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Examining the Rate of the Yolk Uptake Through the Yolk Stalk in the Post-hatch Broiler Chick By Using Cr ^{51} Labelled Microspheres as A Tracer

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ABSTRACT

The trial was conducted with the purpose to investigate the usefulness ^{51}Cr labeled microspheres as tracers in yolk absorption and or its rate absorption through yolk stalk. In the trial, 20 broiler chicks were injected in the yolk sac on Day 0 with with 0.2 ml of radioactive ^{51}Cr labelled microspheres suspended in physiological saline. Injection was made with directly through the abdominal wall into the underlying yolk sac. At 1300 h each day for five days, four chicks were sacrificed at random by cervical dislocation. For each chick, total body weight, and the weight and level of radiation (cpm) from the following organs were determined: total body, carcass, intestine, liver, yolk sac, gizzard, kidney, and excreta. The level of radiation from tissues were obtained with the use of a High Energy Gamma Scintillator Probe containing NaI crystal. Results show body weight, and relative intestine, and excreta weight increased between 1 and 5 days; conversely, relative yolk sac weight decreased between 1 and 5 days. Relative yolk stalk weight peaked on Day 4, but was not significantly different from that at Day 1. Relative cpm of the liver, yolk sac, yolk stalk, and excreta were influenced by day of age. The level of radiation was significantly higher in the yolk sac, kidney, and excreta than in the carcass, intestine, liver, blood, and gizzard. It was concluded that significant amounts of yolk may be absorbed and pass through the yolk stalk into the intestine through 5 days of age in post-hatch chicks and the rate of movement increases through 5 days post-hatch, particularly after Day 3.

(Key words: chick, yolk sac, yolk stalk, ^{51}Cr labelled microspheres, cpm)

INTRODUCTION

In the recent poultry industry, the efficiency of yolk uptake in post-hatch broiler chickens because of its subsequent effects on their viability and growth performance has been a major concern. However, information regarding the role of yolk sac and the yolk stalk in yolk uptake of post-hatch broiler chick is still limited.

The yolk sac membrane acts as a temporary storage area for a considerable proportion of the lipid found within the yolk complex (Noble & Tullet, 1989). Moreover, Freeman and Vince (1974) stated that the yolk sac membrane plays a key role in the nutrition of the developing embryo, not only as a digestive and absorptive organ but also as a site for the synthesis of specific proteins, amino acids, and blood. It is also a source of lymphoid precursor cells for the thymus and bursa of fabricius, is a site for glycogen storage, and is a temporary excretory organ.

In spite of extensive mobilization of yolk during incubation, a large portion of yolk remains unabsorbed at hatching and becomes incorporated into the body of the chick as part of a yolk sac which extends off the small intestine (Romanoff, 1960; Freeman and Vince, 1974). Murakami *et al*. (1992) found that almost all the residual yolk disappears during the first 5 to 7 days after the chick hatches, while Nobel & Ogunyemi (1989) reported that approximately 85% of residual yolk is absorbed by the fifth day post-hatch. However, what the role of yolk stalk, particularly after hatching, in the yolk absorption has been a controversial (Sulaiman, 2005).

There are two possible routes by which yolk may be absorbed in the developing bird. Yolk nutrients may be either absorbed directly across the yolk sac membrane into the blood stream or later in the gut after their conductance through a yolk stalk connected to the duodenum.

In chicks, the yolk sac is connected to the intestine by a thick-walled tubular peduncle referred to as the yolk stalk. Peripherally attached vitelline blood vessels enter the embryo along side the yolk stalk. Furthermore, transverse sections of the yolk stalk of 5 day-old chicks show that their gross histological features are similar to those of the intestine (Kar, 1947; Sturkie, 1976). It is likely that significant

amounts of yolk pass through the yolk stalk into the intestine of the hatched bird and are digested there (Kar, 1947; Romanoff, 1960). The rate of yolk assimilation is assumed much more rapid than during the embryonic period (Romanoff, 1960).

