. Bricka, P. Y. Hester, E. J. Kim, P. D. Gerard, and E. D. Peebles. 2015. Effects of *in ovo* injection of organic trace minerals and post-hatch holding time on broiler performance and bone characteristics. Poult. Sci. 94:2677–2685.

Peebles, E. D., T. M. Barbosa, T. S. Cummings, J. Dickson, and S. K. Womack. 2016. Comparative effects of *in ovo* versus subcutaneous administration of the Marek's disease vaccine and pre-placement holding time on the early post-hatch quality of Ross × Ross 708 broiler chicks. Poult. Sci. 95:2038–2044.

Peebles, E. D., T. M. Barbosa, T. S. Cummings, J. Dickson, S. K. Womack, and P. D. Gerard. 2017. Comparative effects of *in ovo* versus subcutaneous administration of the Marek's disease vaccine and pre-placement holding time on the post-hatch performance of Ross 708 broilers. Poult. Sci. 96:1071–1077.

Peebles, E. D., R. Pulikanti, W. Zhai, and P. D. Gerard. 2014. Relationships of incubational hatching egg characteristics to post-hatch body weight and processing yield in Ross x Ross 708 broilers. J. Appl. Poult. Res. 23:34–40.

Ricks, C. A., A. Avakian, T. Bryan, R. Gildersleeve, E. Haddad, R. Ilich, S. King, L. Murray, P. Phelps, R. Poston, C. Whitfill, and C. Williams. 1998. Poultry Vaccines-*In Ovo* Vaccination Technology. Pages 495–513 in: Advances in Veterinary Medicine. vol. 41. 1st edn. W. J Dodds, and R. D. Schultz, eds. Academic Press, San Diego, CA.

SAS Institute, 2012. SAS Proprietary Software Release 9.4. SAS Institute, Inc. Cary, NC.

Sharma, J. M., L. F. Lee, and P. S. Wakenell. 1984. Comparative viral, immunologic, and pathologic responses of chickens inoculated with herpesvirus of turkeys as embryos or at hatch. Am. J. Vet. Res. 45:1619–1623.

Snedecor, G. W., and W. G. Cochran. 1974. Statistical Methods. 6th ed. Iowa State Univ. Press, Ames.

Stamps, L. K., and L. D. Andrews. 1995. Effects of delayed housing of broiler chicks and three different types of waterers on broiler performance. Poult. Sci. 74:1935–1941.

Vieira, S. L., and E. T. Moran, Jr. 1999a. Effects of delayed placement and used litter on broiler yields. J. Appl. Poult. Res. 8:75–81.

Vieira, S. L., and E. T. Moran, Jr. 1999b. Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. World's Poult. Sci. J. 55:125–142.

Willemsen, H., M. Debonne, Q. Swennen, N. Everaert, C. Careghi, H. Han, V. Bruggeman, K. Tona, and E. Decuypere. 2010. Delay in feed access and spread of hatch: importance of early nutrition. World's Poult. Sci. J. 66:177–188.