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Effect of incubation system on the development of intestinal villi, metabolism, and performance of one- to forty-day-old broiler chickens

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ABSTRACT - An experiment was performed to evaluate the effect of the incubation system — multiple-stage (MS) and single-stage (SS) — on the characteristics at hatching, intestinal morphology, metabolism, and performance of Cobb-500 chickens from 1 to 40 d of age. A total of 1,968 fertile eggs were incubated in two setters under commercial conditions. Birds hatched in SS were longer than those hatched in MS, and the females had higher relative intestinal weight compared with males. However, at hatching, there were no differences in BW and yolk-free body mass from incubation system or sex. In the period from 1-40 d, the birds hatched in SS were heavier, had higher weight gain and better adjusted feed conversion, without differences in feed intake and feed conversion. This result is due mainly to MS females, which always, although in some periods only numerically, exhibited worse responses than the other treatment, lowering the average MS bird performance. Sex influenced the duodenal villi height in chickens at 0 d; females showed larger villi than males. Moreover, the birds hatched in SS had deeper crypts than those hatched in MS. At 7 d, because males had higher feed intake, the differences in duodenal villi height found at hatching disappeared, leaving only a gender effect on jejunum crypt depth: females showed deeper crypts. As regards the metabolism coefficients of nutrients from 5 to 7 d, females were more efficient in metabolizing energy and showed higher apparent metabolizable energy corrected for nitrogen values than males, with no differences caused by the incubation system. The best incubation conditions are obtained with the single-stage system, based on the improved broiler performance (2.98%), especially in the females (5.04%).

Key Words: hatching, intestinal morphology, multi-stage, single-stage

Introduction

The increased weight of broiler chickens over the last 25 years was not due to any change in the incubation time of the eggs. Currently, the incubation time represents between 30% and 40% of the total broiler life, whereas 25 years ago, it represented 20% to 25% (Hulet, 2007). Thus, the impact of the incubation conditions on embryonic growth is more critical for the success of birds and health in their subsequent performance. Under commercial conditions, one of the most common stresses that embryos encounter inside the setter occurs if conditions such as temperature, humidity and ventilation are not adequate (Bruzual et al., 2000; Peebles et al., 2001; De Smit et al., 2006; Leksrisompong et al., 2007).

At present, there are multi-stage (MS) and single-stage (SS) incubation systems. The MS system contains embryos at different stages of development and is operated with average parameters of temperature, humidity, and ventilation, which partially satisfy the embryonic requirements. The SS system employs profiles that change according to the incubation time, providing, in theory, the necessary conditions for embryos.

It has been observed that most multi-stage incubation systems do not provide enough heat to embryos in their early development (Oviedo-Rondón et al., 2009a). Likewise, the increased growth rate of embryos leads to an increase in the metabolic heat production of the eggs, and it accumulates inside the setter (Barri et al., 2011). Therefore, although setters have been programmed to operate at temperatures of 37.4 °C, if there is not adequate ventilation to remove the excess heat generated by embryos, those machines may reach temperatures up to 41.1 to 41.7 °C, particularly in the second half of the incubation (Hulet, 2007). Overheating during this time affects embryonic development, decreasing the yolk-free mass of hatchlings (Lourens et al., 2005; Willemsen et al., 2010) and their subsequent performance (Hulet et al., 2007; Oviedo-Rondón et al., 2009b; Molenaar et al., 2010b).

During the time that embryos are within the hatcher, organ development occurs. The last four days of incubation

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are a decisive point, and any stress in this phase can produce changes in the growth rate of digestive organs (Uni et al., 2003), such as high eggshell temperatures (40.3 °C) (Leksrisompong et al., 2007) or O_2 concentrations lower than 21% (Wineland et al., 2006).

The objective of the present study was to determine the differences in one-day-old chicks, in metabolism coefficients during the first seven days posthatch, in performance and in intestine morphological characteristics from multi-stage and single-stage incubation systems.

Material and Methods

All procedures described were approved by the Ethics Committee in Animal Use of Universidade Federal do Rio Grande do Sul, located in Rio Grande do Sul, Brazil.