Materials such as phenol red (Nitsan *et al*.,1995), blue dextran (Noy *et al*., 1996) and India ink (Sulaiman, 2005) have been used as markers in determining yolk absorption in previous studies. Chromium is a shiny, hard, white metal, belonging to the first series transition elements. Its atomic number and mass are 24 and 52 (51.9961) respectively. Five radioactive isotopes can be produced, but only ^{51}Cr with a half-life 27.8 d is marketed and used in tracer studies. The absorption of ^{51}Cr across epithelial cells in the intestine has been reported to be minimal (0.2 to 2%) (Ducros, 1992). Due to its low absorption across epithelia, ^{51}Cr has potential for use as a radioactive tracer for determining the routes and rate of yolk utilization in the post-hatch broiler chick.

The purpose of this study was to investigate the usefulness ^{51}Cr labeled microspheres as tracers in yolk absorption and or its rate absorption through yolk stalk. The results of this study could be used as base information in order to use the more knowledge of yolk and yolk stalk role in enhancing viability and growth performance of chickens in the poultry industry.

MATERIAL AND METHODS

In this Trial, twenty commercial broiler chicks were injected in the yolk sac at 1300 h on Day 0 with 0.2 ml of radioactive ^{51}Cr labelled microspheres suspended in physiological saline (NEN-TRAC™ Microspheres, NEM-032B ^{51}Cr NEN™, Medical Products Depart., Biotechnology Div., Wilmington, DE 19898)

Each microsphere was 15.5 µm. 0.1 µm in diameter. The radiation concentration of the solution was 6.77 microcuries per gram, and there were 3.66 x 10^5 microspheres per mg of solution. The amount of gamma radiation emitted by the injected solution was 134,000 cpm. Injection was made with a 25 gauge needle directly through the abdominal wall into the underlying yolk sac, approximately 0.6

cm anterior to the umbilicus. Gentle pressure was used to assure that the yolk sac was positioned immediately against the abdominal wall. After injection, the yolk sac was inserted back into the body cavity, and the incision opening was auto-clipped. After treatment, chicks were placed in a heated battery that was divided into 20 hanging basket pens. One chick was placed in each pen. All chicks were exposed to continuous lighting and normal brooding temperatures throughout the experiment. Chicks were offered a standard broiler starter diet which met NRC (1984) recommendations. Feed and water were provided *ad lib.*

At 1300 h each day for five days, four chicks were sacrificed at random by cervical dislocation. Individual chicks represented a replicate. For each chick, total body weight, and the weight and level of radiation (cpm) from the following organs were determined: carcass, intestine, liver, yolk sac, gizzard, kidney, and excreta. The carcass represented the body of the chick after removal of all the above internal organs. Kidney samples were taken from one lobe. The level of radiation from tissues were obtained with the use of a High Energy Gamma Scintillator Probe containing (Probe-Model 44-2, Ludlum Measurements Inc., Sweetwater, TX 79556) 2.5 cm (diameter and thickness) NaI crystal. The probe was connected to a digital Ratemeter (Scaler Model 2200, Ludlum Measurements Inc., Sweetwater, TX 79556). Background counts were determined each day before and after the radiation of all specimens was determined. The average background cpm for that day was subtracted from the total cpm of each sample.

Carcass, intestine, liver, yolk sac, gizzard, and excreta weights were converted to percentages of total body weight and angular transformations (arc sine of the square root of the the proportion affected) were performed on the data prior to analysis. Relative cpm of the following tissues or organs: carcass, intestine, liver, yolk sac, yolk stalk, kidney, blood, and excreta were obtained by dividing the cpm of the sample by its weight. Total and relative cpm for all tissues or organs were ranked prior to analysis to avoid abnormality of the data. Ranked data for total cpm and relative cpm were analyzed by one-way ANOVA to test for differences between

day for each sample and between samples within each day (Steel and Torrie, 1985). Fisher's protected LSD was utilized to separate means when significant main effects were observed. Statements of statistical significance were based on $P< 0.05$ unless otherwise indicated.

