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Effect of fasting or post-hatch diet's type on Intestinal morphology in broilers

S.A. Tabeidian[1*], A. Samie[2], J. Pourreza[2] and Gh. Sadeghi[3]
1. Department of animal science, Agriculture faculty, Islamic Azad University-Khorasgan Branch, Esfahan-Iran.
2. Esfahan University of Technology, Isfahan-Iran
3. University of Kurdistan, Sanadaj-Iran
* Corresponding Author: Department of Animal Science, Islamic Azad University, Khorasgan Branch, Esfahan, Iran. Tel: +983115354038 Fax: +983115354038
Email Address: tabeidian@yahoo.com

Abstract—**An experiment was carried out to evaluate the effects of fasting and early diet composition on broiler chicken's development. A totally of 540 one-day old male broiler chicken were used in this study. The treatments were Control (C), fasted for 24 h (24F), fasted for 48 h (48F), feeding a diet containing 15% egg powder for 24 h (24E) or 48 h (48E), feeding a diet containing 20% glucose syrup for 24 h (24G) or 48 h (48G), and feeding a diet containing 15% egg powder and 20% glucose syrup for 24 h (24EG) or 48 h (48EG). At 21 days of age, feeding G diet for 48 h resulted in longer duodenum and ileum than other treatments. In addition, longer (P<0.05) jejunum length was found in chicks who received the GE diet in the first 48 h. feeding E diet for 48 h did increase ileum weight significantly. At day 42, the 24G diet fed chick was found to have the highest duodenum and ileum weight. The results for morphological parameters show that feeding a diet containing egg powder and glucose syrup for 48 hours resulted in higher duodenum crypt depth (CD) in comparison to chicks fed with diet containing egg powder for 48 hours. No access to feed for 48 hours decreased duodenum villous height (VH) and increased (P<0.05) duodenum CD and subsequently decreased VH: CD ratio on day 7. Feeding a diet containing egg powder and glucose syrup for 24 hours resulted in longer VH and lower CD and subsequently higher VH: CD ratio in duodenum on day 7. On day 21, none of experimental treatments could affect the morphometric parameters of duodenum and ileum. Feeding EG diet for 48 h resulted in longer (P<0.05) ileum VH and higher VH: CD than chicks fasted for 24h on day 7. In conclusion, the present study showed that the diet composition affects chick development post-hatch and feeding a semi-moist diet with high protein and suitable energy levels containing egg powder and glucose syrup for 48 hours post-hatch is beneficial for post-hatch growth and considerable performance benefits than control.**

Keywords: fasting, early diet, broiler, Intestinal morphology

I. INTRODUCTION

In commercial operations, chicks hatch over a 48 hour period and are removed from the incubator only when the maximum number of the birds have completely cleared the shell [1, 2]. In some cases the chicks have to be sexed, vaccinated and etc. which may extend the off-feed time. Indeed, in some cases it normally takes 24-48 hours to deliver the chicks to grow out facility and offer the first feed and water to newly hatched chicks. Immediately after hatching the nutrient intake of chicks can greatly influence their subsequent performance characteristics. It has been shown that early access to feed and/or protein results in more rapid gastro-intestinal and muscular development in the immediate post hatch period [3], investment in the chick's immune system [4] and faster utilization of yolk suck [5].

Diet composition could interact with yolk suck utilization and different sources of energy and protein have variable impact on poultry [6], showing a need for more digestible nutrients. The post-hatch chicks have different physiological conditions in comparison with older chicks and this may impact on nutrient sources and nutritional requirements in first days of age. Suitable feed composition and optimal feed formulations for specifically the first day's post-hatch of broiler chickens are less known. The usual starter diets have complex components and more digestible and simple feed components such as glucose syrup or egg powder may be more suitable for first days post-hatch. Thus, this study was carried out to evaluate the effect of feed type and fasting over the first 48 hours immediately following hatch on gastrointestinal morphology of broiler chickens.

