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Original Research Article

Changes in Yolk Absorption, Embryo Development Patterns and Chick Quality in Small and Large Eggs Obtained from Broiler Breeder Flocks at Different Ages

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ABSTRACT

The aim of the present study was to determine the effects of broiler breeder age and egg weight on yolk absorption, embryo development and chick quality in broilers. A total of 3000 hatching eggs were obtained from two different Ross 308 broiler breeder flocks at 36 and 52 weeks of ages. The eggs were classified into two weight categories as small (S: 58-63 g) and large (L: 64-69 g) eggs. To determine the yolk absorption and embryo development during incubation period, yolk absorption, embryo body weight, yolk free body weight and length, shank length were measured on days 14 and 18 of incubation. At hatch, chicks were sampled for determination of chick body weight and length, residual yolk weight and, yolk-free body weight, body and shank length. Yolk absortion and embryo development during incubation period were affected by both breeder age and egg weight. On day 18 of incubation, a higher yolk absorption and relative yolk absorption were observed in embryos from 52 weeks of age flock (9.2g and 43.0%, respectively) compared to other ones from 36 weeks of age (7.8 g and 39.2%, respectively, P<0.01). A lower relative yolk absorption with a value of 39.3% was observed for embryos obtained from large eggs (P<0.01). The embryo body weight and length were found to be higher for embryos obtained from large eggs from 52 weeks of age flock (32.5 g and 17.3 cm, respectively, P<0.01). At hatch, a lower chick body weight with a shorter body length were observed for chicks from 36 weeks of age flock (43.0 g and 20.2 cm, respectively) compared to the chicks obtained from 52 weeks of age (45.1 g and 20.7 cm, respectively, P<0.01). Also, a higher yolk absorption with a heavier chick body weight and length were found for chicks obtained from large eggs compared to small eggs (P<0.01). In conclusion, yolk absorption and embryo development during incubation and subsequently chick quality at hatch were affected by both breeder age and egg weight in broilers.

Keywords: Breeder age, Egg weight, Yolk absorption, Embryo development, Chick quality

INTRODUCTION

The developing embryo uses the contents of the egg for body growth and organ development during incubation period (Meijerhof, 2009; Ulmer – Franco *et al.*, 2010; Yadgary *et al.*, 2010). The egg yolk is the main energy source for tissue growth, organ development and maintenance during embryonic development (Noble and Cocchi, 1990; Speake *et al.*, 1998). On day 19 of incubation, embryo begins to absorb yolk sac into its abdominal cavity (Mikec *et al.*, 2006) and it comprises approximately 15-25% of the chick's body weight at hatch (Chamblee *et al.*, 1992; Murakami *et al.*, 1992; Noy *et al.*, 1996; Şahan *et al.*, 2014). This residual yolk sac provides required energy and nutrients to the newly hatched chick during the first few days of post-hatch period (Noble and Ogunyemi, 1989; Noy and Sklan, 2001; Uni *et al.*, 2003; Mikec *et al.*, 2006).

During incubation, embryo development is determined by embryo body weight, yolk free body weight, body and shank length (Hill, 2001; Molenaar *et al.*, 2008). The absorption and utilization of nutrients from the yolk sac by the embryo are affected by some factors, the most important factors are breeder age and egg weight (Gous, 2010; Yadgary *et al.*, 2010; Nangsuay etal., 2011; Şahan *et al.*, 2014). Nangsuay *et al.* (2011) found that yolk absorption (certain amount and relative value) of embryos and chicks obtained from old flock (53 weeks of age) on day 18 of incubation and also at hatch were higher than that of the young flock (29 weeks of age). On the other hand, egg weight also affects embryo development due to yolk absorption. It is known that yolk sac utilisation might be insufficient for optimum embryo development in smaller eggs compared to large ones (Gous, 2010).