RESULTS

The effects of day of age on body weight, and relative carcass, intestine, liver, yolk sac, yolk stalk, and excreta weights in post-hatch chicks between 1 and 5 days after being injected in the yolk sac with ^{51}Cr labelled microspheres at Day 0 are provided in Table 1 .

TABLE 1. Body weight (BDW), and relative carcass (RCRW), intestine (RINW), liver (RLVW), yolk sac (RYSW), yolk stalk (RYTW), and excreta (REXW) weights at Days 1, 2, 3, 4 and 5 posthatch in chicks injected in the yolk sac with ^{51}Cr-labelled microspheres at Day 0 in the Trial.

DAY	BDW	RCRW	RINW	RLVW	RYSW	RYTW	REXW
	(g)	----------[(g/g BDW) *100]----------					
1	46.03^{d}	76.78^{a}	5.29^{b}	2.89^{a}	8.19^{a}	0.0147^{ab}	2.30^{c}
2	45.12^{d}	76.22^{a}	6.48^{b}	3.12^{a}	3.94^{b}	0.0116^{b}	8.86^{bc}
3	53.92^{c}	75.47^{a}	9.68^{a}	2.22^{a}	1.70^{c}	0.0091^{b}	18.04^{b}
4	63.99^{b}	77.28^{a}	11.36^{a}	2.66^{a}	1.10^{cd}	0.0228^{a}	37.58^{a}
5	81.81^{a}	74.56^{a}	11.78^{a}	3.07^{a}	0.51^{d}	0.0092^{b}	40.56^{a}
SEM^{+}	2.31	1.12	0.85	0.22	0.73	0.0030	3.40

$^{a-d}$Means within a column with no common superscript differ significantly ($p< 0.05$) by Fisher's Protected LSD.

$^{+}$SEM based on pooled estimate of variance (n=20).

Body weight, and relative intestine, yolk sac, yolk stalk, and excreta weights

changed with day of age. Body weight, and relative intestine, and excreta weights increased between 1 and 5 days. Conversely, relative yolk sac weight decreased between 0 and 5 days. Body weight increased significantly between Days 2 and 3, 3 and 4, and 4 and 5. Significant increases for relative intestinal weight occurred between Days 2 and 3, and for relative excreta weight between Days 3 and 4. Relative yolk sac weight decreased significantly between Days 1 and 2, and then between Days 2 and 3. Relative yolk stalk weight peaked on Day 4, but was not significantly different from that at Day 1.

Total cpm of carcass, intestine, liver, yolk sac, yolk stalk, and excreta in post-hatch chicks between 1 and 5 days after being injected in the yolk sac with ^{51}Cr labelled microspheres at Day 0 their ranked tranformations are provided (in parentheses) in Table 2.

Total cpm of intestine, liver, yolk stalk and excreta changed with age. Total cpm of intestine, liver, yolk stalk, and excreta increased between Days 1 and 5. Total cpm of the intestine increased significantly between Days 1 and 3, and Days 3 and 5. The total cpm of the liver increased significantly between 4 and 5 days; whereas, that of the yolk stalk increased significantly between 1 and 2, and 2 and 3 days, and that of the excreta between 1 and 2, and 3 and 4 days.

After dividing the total cpm of each organ or tissue by its weight, a relative level of radiation (cpm/g) was calculated for each sample and the effects of day of age on ranked transformations (in parentheses) of relative cpm of the carcass, intestine, liver, yolk sac, yolk stalk, kidney and excreta are provided in Table 3.

Relative cpm of the liver, yolk sac, yolk stalk, and excreta increased between Days 1 and 5. A significant increase in relative cpm occurred for the liver between Days 1 and 2, for the yolk sac between Days 2 and 3, and for the excreta between Days 3 and 4. Relative cpm of the yolk stalk increased between Days 1 and 2, and 2 and 3. Relative blood cpm increased significantly between Days 1 and 3, decreased between Days 2 and 3, and increased again between Days 4 and 5.

Differences between ranked transformations for relative cpm of carcass,

intestine, liver, yolk sac, yolk stalk, kidney, blood and excreta within Days 1, 2, 3, 4, and 5 post-hatch in chicks injected in the yolk sac with ^{51}Cr labelled microspheres at Day 0 are provided in Table 4.