II. MATERIAL AND METHODS

A total of newly hatched 540 Ross 308 male broiler chicks were obtained from a commercial hatchery. The chicks were divided into seven groups corresponding with six treatments and one control group included in the study. The average of each group had approximately similar initial weight and weight distribution. The seven groups were identified based on their experimental diets: as: Control - feeding a corn- soybean meal based diet (C), fasted for 24 h (24F), fasted for 48 h (48F), feeding a diet containing 15% egg powder for 24 h (24E) or 48 h (48E), feeding a diet containing 20% glucose syrup for 24 h (24G) or 48 h (48G), and feeding diet containing 15% egg powder and 20% glucose syrup for 24 h (24EG) or 48 h (48EG). All experimental diets (Table 1) were semi-moist with a moisture content of 30%. After 24 or 48 hours all the chicks were fed with commercial starter (up to 21 d) and grower (22-42 d) diets. The diets (Table2) were formulated to meet nutrient requirements according to NRC (1994) [7].

Evaluation of Intestinal Morphology

At 24 h, 48h, 7 d and 21 d one bird from each replicate was killed to collect the small intestine. The intestines were removed and segments of duodenum (from the pylorus to the distal point of entry of the bile ducts), jejunum (from entry of the bile ducts to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileocecal junction) and then were gently flushed twice with physiological saline solution to remove the intestinal content. For morphological analysis, approximately 5 cm of the middle portion of the duodenum, jejunum and ileum was excised and fixed in 10% formalin. Six cross sections of 70% ethanol preserved segments for each sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures [8]. Villus Height (VH) and Crypt Depth (CD) were measured using the Image-Pro Plus as described in details by [9] and VH: CD Ratio (VCR) was calculated.

Statistical Analysis

Data were subjected to analysis of variance procedure using the general linear model procedure of SAS (2001) [10]. Statically different means were separated using Duncan's Multiple Range Test ($P<0.05$).

III. Results & Discussion

The result in this study shows that effects of fasting or type of post-hatch diet on weight and length of small intestine are tabulated in Table 3. At 21 day of age, fasting for 24 or 48 hrs had no significant effect on small intestine lengths, however, small intestine lengths were significantly higher in chicks receiving either the G or EG diet for 48 h compared with chicks receiving the EG diet for 24 h. Feeding G diet for 48 h resulted in longer duodenum and ileum than other treatments. In addition, longer ($P<0.05$) jejunum length was found in chicks who received the GE diet in the first 48 h. These findings are consistent with other researchers [4] that who showed that chicks with access to a semi-moist diet for 48 hrs showed significantly longer intestines compared with both the non-fed chicks and the chicks fed dry feed. It has been suggested that the yolk sac provides 50% of the chick's energy on the first day after hatch and is negligible by the fourth days being only about 2% [11]. During the post-hatch period, the small intestine develops at a faster rate than the body mass [12, 13] Hence, providing a complete diet with high energy content (G 48 diet) or high protein content and suitable protein source such as egg powder with a good profile of amino acids may be explain the better development of the GIT in chicks.

Duodenum and jejunum weight was not influenced by the experimental diets. However, feeding E diet for 48 h did increase ileum weight significantly ($P<0.05$).

At day 42, the 24G diet fed chick was found to have the highest duodenum and ileum weight. Small intestine, duodenum, jejunum and ileum lengths did not differ across treatments. This suggests that GIT development mainly in first weeks of bird's age in the last weeks the GIT growth to body growth is less and dose not affect by diet.

The results for morphological parameters (Tables 4, 5 and 6) show that feed deprivation or feeding different feed type had no effect on duodenum, jejunum and ileum villous height (VH), crypt depth (CD) and VH: CD ratio in chicks during the first 24 hours after hatch. However, feeding a semi-moist diet containing egg powder and glucose syrup for 48 hours resulted in higher duodenum CD in comparison to chicks fed with diet containing egg powder for 48 hours. No access to feed for 48 hours decreased duodenum VH and increased ($P<0.05$) duodenum CD and subsequently decreased VH: CD ratio on day 7. Feeding a semi-solid diet containing egg powder and glucose syrup for 24 hours resulted in longer VH and lower CD and subsequently higher VH: CD ratio in duodenum on day 7. On day 21, none of experimental treatments could affect the morphometric parameters of duodenum and ileum.

Feeding EG diet for 24 h resulted in longer ($P<0.05$) jejunum VH and higher VH: CD than other experimental groups on day 7. Feeding the early diet containing egg powder for 48 h increased the CD in comparison to chicks that fed with EG diet for 48 h.