At the end of the incubation period, embryo development has a crucial effect for chick quality at hatch. Breeder age and egg weight affect embryo development and subsequently chick weight, yolk-free body weight, and chick length at hatch (Suarez *et al.*, 1997; Hill, 2001; O'Dea *et al.*, 2004; Tona *et al.*, 2004; İpek and Sözcü, 2015). Recently, one-day old chick quality has crucial importance for success and productivity of hatcheries and also broiler producers (İpek and Sözcü, 2013). In practice, chick weight and chick length are used to predict the chick quality, because there is a critical relationship between the day old chick quality and posthatch broiler performance (Tona *et al.*, 2003; Meijerhof, 2009). On the other hand, chick weight at hatch includes both absolute chick body weight and also residual yolk sac weight. Therefore, yolk free body weight is more reliable parameter to predict how much of egg content converted into chick growth (Meijerhof, 2009).

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The objective of this study was to investigate the effects of broiler breeder age (young and old) and egg weight (small and large) on yolk sac absorption and embryo development during incubation period (on days 14 and 18 of embryonic development), and chick quality parameters at hatch.

MATERIALS AND METHODS

All procedures of this study for the care and use of animals were in accordance with the laws and regulations of Turkey and were approved by the Ethical Committee of the Uludağ University (License number 2012-07/01).

A total of 3000 hatching eggs were obtained from commercial Ross 308 broiler breeder flocks at 36 wk (Y; young flock) and 52 wk (O; old flock) of age. The breeder flocks received a broiler breeder diet with 2750 kcal ME/kg and 14.50% CP. The flocks were kept under the same management conditions according to the breeding company's recommendations (Anonymous, 2011). Eggs were classified into two weight groups as small group (S; 58-63 g) and large group (L; 64-69 g) in two breeder age groups. Therefore, the experimental groups were designated as; young flock-small eggs (Y x S), young flock-large eggs (Y x L), old flock-small eggs (O x S), old flock-large eggs (O x L).

Before setting process, a total of 100 eggs (n=25 eggs per egg weight/breeder age) were randomly selected to determine the initial yolk weight. Eggs were weighed and then broken for yolk weight measurement. Albumen and yolk were carefully seperated from egg content and yolk was weighed with ±0.1 g precision to determine initial yolk weight. Yolk rate (%) was calculated with the formula (Kontecka *et al.*, 2012).

Yolk rate (%) = [Yolk weight (g) / Egg weight (g)] x 100

The eggs were stored at 18ºC and 65% RH for 3 days and were then warmed to room temperature (21ºC) for 8 h before setting. The eggs were weighed with ±0.1 precision one by one and numbered before incubation. Eggs were incubated in a commercial hatchery in the same incubator (87400 eggs capacity, multi-stage setters-Fix shelf Chickmaster) at 37.5°C and a relative humidity of 55-60% during the first 18 days of incubation. A total of twenty incubator trays (n=5 trays/breeder age/egg weight) with 150 eggs were randomly placed in the incubator. On day 18 of the incubation, eggs were transferred to the hatcher (87400 eggs capacity, hatchers-Fix shelf Chickmaster). The hatcher was operated at 36.8ºC and 60% relative humidity.

A total of 100 eggs from each experimental groups (n= 25 eggs/ egg weight/breeder age) were randomly sampled on days 14 and 18 of incubation period. The eggs were opened and embryos were killed by cervical dislocation. The embryos and yolk sac were carefully separated from each other and then excessive embryonic fluid of embryo was dried off with absorbent paper. The embryos were measured for embryo body weight, yolk sac weight and yolk-free body weight to calculate relative embryo and yolk absorption (Willemsen *et al.* 2010). Embryo length was measured from the tip of the beak to the tip of the middle toe by placing the embryo face down on a flat surface and straightening the right leg (Hill 2001, Nangsuay *et al.* 2011). Shank length was mesured from right knee joint to the tip of the middle toe (Willemsen *et al.* 2008). The yolk absorption was calculated with the mean initial yolk weight follows as (Nangsuay *et al.* 2011):

Yolk absorption (g) = initial yolk weight – yolk sac or residual yolk weight (g)
Relative yolk absorption (%) = (yolk absorption / initial yolk weight) x 100
Relative embryo weight (%) = (embryo weight / initial egg weight) x 100
Yolk-free body weight (g) = embryo weight (g) - yolk absorption (g)
Relative yolk free-body weight (%) = (yolk-free body weight / initial egg weight) x 100

On the hatching day, after completing the hatching process the chicks were pulled out according to standard hatchery procedures. At hatch, a sample of 5 chicks per hatching basket (25 chicks/breeder age/egg weight) were killed by cervical dislocation to determine chick body weight and length, residual yolk sac weight and, yolk-free body weight and shank length. Chick body and shank length were measured in the same way as the embryo (Nangsuay *et al.* 2011).