Table 2. Total counts per minute (cpm) of carcass (CRC), intestine (INC), liver (LVC), yolk sac (YSC), yolk stalk (YTC), and excreta (EXC) and their ranked transformations at days 1, 2, 3, 4, and 5 posthatch in chicks injected in the yolk sac with ^{51}Cr-labelled microspheres at Day 0 in the Trial.

DAY	CRC	INC	LVC	YSC	YTC	EXC
	----------[CPM]----------					
1	703.25 (14.25)^{a}	45.17 (3.25)^{c}	0.00 (6.00)^{b}	337858.44 (12.25)^{a}	0.00 (2.50)^{c}	110.83 (3.25)^{c}
2	82.58 (11.00)^{a}	504.08 (9.00)^{bc}	14.67 (10.75)^{b}	364028.55 (13.00)^{a}	24.08 (7.25)^{b}	1608.08 (7.50)^{b}
3	11.25 (4.00)^{a}	477.20 (9.50)^{b}	3.25 (7.75)^{b}	410383.25 (14.00)^{a}	187.00 (12.75)^{a}	3029.25 (9.25)^{b}
4	173.08 (11.25)^{a}	1780.33 (14.25)^{ab}	85.42 (11.00)^{b}	306719.80 (9.50)^{a}	303.58 (14.00)^{a}	47658.08 (15.75)^{a}
5	106.42 (12.00)^{a}	3311.67 (16.50)^{a}	45.42 (17.00)^{a}	150691.44 (3.75)^{a}	991.67 (16.00)^{a}	55368.93 (16.75)^{a}
SEM $^{+}$	22.19	1032.833	38.55	59763.47	322.23	12009.25
SEM^{++}	(2.85)	(1.92)	(1.74)	(2.59)	(1.32)	(1.14)

$^{a-c}$Mean ranks in parentheses within a column with no common superscript differ significantly ($p< 0.05$) by Fisher's Protected LSD. SEM based on pooled estimate of variance.(n=20).

Table 3. Relative counts per minute (cpm) of carcass (RCRC), intestine (RINC), liver (RLVC), yolk sac(RYSC), kdney (RKDC), blood (RBLC), and excreta (REXC) and their ranked transformations at days 1, 2, 3, 4, and 5 posthatch in chicks injected in the yolk sac with ^{51}Cr-labelled microspheres at day 0 in the Trial.

DAY	RCRC	RINC	RLVC	RYSC	RYTC	RKDC	RBLC	REXC
	----------[cpm/g sample]----------							
1	20.53	15.68	0.00	105102.58	0.00	0.00	0.00	83.62
	(14.50)^{a}	(3.50)^{a}	(6.00)^{c}	(3.50)^{c}	(2.50)^{c}	(7.00)^{a}	(7.00)^{c}	(4.50)^{b}
2	2.43	260.84	11.22	231324.75	4589.28	120.37	3.63	346.35
	(11.75)^{a}	(11.50)^{a}	(12.25)^{ab}	(8.00)^{bc}	(8.25)^{b}	(10.00)^{a}	(13.25)^{b}	(8.50)^{b}
3	0.29	101.99	1.42	516415.11	38791.67	85.72	0.00	346.35
	(4.2)5^{a}	(10.25)^{a}	(8.00)^{bc}	(14.00)^{a}	(15.75)^{a}	(9.75)^{a}	(7.00)^{c}	(8.50)^{b}
4	3.46	232.97	29.72	711934.45	22405.56	0.74	0.00	2167.26
	(11.00)^{a}	(13.50)^{a}	(11.00)^{abc}	(14.75)^{a}	(12.75)^{a}	(8.75)^{a}	(7.00)^{c}	(15.50)^{a}
5	1.76	334.20	13.19	366618.14	31627.84	171.13	27.94	1566.36
	(1.00)^{a}	(13.75)^{a}	(15.25)^{a}	(12.25)^{ab}	(13.25)^{a}	(17.00)^{a}	(18.25)^{a}	(15.00)^{a}
SEM^{+}	6.51	334.83	13.67	148223.10	12909.84	83.42	6.66	470.17
	(2.90)	(2.70)	(1.97)	(1.86)	(1.48)	(2.24)	(1.03)	(1.66)

$^{a-c}$Means ranks in parentheses within a column with no common superscript differ significantly ($p< 0.05$) by Fisher's Protected LSD.