Feeding EG diet for 48 h resulted in longer ($P<0.05$) ileum VH and higher VH: CD than chicks fasted for 24h on day 7.

Although the digestive capacity begins to develop a few days before hatch, most of the development occurs post-hatch when the neonatal chick begins consuming feed [14]. The intestinal crypts are clearly defined several days post-hatch, increasing in both cell numbers and size [15, 16]. In agreement to this finding, previous studies have shown that feeding immediately post-hatch accelerates the morphological development of the small intestine [17]. While, delay access to first feed for 24-48 hours post-hatch have decreased villi length [18], decreased crypt size and crypts per villi, and decreased enterocytes migration rate [19].

IV. Conclusion

This study indicates that the diet composition affects chick development post-hatch. The results of this study showed that feeding a semi-moist diet with high protein and suitable energy levels containing egg powder and glucose syrup for 48 hours post-hatch has effect beneficial on development of intestinal morphology.

References

[1] Noy, Y. and Sklan, D. Yolk utilization in the newly hatched poult. British Poultry Science, 1998a; 39, 446-451.

[2] Batal, A. B. and Parsons, C. M. Effects of fasting versus feeding oasis after hatching on nutrient utilization in chicks. Poultry Science, 2002; 81, 853–859.

[3] Noy, Y. and Sklan, D. Yolk and exogenous feed utilization in the posthatch chick. Poultry Science, 2001; 80, 1490–1495.

[4] Brink, M.V.D. and Rhee, W.V. Semi-moist diets to improve day old chick's performance. World Poultry, 2007; 23, 17-19.

[5] Noy, Y., Uni, Z. and Sklan, D. Routes of yolk utilization in the newly hatched chicks. British Poultry Science, 1996; 37, 987-996.

[6] Lilburn, M.S. Practical aspects of early nutrition for poultry. Journal of Applied Poultry Research, 1998; 7, 420- 424.

[7] National Research Council. Nutrient Requirements of Poultry. 9th Rev. ed. National Academy Press, 1994; (Washington DC. USA).

[8] Uni,Z., Noy, Y. and Sklan, D. Posthatch changes in morphology and function of the small intestines in heavy and light strain chicks. Poultry Science, 1995; 74, 1622–1629.

[9] Touchette, K. J., Carroll, J. A., Allee, G. L., Matteri, R. L., Dyer, C. J., Beausang, L. A. and Zannelli, M. E. Effect of spray-dried plasma and lipopolysaccharide exposure on weaned pigs: I. Effects on the immune axis of weaned pigs. Journal of Animal Science, 2002; 80, 494–501.

[10] SAS Institute Inc. SAS STAT User's Guide Release 8.2. 2001; SAS Institute Inc., Cary, NC.

[11] Nitsan, Z., Ben-Avraham, G., Zoref, Z. and Nir, I. Growth and development of the digestive organs and some enzymes in the broiler chick after hatching. British Poultry Science, 1991; 32, 515-523.

[12] Sell, J. L., Angel, C. R., Piquer, F. J., Mallarino, and al-Batshan, H. A. Developmental patterns of selected characteristics of the gastrointestinal tract of young turkeys. Poultry Science, 1991; 70, 1200-1205.

[13] Sklan, D. Development of the digestive tract of poultry. World's Poultry Science Journal, 2001; 57, 415-427.

[14] Ferket, P., Uni, Z. Early feeding – in ovo feeding enhances of early gut development and digestive capacity of poultry. 2009; http://iba.zwans.com/fullpapaer/10636.pdf

[15] Geyra, A., Uni, Z. and Sklan, D. Enterocyte dynamics and mucosal development in the posthatch chick. Poultry Science, 2001a; 80, 776-782.

[16] Uni, Z., Geyra, A., Ben-Hur, H. and Sklan, D. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. British Poultry Science, 2000; 41, 544-551.

[17] Noy, Y. and Sklan, D. Are metabolic responses affected by early nutrition? Journal of Applied Poultry Research. 1998b; 7, 437-451.

[18] Yamauchi, K. E., Kamisoyama, H. and Isshiki, Y. Effects of fasting and refeeding on structure of the intestinal villi and epithelial cells in white leghorn hens. British Poultry Science, 1996; 37, 909-921.