Relative chick weight (%) = (chick weight / initial egg weight) x 100
Yolk-free body weight (g) = chick weight (g) – residual yolk sac weight (g)
Relative yolk-free body weight (%) = (yolk-free body weight / initial egg weight) x 100
All data were analyzed using the General Linear Model with Minitab 16 (Minitab, 2010). The statistical model for the measured parameters was used as follows:
$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$
Y_{ijk}: dependent variable;
μ: overall mean;

Ai: effect of breeder age (i = 36 or 52 wk);
Bj: effect of egg weight (j = small or large);
ABij: interaction between breeder age and egg size;
eijk: error term.

Data given as percentages was analysed by transforming to an arcsine format, and the actual means were given in results. Significant differences among treatment groups were classified with Tukey test. The probability level was set at $P < 0.05$.

RESULTS AND DISCUSSION

The differences in yolk absorption and embryo development pattern and chick quality were measured in small and large eggs obtained from 36 and 52 wk of age broiler breeders. The mean egg weight was found to be 62.5 g and 63.1 g in eggs from breeders of 36 and 52 wk of age, and 60.5 g and 65.1 g in small and large eggs, respectively (P<0.01). Yolk weight and yolk rate were found to be 19.9 g and 31.8%, for eggs from young flock, 21.4 g and 33.9% in eggs from old flock, respectively. On the other hand, the yolk weight and yolk rate were determined as 20.2 g and 33.3% in small eggs, 21.1 g and 32.4% in large eggs, respectively (P<0.01).

The effects of broiler breeder age and egg weight on yolk absorption (g and %) during incubation and at hatch are shown on Table 1. During incubation period, it was found that breeder age affected yolk absorption (g and %) except day 14 of incubation. On day 18 of incubation, yolk absorption and relative yolk absorption were found to be higher in embryos obtained from the old flock compared the other ones obtained from young flock (9.2 g vs. 7.8 g; 43.0% vs. 39.2%; P<0.01). Similar difference was observed for yolk absorption at hatch. A higher yolk absorption was observed in embryos obtained from old fock than other ones from young flock (16.9 g vs. 15.9 g, P<0.01), whereas relative yolk absorption was found to be similar in two breeder age groups. It is known that broiler breeder age affects yolk absorption and subsequently nutrient utilisation and also embryo development (Latour *et al.*, 2000; Yadgary *et al.*, 2010; Nangsuay *et al.*, 2011, 2013; Şahan *et al.*, 2014). In this study, observed differences for yolk absorption is consistent with results of other studies performed by Sklan *et al.* (2003), Hamidu *et al.* (2007), Nangsuay *et al.* (2011), Şahan *et al.* (2014).

Table 1. The effects of breeder age and egg weight on yolk absorption (g) and relative yolk absorption (%) during incubation and at hatch.

Main effects	Days of incubation						
	0[1]	14		18		At hatch	
		YA	RYA	YA	RYA	YA	RYA
BA							
Y	19.9 ± 0.12 [b]	3.8 ± 0.19	19.1 ± 0.93	7.8 ± 0.20 [b]	39.2 ± 1.08 [b]	15.9 ± 0.13 [b]	79.9 ± 0.76
O	21.4 ± 0.12 [a]	4.1 ± 0.19	19.2 ± 0.93	9.2 ± 0.20 [a]	43.0 ± 1.08 [a]	16.9 ± 0.13 [a]	79.0 ± 0.76
EW							
S	20.2 ± 0.12 [b]	4.2 ± 0.19 [a]	20.8 ± 0.93 [a]	8.7 ± 0.20	43.0 ± 1.08 [a]	16.2 ± 0.13 [b]	80.2 ± 0.76 [a]
L	21.1 ± 0.12 [a]	3.7 ± 0.19 [b]	17.5 ± 0.93 [b]	8.3 ± 0.20	39.3 ± 1.08 [b]	16.6 ± 0.13 [a]	78.7 ± 0.76 [b]
BA x EW							
Y x S	19.5 ± 0.17	4.0 ± 0.28	20.5 ± 1.32	8.1 ± 0.29	41.5 ± 1.53	15.8 ± 0.19	81.0 ± 1.08
Y x L	20.3 ± 0.17	3.6 ± 0.28	17.7 ± 1.32	7.5 ± 0.29	36.9 ± 1.53	16.0 ± 0.19	78.8 ± 1.08
O x S	20.9 ± 0.17	4.4 ± 0.28	21.1 ± 1.32	9.3 ± 0.29	44.5 ± 1.53	16.6 ± 0.19	79.4 ± 1.08
O x L	21.9 ± 0.17	3.8 ± 0.28	17.4 ± 1.32	9.1 ± 0.29	41.6 ± 1.53	17.2 ± 0.19	78.5 ± 1.08
P-Value							
BA	<0.001	0.08	0.83	<0.001	<0.001	<0.001	0.13
EW	<0.001	0.02	<0.001	0.09	<0.001	0.01	0.01
BA x EW	0.43	0.52	0.48	0.42	0.59	0.44	0.78