We performed three experiments in the hatchery, experimental farm, and metabolism room to evaluate the incubation process and hatchlings, broiler performance, and chicken metabolism.

A total of 1,968 fertile eggs stored for three days, weighing between 65 and 68 g, from a 44-wk-old Cobb-500 broiler breeder flock, were used. In a commercial hatchery, 1,200 eggs were incubated in a SS setter (Airstreamer A12S model, Petersime NV, Zulte, Belgium) distributed into eight trays of 150 eggs each, and 768 eggs were incubated in a MS setter (MG125 model, Casp, São Paulo, Brazil) distributed into eight trays of 96 eggs each. Each tray was considered an experimental unit, and all trays were distributed in a completely randomized design. The setter capacities were 57,600 and 126,000 eggs for SS and MS, respectively. The incubation profiles used in this experiment were those commonly used for broiler chickens in the industry (Table 1). The transfer time from the setter to the hatcher and the total incubation time were 456 and 504 h, respectively, for both systems.

At hatching, five chicks of each sex were randomly selected per experimental unit. The evaluations were as follows: the selected animals were weighed individually and then killed by cervical dislocation. The yolk was carefully separated and weighed to determine yolk-free body mass. The chicks were measured with a ruler from the beak tip to middle finger tip of the right leg.

Similarly, two chicks of each sex were randomly selected from each experimental unit for morphological intestine evaluation: the animals were weighed alive and then killed by cervical dislocation to weigh the intestine (small intestine, pancreas, and cecum). The relative intestine weight (RIW) was calculated using the following formula:

$$RIW(\%) = \frac{\text{intestine weight}}{\text{body weight}} \times 100$$

Five centimeters of the duodenum (middle section of the pancreatic loop) and 6 cm of jejunum (midpoint between the end of the duodenal loop and Meckel's diverticulum) were taken from each sampled bird to evaluate the villus height (VH) and crypt depth (CD). The collected intestines were gently flushed with 10% buffered formalin and stored in Falcon tubes containing the same solution until further analysis. The composition of 1 L of solution was 100 mL of 40% formaldehyde, 4 g of monobasic sodium phosphate, 6.5 g of dibasic sodium phosphate, and 900 mL of distilled water.

For the preparation of histological slides, the samples were dehydrated in an ascending series of alcohol (70°, 80°, 95°, and 99°) with a total of six baths. Then, they were placed in xylol solution (three times), immersed in paraffin, and embedded in paraffin to form blocks. Subsequently, they were microtomized in 3 µm sections, and three sections from each segment were placed on one slide and stained with hematoxylin-eosin (Luna, 1968).

All measurements of intestinal segments were made using images obtained by optical microscopy (Nikon).

Table 1 - Incubation profiles in multi-stage and single-stage systems

Item	Multi-stage		Single-stage			
	MT	WBT	AT_{min}	AT_{max}	EST	WBT
In setter						
Pre-heating, ºF	86.0	-	76.0	97.0	-	-
Initial temperature, ºF	99.4	82.0	99.7	99.7	100.2	96.0
Final temperature, ºF	99.4	82.0	96.8	98.3	100.2	80.0
N of steps	1			14		
In hatcher						
Initial temperature, ºF	98.5	85.0	99.2	-	-	85.0
Final temperature, ºF	98.5	85.0	99.2	-	-	82.0
N of steps	3			5		

MT - incubator air temperature; WBT - wet bulb temperature; AT_{min} - minimum incubator air temperature; AT_{max} - maximum incubator air temperature; EST - eggshell temperature.

The images were captured with a camera attached to the microscope (Sony Super Steady Shot 9.1 megapixels) and transferred to an image analyzer (Image-Pro Plus, version 4.5.0.29, 2001). Villus height and CD were determined from villi in the first development stage (V1). Ten readings of VH and CD were performed per slide. One slide was prepared for each of the four birds selected from each experimental unit. The VH was measured from the apical to the basal region (crypt upper portion). The CD was measured from the base to the transition region between the crypt and villi.