[19] Geyra, A., Uni, Z. and Sklan, D. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. British Journal of Nutrition, 2001b; 86, 53-61.

TABLE 1:.COMPOSITION AND CALCULATED NUTRIENT CONTENT OF EXPERIMENTAL DIETS THAT FED FOR 24 OR 48 HOURS POST-HATCH.

Feed ingredients	Control	E	G	EG
Corn	550.5	526.0	438.0	230.0
Soybean meal	349.5	296.5	267.5	378.0
Fish meal	66.0	0.0	60.0	0.0
Egg powder	0.0	150.0	0.0	150.0
Glucose Syrup	0.0	0.0	200.0	200.0
Mono-calcium phosphate	10.0	11.5	9.0	11.5
CaCo3	9.0	10.5	8.0	10.5
Soybean oil	10.0	0.0	0.0	0.0
NaCl	0.0	0.0	2.5	3.0
Mineral premix	2.5	2.5	2.5	2.5
Vitamin premix	2.5	2.5	2.5	2.5
Calculated nutrients				
Metabolizable energy	2900	2700	3050	2700
Crude protein (g/kg)	230	240	183	250
Met (g/kg)	7.7		6.1	
Lys (g/kg)	14.4		11.5	
Ca (g/kg)	9.4	7.5	8.5	8.0
AP (g/kg)	5.2	3.5	4.5	3.5

TABLE 2: COMPOSITION AND CALCULATED NUTRIENT CONTENT OF STARTER AND GROWER DIETS

Feed ingredients	Starter (up to 21d)	Grower (22 to 42 d)
Corn	535	583
Soybean meal	357	320
Fish meal	50	30
Mono-calcium phosphate	5.7	5.0
CaCo3	12.5	14.1
NaCl	3.5	3.0
Soybean oil	30	40
DL- methionin	0.8	0.1
Mineral premix[1]	2.5	2.5
Vitamin premix[2]	2.5	2.5
Calculated nutrients		

Metabolizable energy (MJ/kg)	12.55	12.97
Crude protein (g/kg)	215.6	193.7
Met (g/kg)	4.4	3.4
Met+Cys (g/kg)	8.4	7.0
Lys (g/kg)	13.2	11.5
Ca (g/kg)	9.3	8.7
AP[3] (g/kg)	4.2	3.4

[1]supplemented (mg kg^{-1} of diet): Mn, 1200; Fe, 60; Zn, 120; Cu, 12; I, 1.2; Se, 0.24

[2] supplemented (mg or IU kg^{-1} of diet): Vit. A, 10800 IU; D_3, 2400 IU; E, 21.6 IU; K_3, 2.4 IU;B_1, 2.16; B_2, 7.9; B_3, 12; B_5, 3.6; B_9, 1.2; B_{12}, 0.015; Biotin, 0.12; choline chloride, 600; and adequate anti oxidant

[3]AP: available phosphorus

TABLE 3: EFFECT OF FASTING A OR TYPE OF POST-HATCH DIET ON WEIGHT (% OF BODY WEIGHT) AND LENGTH (CM) OF SMALL INTESTINE AT 21 AND 42 DAYS OF CHICK'S LIFE.