$^{+}$SEM based on pooled estimate of variance (n=20).

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Table 4. Ranked Transformations of relative counts per minute of carcass (RCRC), intestine (RINC), liver (RLVC), yolk sac (RYSC), yolk stalk (RYTC), kidney (RKDC), blood (RBLC), and excreta (REXC) within days 1, 2, 3, 4, and 5 posthatch in chicks injected in the yolk sac with ^{51}Cr-labelled microspheres at Day 0 in the Trial.

DAY	RCRC	RINC	RLVC	RYSC	RYTC	RKDC	RBLC	REXC	SEM^{+}
1	21.12^{B}	14.87^{BC}	11.50^{C}	30.50^{A}	11.50^{C}	11.50^{C}	11.50^{C}	21.12^{B}	2.17
2	10.75^{CDE}	18.00^{BCD}	10.00^{DE}	30.50^{A}	26.25^{AB}	8.12^{E}	9.62^{E}	18.75^{BC}	2.83
3	10.00^{C}	18.75^{B}	8.87^{C}	30.50^{A}	26.50^{A}	10.37^{C}	6.50^{C}	22.50^{B}	1.98
4	12.75^{C}	19.50^{B}	9.50^{CD}	30.50^{A}	26.50^{A}	7.00^{DE}	5.00^{E}	21.75^{B}	1.47
5	2.50^{F}	17.50^{C}	9.00^{E}	30.50^{A}	26.25^{B}	13.75^{CD}	10.00^{DE}	20.50^{BC}	1.39

$^{A-F}$Means within a row with no common superscript differ significantly ($P< 0.05$) by Fisher's Protected LSD.

$^{+}$SEM based on pooled estimate of variance (n=4).

At Day 1, relative cpm of the yolk sac was the highest. Relative cpm of the excreta and carcass were higher than those of the liver, yolk stalk, kidney, and blood, but the relative cpm of the intestine was intermediate. At Day 2, relative cpm of the yolk sac was higher than those all other organs or tissues except that of the yolk stalk; however, the yolk stalk had cpm that exceeded those of the carcass, liver, kidney, and blood. Excreta cpm were higher than the liver, kidney, and blood, and the intestine was higher than that of the kidney and blood. At Day 3, relative cpm of the yolk sac and yolk stalk were greater than those of all other organs or tissues. However, the intestine and excreta exceeded those of the carcass, liver, kidney, and blood. At Day 4, cpm of the yolk sac, yolk stalk, intestine, excreta and carcass exhibited differences similar to those at Day 3. However, at Day 4, relative blood cpm was lower than that of the liver with the relative cpm of the kidney intermediate. At Day 5, relative yolk sac cpm was highest. Relative yolk stalk and excreta cpm were also higher than the carcass, intestine, liver, kidney, and

blood. The relative cpm of the kidney was higher than those of the carcass and liver , and the relative blood and liver cpm were higher than the carcass.

DISCUSSION

In this trial, body weight, relative yolk sac and supply organs weights were change insignificantly, although the changes in body weight and relative yolk sac weight were significant in this trial. Relative yolk stalk weight also changed during the first 5 days post-hatch. Relative yolk stalk weight peaked on Day 4, but was not significantly different from that at Day 1. The first week after hatch is a crucial period for broiler chicks, because of the major changes that occur. The bird experience's a transition from an endogenous supply of nutrients from the yolk to the ingestion and digestion of exogenous food. In this study, body weight increased steadily from Day 0 to Day 5, and relative intestine and liver weights increased steadily between Days 0 and 4 before stabilizing through Day 5. Conversely, relative yolk sac weight decreased steadily from Day 0 to Day 5. Also, during this period residual yolk, which makes up about 10% of body weight of chicks at hatch becomes negligible in size (Romanoff, 1960; Murakami *et al*., 1992) and the decrease is being most pronounced by 3 days of age (Latour *et al*., 1994).