Three hundred and thirty-six chicks from the hatchings described above (168 males and 168 females, 1-d-old) were randomly allocated to 28 floor pens (experimental unit, 1 m × 1 m) with nipple drinkers and feeders in a temperature-controlled room under a continuous light system. In the hatchery, the birds were vaccinated against Marek's disease. The transfer time of birds from the hatchery to the experimental unit was 13 h, during which time the birds were kept without access to food and water. The chicks were distributed into four experimental groups with seven replicates of 12 birds each in a completely randomized design with a 2 × 2 factorial arrangement, with sex and incubation system (MS and SS) as the factors.

The diets were corn-soybean meal-based and were formulated to exceed NRC recommendations (NRC, 1994) (Table 2). The dietary phases were pre-starter (1 to 7 d), starter (8 to 21 d), grower (22 to 35 d), and finisher (36 to 40 d). All birds were provided with feed in mash form and water *ad libitum* throughout the grow-out period.

During the experimental period (1 to 40 d), body weight (BW), weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were determined weekly. For the whole period, the adjusted feed conversion ratio (AFCR) for a weight of 3 kg was calculated. Dead birds were weighed and removed from the pens daily, and the data were used to correct the FCR. At 7 d of age, one bird per replicate with a weight close to the average weight of the pen was sacrificed by cervical dislocation to determine the RIW, VH, and CD of duodenum and jejunum. The selected birds were fasted for 3 h and then were killed. The procedure for the collection of intestinal sections was as described in the previous section.

A total of 250 chicks from the hatching described above (130 males and 120 females, 1-d-old) were randomly allocated in 25 cages with wire floors (experimental unit, 0.95 × 0.87 × 0.27 cm) in a temperature-controlled room with constant illumination. The evaluated treatments consisted of groups of birds from each sex and two incubation systems (MS and SS). The treatment groups had 6 replicates of 10 birds each, except for the males from MS system treatment, which consisted of seven replicates of the same number of animals. The experimental design was completely randomized with a 2 × 2 factorial arrangement, with sex and incubation system (MS and SS) as the factors. A pre-starter diet (Table 2) was used throughout the grow-out period (1 to 7 d). Feed in mash form and water were provided *ad libitum*. The total collection method was used to determine metabolism. An initial adaptation period of four days was observed for the birds to adapt to the diet and environment, after which period total excreta collection was performed for three days (Cortés et al., 2009). The excreta were collected in plastic bags twice each day (09.00 and 16.00 h) to prevent fermentation and stored at –10 °C until further analysis. The dry matter (DM), nitrogen (N) and gross energy (GE) in diets and excreta were determined in agreement with the AOAC (1996) to determine the coefficients of metabolization of dry matter, crude protein, gross energy, and apparent metabolizable energy corrected

Table 2 - Ingredient and nutrient composition of the experimental diets (as-is basis)

Ingredient	Pre-starter (g kg^{-1})	Starter (g kg^{-1})	Grower (g kg^{-1})	Finisher (g kg^{-1})
Corn	565.3	578.5	608.2	620.8
Soybean meal 47%	367.2	347.6	315.9	304.5
Limestone	13.7	12.1	11.3	11.5
Dicalcium phosphate	10.1	8.6	9.4	7.8
Soybean oil	29.8	42.2	43.4	45.9
Salt	5.1	4.5	4.6	4.3
Vitamin premix[1]	0.4	0.4	0.4	0.4
Mineral premix[2]	0.7	0.7	0.7	0.7
DL-methionine	3.7	3.1	3.1	2.4
L-lysine	1.9	0.7	1.2	0.6
L-threonine	1.7	1.2	1.4	0.7
Monensin 40%	0.3	0.3	0.3	0.3
Ronozyme NP	0.1	0.1	0.1	0.1
Nutrient profile				
AME (kcal/kg)	3,000	3,100	3,104	3,170
Crude protein (%)	22.00	21.00	19.80	19.20
Digestible lysine (%)	1.32	1.18	1.13	1.05
Digestible met + cys (%)	0.93	0.86	0.83	0.75
Digestible threonine (%)	0.86	0.78	0.76	0.67
Digestible tryptophan (%)	0.25	0.24	0.22	0.22
Calcium (%)	1.00	0.90	0.88	0.85
Total P (%)	0.57	0.53	0.53	0.50
Available P (%)	0.45	0.42	0.42	0.40
Sodium (%)	0.22	0.20	0.20	0.19
Chloride (%)	0.38	0.33	0.34	0.31
Potassium (%)	0.91	0.88	0.82	0.80