[a,b] Means in column that possess different superscripts differ significantly
[1] It shows initial yolk weight.
YA: yolk absorption (g), RYA: relative yolk absorption (%)
BA: breeder age, EW: egg weight
Y: young (36 weeks), O: old (52 weeks), S: small (58-63 g), L: large (64-69 g)

In this study, egg weight affected yolk absorption and relative yolk absorption of embryos during incubation period and at hatch (P<0.01). On day 14 of incubation, yolk absorption and relative yolk absorption were interestingly found to be higher with a value of 4.2 g and 20.8% in embryos of small eggs compared to embryos from the large eggs (3.7 g and 17.5%, respectively, P<0.01). On day 18 of incubation, yolk absorption was similar in two egg weight groups, whereas a higher relative yolk absorption was observed in embryos obtained from small eggs compared to other (43.0% vs. 39.3%, P<0.01). Embryos from large eggs had a higher yolk absorption but a lower relative yolk absorption than the embryos from small eggs (P<0.01). The findings are supported by previous results explained by Ulmer – Franco *et al.* (2010), Nangsuay *et al.* (2011) who reported that as egg weight increased, yolk absorption showed an increment.

The effects of breeder age and egg weight on embryo development during incubation period are shown on Table 2. On day 14 of incubation, significant breeder age x egg weight interactions were observed embryo development parameters, except embryo body length. Embryos obtained from large eggs of old flock had a higher body weight with a value of 12.2 g, compared to other groups (P<0.01). Relative embryo weight was found to be the lowest in embryos from large eggs of young flock (17.5% vs. 18.6-19.0%), whereas embryo the shortest shank length was observed in embryos from small eggs from young flock (2.2 cm vs. 2.4-2.5 cm, P<0.01). On day 18 of incubation, all of embryo development parameters were affected by breeder age and egg weight interaction. Embryo body weight, body and shank legth were found to be the highest for the embryos obtained from large eggs of old flock (32.5 g, 17.3 cm and 4.9 cm, respectively), whereas relative embryo weight was the highest for the embryos obtained from small eggs of old flock (52.1%, P<0.01).

Table 2. The effects of breeder age and egg weight on embryo development parameters during incubation period.