	21d								42d							
	SIW	DW	JW	IW	SIL	DL	JL	IL	SIW	DW	JW	IW	SIL	DL	JL	IL
Control		1.80	3.45^{b}	2.61^{b}	109.50^{ab}	2.00^{b}	4.75^{ab}	4.75^{ab}		0.92^{ab}	1.96	1.89^{b}	1.87.50	2.9.50	7.6.50^{ab}	8.1.50
F24*		2.14	4.81^{a}	4.25^{ab}	1.18.00^{ab}	2.00^{b}	5.0.00^{ab}	4.6.00^{ab}		0.90^{ab}	1.90	1.88^{b}	1.96.25	3.2.50	7.9.75^{ab}	8.4.00
EG24		1.76	4.33^{ab}	3.39^{ab}	9.6.25^{b}	2.3.50^{b}	3.6.25^{b}	3.6.50^{b}		0.81^{ab}	1.97	1.86^{b}	1.96.75	2.9.75	8.5.50^{a}	8.1.50
E24		1.77	4.33^{ab}	3.64^{ab}	1.15.00^{ab}	2.1.50^{b}	4.6.00^{ab}	4.7.50^{ab}		0.80^{b}	1.60	1.61^{b}	1.86.50	2.9.75	7.7.75^{ab}	7.9.00
G24		2.26	4.03^{ab}	4.06^{ab}	1.01.25^{ab}	2.0.50^{b}	3.7.50^{ab}	4.3.25^{ab}		1.00^{a}	2.20	2.49^{a}	1.99.25	3.2.25	8.1.50^{ab}	8.5.50
F48		2.01	4.64^{ab}	3.98^{ab}	1.15.75^{ab}	2.1.00^{b}	4.6.00^{ab}	4.8.75^{ab}		0.87^{ab}	1.73	1.74^{b}	1.83.50	3.2.50	7.4.00^{ab}	7.7.00
EG48		1.86	3.98^{ab}	4.14^{ab}	1.27.00^{ab}	2.2.50^{b}	5.3.50^{a}	5.1.00^{ab}		0.86^{ab}	1.77	1.63^{b}	1.87.00	3.1.00	7.7.75^{ab}	7.8.25
E48		1.88	4.52^{ab}	4.93^{a}	1.16.75^{ab}	1.9.75^{b}	4.8.00^{ab}	4.9.00^{ab}		0.98^{ab}	1.76	1.86^{b}	1.84.25	3.2.50	7.0.00^{b}	8.1.75
G48		2.31	4.11^{ab}	3.85^{ab}	1.28.00^{a}	2.8.50^{a}	4.4.50^{ab}	5.5.00^{a}		0.90^{ab}	1.77	1.54^{b}	1.83.25	3.2.00	7.3.75^{ab}	7.7.50
SEM		0.069	0.127	0.210	3.2320	0.660	1.699	1.665		0.020	0.073	0.073	2.3994	0.525	1.433	1.103

*F24: fasted for 24 h ; EG24; diet containing 15% egg powder and 20% glucose syrup that fed for 24 h ; E24: diet containing 15% egg powder that fed for 24 h; G24: diet containing 20% glucose syrup that for 24 h; F48: fasted for 48 h ; EG48: diet containing 15% egg powder and 20% glucose syrup that fed for 48 h; E48: diet containing 15% egg powder that fed for 48 h; and G48: diet containing 20% glucose syrup that fed for 48 h.

SEM: Standard error of means.

TABLE 4 .EFFECTS OF FASTING OR TYPE OF POST-HATCH DIET ON MORPHOLOGICAL PARAMETERS OF DUODENUM IN BROILER CHICKENS

	24 hours			48 hours			7 day			21 day		
	VH	CD	VH/CD	VH	CD	VH/CD	VH	CD	VH/CD	VH	CD	VH/CD
Control	300	65	5.49	565	75ab	7.53b	1049ab	130b	8.07abc	1585	190	8.34
F24*	620	50	12.44	-	-	-	1029ab	165ab	6.31bc	1690	265	6.57
EG24	695	90	9.18	-	-	-	1243a	120b	11.04a	1720	215	8.07
E24	895	105	9.25	-	-	-	1180ab	110b	10.80ab	1650	235	7.38
G24	625	45	14.12	-	-	-	1114ab	145b	7.93abc	1905	200	9.54
F48	-	-	-	6	80	7.9	99	21	4.7	1	2	7.5

						15	ab	0b		7b	0a	0c		615	15	2	
EG 48		-	-	-		8 60	95 a	9.0 8b		10 03b	11 0b	9.1 1abc		1 525	1 90	8.0 5	
E48		-	-	-		7 85	55 b	14. 36a		11 23ab	12 0b	9.7 0ab		1 700	2 20	7.7 1	
G48		-	-	-		4 80	65 ab	6.8 2b		10 64ab	12 0b	8.8 6abc		1 680	1 55	11. 74	
SE M		7 0.2	1 1.4	1.3 55		6 1.4	5. 4	1.0 10		24. 3	8. 6	0.5 64		3 8.4	1 0.7	0.5 23	

*F24: fasted for 24 h ; EG24; diet containing 15% egg powder and 20% glucose syrup that fed for 24 h ; E24: diet containing 15% egg powder that fed for 24 h; G24: diet containing 20% glucose syrup that for 24 h; F48: fasted for 48 h ; EG48: diet containing 15% egg powder and 20% glucose syrup that fed for 48 h; E48: diet containing 15% egg powder that fed for 48 h; and G48: diet containing 20% glucose syrup that fed for 48 h. SEM: Standard error of means.