AME - apparent metabolizable energy.
[1] Supplied per kilogram of feed: vitamin A - 9,280 IU from retinyl acetate; vitamin D - 2,240 IU from cholecalciferol; vitamin E - 20.8 IU from DL-α-tocopheryl acetate; vitamin K - 2.4 mg from menadione bisulfite; thiamin - 2.4 mg from thiamine mononitrate; riboflavin - 7.2 mg; pyridoxine - 3.6 mg from pyridoxine hydrochloride; cobalamin - 0.016 mg from cyanocobalamin; pantothenic acid - 17.6 mg from D-pantothenic acid; niacin - 52.8 mg from nicotinic acid; folic acid - 0.96 mg; biotin - 0.08 mg from D-biotin.
[2] Supplied per kilogram of feed: Mn - 105 mg from $MnSO_4$; Zn - 70 mg from ZnO; Fe - 56 mg from $FeSO_4$; Cu - 10.5 mg from CuO; I - 0.84 mg from KI; Se - 0.3 mg from Na_2SeO_3.

for nitrogen (AMEn). For N analysis of excreta, HCl was added (prior to drying) to prevent nitrogen loss during the drying process (Ribeiro et al., 2001).

All data collected were processed using the statistical software package SAS (Statistical Analysis System, version 9.0). Analyses of variance were performed using the GLM procedure (General Linear Models). When the means of the GLM were significantly different, they were further compared by Tukey's test. Only data on the intestinal morphology of 0-d-old chicks were logarithm-transformed due to the high coefficient of variation.

Results and Discussion

The chicks hatched in the SS system exhibited the highest CL (Table 3), with a length approximately 1.06% and 2.56% higher than the males and females of the MS system, respectively. These results indicate that the incubation system influenced the CL (P<0.001) but did not affect the other characteristics. The difference in favor of the SS system can be explained because this system works with a varying incubation profile, meeting the embryo requirements as the incubation time progresses, whereas the MS system operates with average parameters of temperature and humidity due to the presence of eggs at different incubation times. Oviedo-Rondón et al. (2008) reported that embryos are sensitive to incubation conditions at any age of development but especially during the last four days of incubation, when most of the organs, including the skeletal system, are rapidly maturing. They concluded that high air temperatures and low oxygen concentrations in the machines significantly decreased the length of long bones of chickens (femur and tibia). French (1997) reported that when the eggs are maintained at a constant machine air temperature (MT), as in the MS incubator, the embryo temperature or the EST are below the MT in the first half of the incubation period and above the MT in the other half, which affects embryonic development. Molenaar et al. (2010b) indicated that a constant EST throughout the incubation period can only be achieved by the SS setters because the parameters can be adjusted to compensate for excess embryo heat production. Therefore, if we consider that the main difference between the systems is incubation temperature control, these results agree with those obtained by Lourens et al. (2005) and Molenaar et al. (2010a), who found a lower CL in birds subjected to higher EST (38.9 °C) compared with those incubated under normal EST (37.8 °C).