Main effects	Day 14				Day 18			
	EBW	REBW	EBL	ESL	EBW	REBW	EBL	ESL
BA								
Y	11.4 ± 0.09 ^{b}	18.2 ± 0.15 ^{b}	10,5 ± 0.06 ^{b}	2.3 ± 0.01 ^{b}	30.2 ± 0.26 ^{b}	48.3 ± 0.50 ^{b}	16.3 ± 0.06 ^{b}	4.7 ± 0.02 ^{b}
O	11.9 ± 0.09 ^{a}	18.8 ± 0.15 ^{a}	10,7 ± 0.06 ^{a}	2.5 ± 0.01 ^{a}	32.1 ± 0.26 ^{a}	50.9 ± 0.50 ^{a}	17.0 ± 0.06 ^{a}	4.9 ± 0.02 ^{a}
EW								
S	11.5 ± 0.09 ^{b}	19.0 ± 0.15 ^{a}	10,6 ± 0.06	2.4 ± 0.01 ^{b}	30.2 ± 0.26 ^{b}	49.9 ± 0.50	16.6 ± 0.06 ^{b}	4.7 ± 0.02 ^{b}
L	11.8 ± 0.09 ^{a}	18.1 ± 0.15 ^{b}	10,5 ± 0.06	2.5 ± 0.01 ^{a}	32.0 ± 0.26 ^{a}	49.2 ± 0.50	16.7 ± 0.06 ^{a}	4.9 ± 0.02 ^{a}
BA x EW								
Y x S	11.5 ± 0.13 ^{b}	19.0 ± 0.22 ^{a}	10,5 ± 0.09	2.2 ± 0.02 ^{c}	28.8 ± 0.36 ^{c}	47.8 ± 0.70 ^{b}	16.4 ± 0.09 ^{b}	4.6 ± 0.03 ^{c}
Y x L	11.3 ± 0.13 ^{b}	17.5 ± 0.22 ^{b}	10,4 ± 0.09	2.4 ± 0.02 ^{b}	31.5 ± 0.36 ^{b}	48.7 ± 0.70 ^{b}	16.1 ± 0.09 ^{c}	4.8 ± 0.03 ^{ab}
O x S	11.5 ± 0.13 ^{b}	18.9 ± 0.22 ^{a}	10,7 ± 0.09	2.5 ± 0.02 ^{a}	31.6 ± 0.36 ^{ab}	52.1 ± 0.70 ^{a}	16.7 ± 0.09 ^{b}	4.8 ± 0.03 ^{ab}
O x L	12.2 ± 0.13 ^{a}	18.6 ± 0.22 ^{a}	10,6 ± 0.09	2.5 ± 0.02 ^{a}	32.5 ± 0.36 ^{a}	49.6 ± 0.70 ^{b}	17.3 ± 0.09 ^{a}	4.9 ± 0.03 ^{a}
P-Value								
BA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EW	<0.001	<0.001	0.20	<0.001	<0.001	0.08	0.04	<0.001
BA x EW	<0.001	<0.001	0.63	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Means in column that possess different superscripts differ significantly
EBW: embryo body weight (g), REBW: relative embryo body weight (%), EBL: embryo body length (cm), ESL: embryo shank length (cm)
BA: breeder age, EW: egg weight
Y: young (36 weeks), O: old (52 weeks), S: small (58-63 g), L: large (64-69 g)

In this study, embryo development was identified by yolk absorption and embryo development parameters. It is known that breeder age is related with embryo development including body weight (Wilson, 1991; Pearson *et al.*, 1996; Suarez *et al.*, 1997; Tona *et al.*, 2004; Ulmer – Franco *et al.*, 2010; Yadgary *et al.*, 2010; Nangsuay *et al.*, 2013) and body length (Hill, 2001). In this study, a higher embryo body weight, and body and shank length were observed in embryos from 52 wk of age flock during incubation and also at hatch. The current results are similar with previous findings of Latour *et al.* (1996), Peebles *et al.* (2001), Yadgary *et al.* (2010), Nangsuay *et al.* (2011, 2013) who reported that broiler breeder age and egg weight affected embryo development parameters during incubation period. Also, this could be related with a lower nutrient contents of eggs obtained from young flocks than older flocks and therefore it might cause a lower development of embryos in eggs from younger flocks (Mcloughlin and Gous, 1999).

Furthermore, smaller eggs that are obtained from sama age breeder flock have a lower yolk weight and subsequently yolk sac memebranes compared to the large ones. Therefore, it might be a restricting factor for embryo development in smaller eggs (Noble and Cocchi, 1990; Gous, 2010). Thus, Lourens *et al.* (2006) concluded that embryos in large eggs had more nutrients for gaining body weight than embryos in small eggs.

