

Effect of egg storage duration on spread of hatch, chick quality and organ development in FUNAAB-alpha chickens

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Effect of egg storage duration (0, 4, 8, 12 vs. 16 days) on spread of hatch, chick quality and organ development in FUNAAB-alpha chickens was determined. Hatching eggs ($n=200$; 53.2 ± 4.67 g) were used. Hatchability was significantly ($P\leq 0.001$) affected by storage duration. Hatchability of eggs stored for 0, 4 and 8 days was higher than for eggs stored for 12 and 16 days. Eggs stored for 4 days hatched earlier than the rest. Hatching commenced after 466 hours incubation time in eggs stored for 4 days, after 472 hours in 0 and 12 days, and 478 hours in 8 and 16 days storage duration. Time delay of 50% hatch resulting from additional storage after 4 days was 60, 45, 37.5 and 60 minutes/day for 0, 8, 12 and 16 day storage, respectively. Storage length had a significant ($P\leq 0.05$) effect on chick activity and closeness of navel. Chicks from 16 day stored eggs were less active than others. Navel closeness was higher in 8 day storage than those from fresh eggs. Chick length was significantly ($P\leq 0.001$) affected by egg storage duration. Longest chicks were obtained from 8 day storage eggs, while the shortest were from 16 day storage. The weights of gizzard ($P\leq 0.01$) and yolk remnant ($P\leq 0.05$) were affected by storage length. Gizzards in 8 and 12 day egg storage chicks were heavier than in 16 day eggs. Weights of gizzards in 0 and 4 day groups were similar to the other three groups. Yolk remnant was lightest in 8 day egg storage chicks and heaviest in 16 day egg storage chicks. In conclusion, egg storage for 4 days yielded better and earlier hatchability. To obtain chicks with good quality, egg storage should not be extended beyond 8 days.

Keywords: Chick quality, FUNAAB-alpha chicken, egg storage, environment, hatching time, hatchability

The quest for thermotolerant strains of chicken has been on-going for a long time in the tropics. One of the locally developed strains within Nigeria that was specially bred for tropical conditions is the newly registered FUNAAB-alpha chickens (Adebambo 2015). The birds are well adapted to hot weather with promising productivity (Abioja et al. 2020). The breed is being tested in all agro-ecological zones of Nigeria and elsewhere in the west coast of Africa (Adebambo 2015). FUNAAB-alpha hens have similar body weight to ISA, an exotic commercial laying breed that is commonly reared in this environment, but with higher weight than Nigerian local hens (Okafor and Omeje 2018). FUNAAB-alpha is a newly registered breed which is gaining popularity and widespread adoption among breeder farmers (Bamidele et al. 2019; Sokoya et al. 2019; Yakubu et al. 2020), with much ongoing research (Saleh et al. 2017; Wheto et al. 2017; Odutayo et al. 2020) in different areas of production. Currently, breeder farms raising

FUNAAB-alpha chickens operate on small scale and it is necessary to collect fertile eggs from scattered small scale farmers before setting.

It is known that extended storage of chicken eggs impact negatively on hatchability, chick quality and post-hatch performance (Tona et al. 2003; Rejrink et al. 2008; Pawlowska and Sosnówka-Czajka 2019). It is therefore necessary to establish the optimum length of storage time for eggs (Dereje et al. 2015). The main purpose of hatchery management is to obtain viable chicks after the operation. In a review of factors that influence egg fertility and hatchability in poultry, it was reported that the length of storage should not exceed 10-14 days in order to maintain good hatchability (King'ori 2011). Goliomytis et al. (2015), however, reported that storage of broiler hatching eggs up to 16 days had no effect on most developmental and growth parameters with the exception of day-old chick weight and length. Eggs from both meat and egg-type quail did not retain good hatchability beyond 10 days of storage

(Romao et al. 2008). Traditionally, the hatchery operators keep chicken eggs for 7 days before setting. However, some researchers have recommended that storage duration should not exceed 3 to 5 days (Khan et al. 2014; Senbeta 2016). Therefore it is likely that there are interactions between egg storage duration, storage temperature and breeder factors which affect outcomes of incubation. Consequently, it is important to determine the effect of egg storage duration on hatchability, spread of hatch, chick quality and organ development in the FUNAAB-alphabreed.

Materials and methods

Experimental materials

Hatching eggs collected from a flock of FUNAAB-alpha breeder hens aged 32 weeks were macroscopically examined to select eggs with no cracks. Selected eggs (n= 200; average weight 53.1 g) were used for the experiment.