Table 3 - Body weight, relative yolk-sac weight, yolk-free body mass, relative intestine weight, and chicken length at hatching

Treatment	BW (g)	RYW (g/100 g)	YFBM (g)	RIW (g/100 g)	CL (cm)
Sex					
Females	44.56	10.76	39.76	5.30a	20.01
Males	44.86	11.20	39.83	4.91b	19.81
Incubator					
Multiple	44.83	11.21	39.80	4.96	19.73
Single	44.58	10.75	39.79	5.25	20.09
Interaction					
Female × Multiple	44.71	11.04	39.76	5.17	19.90b
Female × Single	44.41	10.49	39.76	5.43	20.11a
Male × Multiple	44.96	11.39	39.83	4.74	19.56c
Male × Single	44.76	11.00	39.82	5.07	20.06a
P-value					
Sex	0.152	0.172	0.731	0.030	<0.001
Incubator	0.233	0.121	0.960	0.101	<0.001
Interaction	0.822	0.804	0.984	0.832	0.010
SEM	0.15	0.23	0.14	0.12	0.04

a-c - means with different letters differ (P<0.05) based on Tukey's honestly significant difference test.
SEM - standard error of the mean; BW - body weight; RYW - relative yolk-sac weight; YFBM - yolk-free body mass; RIW - relative intestine weight; CL - chicken length.

Regarding BW and yolk-free body mass, no differences were observed between the incubation systems, confirming the results of Oviedo-Rondón et al. (2008), who reported that the BW and yolk sac absorption were not affected by incubation temperatures. By contrast, various experiments indicated that chicks hatched in incubation systems managed with high EST exhibited lower yolk-free body mass at hatching (Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2010a; Molenaar et al., 2011; Shim and Pesti, 2011). The differences between these results may be due to other factors in addition to temperature, such as the interaction with batches of eggs with different ages that were incubated inside the machine.

The incubation system did not influence the RIW, confirming the results obtained by Leksrisompong et al. (2007), who observed no differences in this variable when standard EST (38.2 ºC) and high EST (40 ºC) were used on incubation days 19 and 20. However, in this study, there was an effect of sex on the RIW (P<0.03), with females showing a higher RIW than males. This result can be explained by the fact that females hatch before males, giving females more time to develop their organs.

We observed an interaction between sex and incubation system for BW and WG (P<0.01) and FCR at 7 d of age (P<0.06). Multiple-stage females were lighter and showed the lowest WG and worst FCR compared with the other treatment (Table 4). This interaction continued during the 8 to 21 d phase (P<0.02, Table 4) for BW and WG, with MS females staying lighter, but the pattern was not maintained for FCR. In the period from 22 to 40 d, there was an effect

Table 4 - Performance of broiler chickens during the periods of 1 to 7, 8 to 21, 22 to 40, and 1 to 40 days of age

Treatment	1 to 7 d				8 to 21 d			22 to 40 d			1 to 40 d			
	BW	WG	FI	FCR	WG	FI	FCR	WG	FI	FCR	BW	WG	FI	FCR	AFCR
	----------g/bird----------			---g/g---	------g/bird-----		---g/g---	-----g/bird-----		---g/g---	----------g/bird----------			-----g/g-----	
Sex															
Female	196	153	168b	1.102	792	1,134b	1.431	1,612b	2,859b	1.774b	2,603b	2,558b	4,163b	1.627b	1.671b
Male	205	162	175a	1.081	918	1,294a	1.411	1,991a	3,335a	1.676a	3,116a	3,070a	4,810a	1.566a	1.525a
Incubator															
Multiple	199	156	170	1.091	853	1,205	1.415	1,764b	3,065	1.745	2,818b	2,772b	4,448	1.606	1.615b
Single	202	159	173	1.092	857	1,223	1.427	1,893a	3,129	1.706	2,902a	2,856a	4,525	1.587	1.581a
Interaction															
Female × Multiple	192c	149c	166	1.116b	779c	1,119	1.437	1,565	2,781	1.780	2,539	2,492	4,070	1.631	1.687
Female × Single	200b	157b	171	1.089ab	806b	1,149	1.425	1,660	2,936	1.769	2,667	2,624	4,256	1.623	1.655
Male × Multiple	206a	163a	174	1.066a	927a	1,290	1.392	1,963	3,349	1.710	3,096	3,052	4,825	1.580	1.543
Male × Single	204ab	161ab	175	1.096ab	909a	1,298	1.429	2,018	3,321	1.643	3,136	3,088	4,795	1.551	1.508
P-value															
Sex	<0.0001	0.0001	0.009	0.150	<0.0001	<0.0001	0.211	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	0.001	<0.0001
Incubator	0.112	0.111	0.162	0.920	0.622	0.271	0.452	0.012	0.270	0.11	0.022	0.023	0.271	0.261	0.052
Interaction	0.011	0.013	0.410	0.062	0.023	0.511	0.132	0.473	0.121	0.262	0.180	0.150	0.131	0.542	0.930
SEM	1.30	1.30	1.52	0.010	6.39	11.61	0.012	19.29	40.56	0.017	22.96	22.59	48.43	0.011	0.012