TABLE5. EFFECTS OF FASTING OR TYPE OF POST-HATCH DIET ON MORPHOLOGICAL PARAMETERS OF ILEUM IN BROILER CHICKENS

		24 hours				48 hours				7 day				21 day		
		VH	CD	VH /CD		VH	CD	VH /CD		VH	CD	VH /CD		VH	CD	VH /CD
Control		220	35	6.28		285	55ab	5.20		367 bcd	80	4.59b		525	165	3.67
F24*		280	45	6.80		-	-	-		527 b	70	8.19ab		490	145	3.48
EG24		295	55	5.49		-	-	-		367 bcd	75	4.98b		620	170	3.62
E24		315	50	6.09		-	-	-		473 bc	110	4.30b		560	120	4.63
G24		410	45	9.12		-	-	-		281 d	75	3.80b		795	140	5.57
F48		-	-	-		230	45b	5.11		312 cd	75	4.55b		445	120	3.71
EG48		-	-	-		325	60ab	5.42		106 9a	110	10.56a		690	145	4.93
E48		-	-	-		290	45b	6.37		465 bc	95	4.88b		700	100	6.98
G48		-	-	-		290	75a	4.10		393 bcd	75	5.24b		665	90	7.67
SEM		27.6	3.7	0.560		21.4	4.5	0.405		55.4	5.4	0.612		45.5	10.4	0.458

*F24: fasted for 24 h ; EG24; diet containing 15% egg powder and 20% glucose syrup that fed for 24 h ; E24: diet containing 15% egg powder that fed for 24 h; G24: diet containing 20% glucose syrup that for 24 h; F48: fasted for 48 h ; EG48: diet containing 15% egg powder and 20% glucose syrup that fed for 48 h; E48: diet containing 15% egg powder that fed for 48 h; and G48: diet containing 20% glucose syrup that fed for 48 h.

SEM: Standard error of means.

TABLE 6: EFFECTS OF FASTING OR TYPE OF POST-HATCH DIET ON MORPHOLOGICAL PARAMETERS OF JEJUNUM IN BROILER CHICKENS

		24 hours				48 hours				7 day				21 day		
		VH	CD	VH/CD		VH	CD	VH/CD		VH	CD	VH/CD		VH	CD	VH/CD
Control		385	40	9.61		315	65b	4.87		321 f	90ab	3.86b		925ab	165	5.89
F24*		420	60	7.04		-	-	-		355 ef	110ab	3.22b		1075ab	145	9.03
EG24		305	50	6.07		-	-	-		913 a	110ab	8.71a		1025ab	170	6.12
E24		400	55	7.44		-	-	-		624 bc	100ab	6.30ab		900ab	150	6.26
G24		335	45	7.76		-	-	-		546 bcd	110ab	5.49ab		1150a	0.175	6.52
F48		-	-	-		3	5	7.0		502	1	5.2		95	1	6.1

8						50	5b	2		bcde	00ab	8ab		5ab	60	1		
EG48		-	-	-		455	80b	5.84		462def	80ab	6.22ab		665b	175	3.74		
E48		-	-	-		500	175a	2.94		639b	120a	5.35ab		810ab	160	5.05		
G48		-	-	-		275	50b	5.42		472cdef	50b	6.26ab		975ab	200	4.54		
SEM		19.4	2.3	0.575		65.2	15.6	0.688		41.8	6.8	0.455		45.4	9.0	2.831		

*F24: fasted for 24 h ; EG24; diet containing 15% egg powder and 20% glucose syrup that fed for 24 h ; E24: diet containing 15% egg powder that fed for 24 h; G24: diet containing 20% glucose syrup that for 24 h; F48: fasted for 48 h ; EG: diet containing 15% egg powder and 20% glucose syrup that fed for 48 h; E48: diet containing 15% egg powder that fed for 48 h; and G48: diet containing 20% glucose syrup that fed for 48 h.

SEM: Standard error of means.