In all birds examined, the yolk stalk was present as a finger-shaped projection on the anterior portion of the duodenum. The average weight of the yolk stalk in this study was .027 g or .01% of chick body weight, however, relative yolk stalk weight increased significantly during the first 5 days post-hatch (Kar, 1947; Sulaiman, 2005). Sulaiman (2005) using india ink suggested that the movement of material through the yolk stalk in post-hatch broiler chicks through 5 d of age is not bidirectional, but that passage is restricted to a one-way direction from the yolk sac to the intestine.

The total cpm of the intestine, yolk stalk, and excreta tended to increase during the first 5 days of post-hatch growth, which would suggest that radioactive material was moving from the yolk sac via the yolk stalk into the gut, and that the

rate of movement increased with age through Day 5. The inhibition of yolk sac membrane absorption of radioactive ^{51}Cr through the use of ^{51}Cr bound to microspheres would support this contention. Comparisons of relative cpm among organs and tissues indicate that ^{51}Cr was more detectable in the yolk sac, yolk stalk, intestine, and excreta than in all other organs or tissues. Only small amounts of ^{51}Cr were detected in the carcass, liver, blood, and kidney. The small amounts of radiation in those tissues may have been due to the absorption of some unbound ^{51}Cr into the blood stream through the yolk sac membrane. However, the total and relative cpm of the carcass, liver, blood and kidney were negligable in comparison to the those of the yolk sac, yolk stalk, intestine and excreta. The ^{51}Cr in the blood was then secreted by the kidney into the excreta because the kidneys are a major route of elimination (Florkin & Stotz, 1971).

It is also interesting to note that relative cpm of the yolk stalk increased significantly between Days 1 and 2, and between Days 2 and 3, and that of the excreta increased between Days 3 and 4. Once in the gut it was eliminated with the excreta during the five day post-hatch period. Because 98% or more of dietary chromium is not absorbed by the gut (Offenbacher *et al*, 1986), once the chromium passed into the gut most of it would have been incorporated into the excreta.

The total cpm of the yolk sac did not change during the post-hatch period; whereas, the relative cpm of the yolk sac tended to increase. These data would further suggest that the movement of radioactive Cr through the yolk stalk must have been small enough so that the total cpm of the yolk sac did not change significantly, and that as yolk sac size was decreasing, the concentration of ^{51}Cr actually increased to cause the relative cpm of the yolk sac to also increase. Yolk absorption evidently occurs primarily through the yolk sac membrane but significant amounts of yolk do appear to move into the gut through the yolk stalk. The rate of movement also increases with the age of the post-hatch chick through 5 days of age, with the increase becoming most noticeable in the excreta after 3 days. The possible movement of significant amounts of ^{51}Cr into the excreta via the yolk sac

membrane, circulation, and kidney, led to the utilization of ^{51}Cr bound to 15 μm microspheres to help eliminate that particular route to the gut in this trial.

CONCLUSION

Results show body weight, and relative intestine, and excreta weight increased between 1 and 5 days; conversely, relative yolk sac weight decreased between 1 and 5 days. Relative yolk stalk weight peaked on Day 4, but was not significantly different from that at Day 1. Relative cpm of the liver, yolk sac, yolk stalk, and excreta were influenced by day of age. Comparisons of relative cpm among organs and tissues indicated that ^{51}Cr was more detectable in the yolk sac, yolk stalk, intestine and excreta than in all other organs or tissues. These data would suggest that radioactive material was moving from the yolk sac via the yolk stalk into the excreta. However, the movement of radioactive Cr through the yolk stalk was small enough so that the total cpm of the yolk sac did not change significantly with age. Increased relative cpm of excreta after 3 days also suggested that the rate of movement of yolk through the yolk stalk may increase with age during the first 5 days post-hatch, particularly after 3 days.

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