a-c - means with different letters differ (P<0.06) based on Tukey's honestly significant difference test.
SEM - standard error of the mean; BW - body weight; WG - weight gain; FI - feed intake; FCR - feed conversion ratio; AFCR - feed conversion rate adjusted to 3.00 kg.

of gender on all variables (P<0.01; Table 4), in which males were heavier, gained more weight, consumed more feed, and were more efficient in converting feed. In the same period, SS chickens gained more weight (P<0.01) regardless of sex; nevertheless, there were no differences in FI and FCR.

In the total period (1-40 d), no interaction between sex and incubation system was found. The results indicated that the incubation system affected BW and WG (P<0.02; Table 5) as well as AFCR (P<0.05; Table 5). Birds hatched in SS were heavier and had higher WG as well as better AFCR compared with those hatched in MS machines for both sexes. The WG was also influenced by gender (P<0.01), with males gaining more weight than females. Similarly, Silva et al. (2011) indicated that birds hatched in SS setters showed higher daily weight gain and better FCR in relation to those hatched in MS machines. Likewise, data from a study carried out by Oviedo-Rondón et al. (2009a) showed that females hatched in SS systems were heavier at 56 d of age than females hatched in MS (P<0.05), with no difference for males. The differences between the incubation systems in relation to BW and WG may be due to differences in EST and ventilation rates. The SS system has a different MT for each embryonic incubation phase in order to maintain a constant EST and has different ventilation rates during the incubation process, particularly regulating the CO_2 levels. Studies indicate that birds hatched in incubation systems that had higher CO_2 levels in the first half of the hatching process, as occurs in SS systems, had better performance at 28 d of age (De Smit et al., 2006). For FI and FCR (1 to 40 d), no differences due to incubation system were found. The FI was different in all periods for each sex (P<0.01); females consumed less feed than males, independent of the incubation system.

No differences in the morphology of the duodenum due to incubation system were observed (Table 5). The VH and CD of duodenum from 0-d-old chickens (Table 5) indicated a gender effect on VH, in which the females exhibited larger villi relative to males (P<0.01), without differences in CD. Once again, these results can be explained by the fact that females hatch before males, giving females more time to develop intestinal villi. However, the same did not happen with the development of the jejunal villi, which showed no differences due to sex. In this segment, chickens hatched in the SS system showed higher CD (P<0.04) with no differences for VH. The differences in the VH of the duodenum due to sex that were observed at 0 days of age were not maintained for the first week of life (Table 5). These results are likely due to the significantly higher feed intake by the males. Studies carried out by Potturi et al.

Table 5 - Intestinal morphology of duodenum and jejunum from chickens of 0 and 7 days of age

Treatment	0 days				7 days			
	Duodenum		Jejunum		Duodenum		Jejunum	
	VH (μm)	CD (μm)	VH (μm)	CD (μm)	VH (μm)	CD (μm)	VH (μm)	CD (μm)
Sex								
Females	518a	56.20	381	56.53	1,339	143.77	782	128.50a
Males	425b	53.34	407	53.94	1,343	148.20	746	112.23b
Incubator								
Multiple	454	54.27	398	52.34b	1,337	140.74	778	117.22
Single	481	55.27	391	58.13a	1,345	151.23	749	
Interaction								
Female × Multiple	499	56.49	383	54.83	1,290	135.68	778	122.47
Female × Single	521	55.92	380	58.23	1,388	151.87	785	134.53
Male × Multiple	408	52.04	412	49.84	1,384	145.80	779	111.97
Male × Single	442	54.63	402	58.03	1,302	150.60	712	112.48
P-value								
Sex	<0.010	0.181	0.312	0.521	0.940	0.540	0.462	<0.050
Incubator	0.320	0.691	0.782	0.040	0.882	0.152	0.541	0.412
Interaction	0.691	0.592	0.942	0.374	0.080	0.430	0.451	0.451
SEM	19.07	1.59	15.94	1.84	34.93	5.02	34.24	5.35