The effects of breeder age and egg weight on chick quality parameters at hatch are shown on Table 3. As seen on table, a significant breeder age x egg weight interaction was only observed for chick body length. Chick body weight (43.0 vs. 45.1 g), relative chick body weight (68.8 vs. 71.5%), yolk free body weight (39.0 vs. 40.6 g) and shank length (5.1 vs. 5.3 cm) were found to be higher in chicks from old flock whereas yolk free body weight rate (90.7 vs. 90.0%) was higher in chicks from young flock (P<0.01). On the other hand, chick body weight (41.5 vs. 46.8 g), relative chick body weight (68.5 vs. 71.8%), yolk free body weight (37.5 vs. 42.3 g), chick body length (20.0 vs. 20.9 cm) and shank length (5.0 vs. 5.4 cm) were found to be higher in chicks from large eggs than the ones from small egss. Egg weight did not only affect the relative yolk free body weight. The highest mean for chick body length was found for the chicks obtained from large eggs of old flock compared to other experimental groups.

Table 3. The effects of breeder age and egg weight on chick quality parameters at hatch.

Main effects	CBW	RCBW	YFBW	RYFBW	CBL	CSL
BA						
Y	43.0 ± 0.34 [b]	68.8 ± 0.63 [b]	39.0 ± 0.38 [b]	90.7 ± 0.39 [a]	20.2 ± 0.05 [b]	5.1 ± 0.04 [b]
O	45.1 ± 0.34 [a]	71.5 ± 0.63 [a]	40.6 ± 0.38 [a]	90.0 ± 0.39 [b]	20.7 ± 0.05 [a]	5.3 ± 0.04 [a]
EW						
S	41.5 ± 0.34 [b]	68.5 ± 0.63 [b]	37.5 ± 0.38 [b]	90.4 ± 0.39	20.0 ± 0.05 [b]	5.0 ± 0.04 [b]
L	46.8 ± 0.34 [a]	718 ± 0.63 [a]	42.3 ± 0.38 [a]	90.2 ± 0.39	20.9 ± 0.05 [a]	5.4 ± 0.04 [a]
BA x EW						
Y x S	40.4 ± 0.48	67.0 ± 0.89	36.8 ± 0.51	90.8 ± 0.56	19.6 ± 0.07 [d]	4.9 ± 0.07
Y x L	45.7 ± 0.48	70.6 ± 0.89	41.4 ± 0.51	90.6 ± 0.56	20.7 ± 0.07 [b]	5.2 ± 0.07
O x S	42.5 ± 0.48	70.0 ± 0.89	38.3 ± 0.51	89.9 ± 0.56	20.3 ± 0.07 [c]	5.1 ± 0.07
O x L	47.8 ± 0.48	73.0 ± 0.89	42.9 ± 0.51	90.0 ± 0.56	21.0 ± 0.07 [a]	5.5 ± 0.07
			P-Value			
BA	<0.001	<0.001	<0.001	0.06	<0.001	<0.001
EW	<0.001	<0.001	<0.001	0.64	<0.001	<0.001
BA x EW	0.96	0.67	0.95	0.89	<0.001	0.93

[a,b,c] Means in column that possess different superscripts differ significantly
CBW: chick body weight (g), RCBW: relative chick body weight (%), YFBW: yolk free body weight (g), RYFBW: relative yolk free body weight (g),
CBL: chick body length (cm), CSL: chick shank length (cm)
BA: breeder age, EW: egg weight
Y: young (36 weeks), O: old (52 weeks), S: small (58-63 g), L: large (64-69 g)

The day-old chick quality is a major criteria for determination of livability, post-hatch growth performance and health status of newly-hatched chick. At hatch, chick weight, yolk-free body weight, body and shank length are measured as indicator parameters of chick quality (Deeming, 2000; Wolanski *et al.*, 2003, 2006; Willemsen *et al.*, 2008). In the study, the chick quality parameters were affected by both breeder age and egg weight. Chick weight at hatch is accepted as a predictor parameter for growth performance and final weight of broilers at slaughter age. A higher chick weight, yolk free body weight and body length observed for the chicks obtained from both old flock and large eggs are consistent with a higher rate of yolk absorption and embryo development during incubation.

CONCLUSIONS

In conclusion, in our study, broiler breeder age and egg weight affected yolk absorption and embryo development during incubation and chick quality at hatch. Producing high quality chicks with maximum number is related with quality of hatching eggs. In that respect, changes in egg quality depending on broiler breeder age is essential for regulation of breeder flock managements and incubation techniques.

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