Egg storage

The eggs were stored in egg tray with broad end up (under 16 °C and 75 % relative humidity) for 0, 4, 8, 12 or 16 days. There were 40 eggs in each group and 10 eggs per replicate.

Incubation

All eggs (40 eggs per group) were set in an incubator (N.V. Petersime® EV1/EN2 Incubator, Belgium) under identical conditions maintained at 37.5 °C dry bulb and 29.5 °C wet-bulb temperature. The candling was done on day 12 of incubation.

Data collection

Hatchability and hatching time: As the predicted hatching time (501 hours) was approaching, eggs in the hatcher were monitored every 6 hours starting from 460 hours incubation time. The number of chicks hatched at every interval was recorded. Hatchability of eggs was calculated

from the data of number hatched in egg storage group. The hatch was concluded 48 hours post predicted time.

Chick weight and yield: Chicks that were completely dried were removed from the hatcher and weighed. Chick yield was calculated as the ratio of chick weight to egg weight, expressed as a percentage.

Chick quality: The Tona hedonic scale (Table 1) was adopted to grade the chick quality traits. Traits observed included chick activity, downs and appearance, retracted yolk, eye, leg, navel, remnant membrane, remnant yolk sac, total Tona grade, and chick length.

Relative weight of some organs in day-old chicks: Chicks were euthanized immediately following weighing. The chicks were dissected to harvest organs: heart, lungs, gizzard, proventriculus, liver, whole intestine and remnant yolk sac. Relative weight of organs was expressed as a percentage of the chick weight.

Table 1: Allocation of scores to different parameters

Parameter	Characteristics	Score
Activity	Good	6
	Weak	0
Downs and appearance	Clean and dry	10
	Wet	8
	Dirty and wet	0
Retracted yolk	Body with normal swallowed yolk	12
	Body with swallowed large yolk and rather hard to touch	0
Eyes	Opened and bright	16
	Opened and not bright	8
	Closed eyes	0
Legs	Normal legs and toes	16
	One infected leg	8
	Two infected legs	0
Navel	Completely closed and clean	12
	Not completely closed and not discoloured	6
	Not closed and discoloured	0
Remaining membrane	No membrane	12
	Small membrane	8
	Large membrane	4
Remaining yolk	Very large membrane	0
	No yolk	16
	Small yolk	12
Remaining yolk	Large yolk	8
	Very large yolk	0
Total		100

Source: Tona et al. (2003)

Statistical analysis

Data obtained were subjected to one-way analysis of variance in a completely randomised design using Minitab 17 computer statistical software.

Statistical model: $Y_{ij} = \mu + D_i + \epsilon_{ij}$

where Y_{ij} is dependent variable; μ is population mean; D_i is i^{th} effect due to storage duration; and ϵ_{ij} is random error.

Significantly different means were separated with Tukey's HSD test. Means were taken to be significantly different when probability was less than or equal to 0.05.

Results

The hatchability of eggs stored for varying lengths of time before incubation is presented in Figure 1. Storage length had a significant

($P \leq 0.001$) effect on hatchability. Mean hatchability was similar among eggs stored for 0 (57.5%), 4 (67.5%) and 8 days (62.5%); these were higher than in eggs stored for 12 (35.0%) and 16 days (30.0%).

Incubation time and calculated time delay of hatch resulting from additional storage after 4 days in FUNAAB-alpha chickens are shown in Figure 2 and Table 2. Eggs stored for 4 days hatched earlier than the rest. Hatching commenced after 466 hours incubation time in 4 day, 472 hours in 0 and 12 day, and 478 hours in 8 and 16 day storage durations. Hatch windows were 24 hours (472 – 496), 24 hours (466 – 490), 12 hours (478 – 490), 18 hours (472 – 490) and 24 hours (472 – 496) in 0, 4, 8, 12 and 16 day egg storage times respectively. The time delay of 50% hatch resulting from differing storage times from 4 days was 60, 45, 37.5 and 60 minutes/day for 0, 8, 12 and 16 day storages, respectively. To achieve 95% hatchability, the delay was 60, 30, 7.5 and 65 minutes/day, respectively.