a-b - means with different letters differ (P<0.05) based on Tukey's honestly significant difference test.
SEM - standard error of the mean; VH - villus height; CD - crypt depth.

(2005) suggest that feed intake is one of the factors that most influence the proliferation of enterocytes and therefore the growth of the villi after hatching. At seven days of age, an influence of gender on the CD of the jejunum was observed (P<0.05; Table 5), and females had deeper crypts than males. No explanation was found in the literature for these results. In the other variables studied in this period, no effects due to incubation system and sex were observed.

Considering the metabolic responses (Table 6), we can state that during the first week of bird life, the metabolic rate was not influenced by incubation system. However, it was observed that females presented higher metabolization coefficients for gross energy and consequently higher AMEn values (P<0.05), without differences for coefficients of metabolization of dry matter or crude protein. These results agree with those obtained by Ten Doeschate et al. (1993), who reported that females showed 3% higher digestibility coefficients compared with males. Similarly, Hughes (2003) reported that the AME of wheat-based diets were influenced by sex, with females exhibiting higher AME (3,500 vs. 3,360 kcal/kg DM). On the other hand, some researchers have reported that sex has no effect on the AMEn (Ravindran et al., 2004) and AME values (Wallis and Balnave, 1984).

The differences between genders found in this experiment may be explained by possible differences in energy costs for repair and maintenance of the gut as well as differences in endogenous losses (Hughes, 2003). Because females hatch before males and the small intestine of birds is immature during the first week of life, in addition to the fact that females showed larger RIW and VH in the duodenum, it is possible that they are in a more advantageous position because there is more space to retain undigested feed and greater absorptive area. However, further research

Table 6 - Coefficients of metabolization of dry matter, crude protein, gross energy, and apparent metabolizable energy corrected for nitrogen of chickens from 5 to 7 days of age

Treatment	Coefficient of metabolization			
	CMDM ($g\ g^{-1}$)	CMCP ($g\ g^{-1}$)	CMGE ($g\ g^{-1}$)	AMEn ($kcal\ kg^{-1}$)
Sex				
Female	0.724	0.689	0.773a	2,960a
Male	0.712	0.673	0.758b	2,903b
Incubator				
Multiple	0.723	0.686	0.770	2,946
Single	0.712	0.676	0.604	2,914
Interaction				
Female × Multiple	0.727	0.688	0.776	2,971
Female × Single	0.722	0.690	0.770	2,949
Male × Multiple	0.720	0.684	0.765	2,925
Male × Single	0.702	0.661	0.751	2,878
P-value				
Sex	0.071	0.102	0.021	0.012
Incubator	0.121	0.301	0.122	0.123
Interaction	0.391	0.230	0.51	0.561
SEM	0.38	0.52	0.34	12.09

a-b - means with different letters differ (P<0.05) based on Tukey's honestly significant difference test.
SEM - standard error of the mean; CMDM - coefficient of metabolization of dry matter; CMCP - coefficient of metabolization of crude protein; CMGE - coefficient of metabolization of gross energy, AMEn - apparent metabolizable energy corrected for nitrogen.

is needed to evaluate the concentrations of enzymes in the small intestine of females and males to confirm the results obtained in this study.

Conclusions

The incubation conditions employed in the single-stage incubation system improve the chicken length and crypt depth of jejunum at hatching, resulting in an improvement in broiler performance (2.98%), especially in females (5.04%). However, unlike the incubation system, sex affects nutrient metabolization coefficients in the first week of the life of the bird.

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