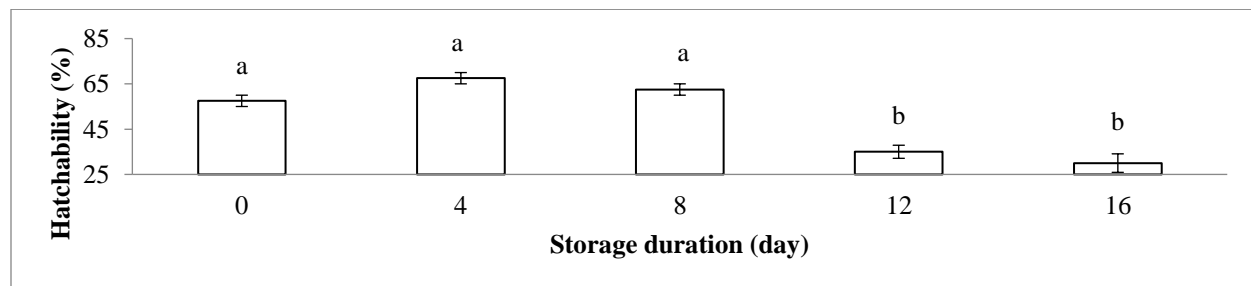


Figure 1: Effect of egg storage duration on hatchability in FUNAAB-alpha chickens

^{a,b} Means represented by bar with different letters differ significantly ($P \leq 0.001$)

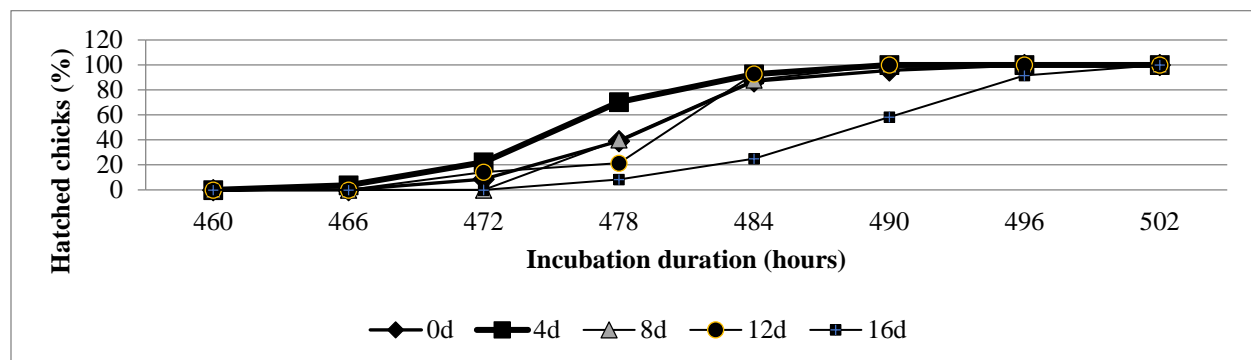


Figure 2: Spread of hatch in relation to egg storage duration time in days (d) for FUNAAB-alpha chickens

Table 2: Incubation time (hours) and calculated time delay of hatch resulting from additional storage after 4 days (d) in FUNAAB-alpha chickens

Spread of hatch (%)	Incubation time					Delay (minutes/d)			
	0d	4d	8d	12d	16d	0d	8d	12d	16d
25	475 ^b	473 ^b	476 ^b	479 ^{ab}	484 ^a	30	45	45	55
50	480 ^{bc}	476 ^c	479 ^{bc}	481 ^b	488 ^a	60	45	37.5	60
75	482 ^b	479 ^b	483 ^b	482 ^b	493 ^a	45	60	22.5	70
95	490 ^b	486 ^b	488 ^b	487 ^b	499 ^a	60	30	7.5	65

^{a,b,c} Means within the same row with different superscripts differ significantly ($P \leq 0.01$)

Table 3 shows the effect of egg storage duration on egg weight, chick weight and yield in FUNAAB-alpha chickens. These were not significantly different ($P > 0.05$) for the various storage times.

Chick quality indices are presented in Table 4. Egg storage length had a significant ($P \leq 0.05$) effect on chick activity and closeness of navel. Chicks from 16 day stored eggs were less active than others. The degree of navel closeness was higher in 8 day storage than those from fresh eggs. Chick length was significantly ($P \leq 0.001$) affected by egg storage duration. Longest chicks

(17.0cm) were obtained from 8 day stored eggs while the shortest (15.7cm) were obtained from 16 day storage.

Table 5 presents the effect of egg storage duration on relative weight of some body organs in FUNAAB-alpha chicks. Relative weights of gizzard ($P \leq 0.01$) and yolk remnant ($P \leq 0.05$) were affected by storage length. Gizzards were heaviest in chicks from eggs stored for 8 and 12 days and lightest in those from eggs stored for 16 days. Yolk remnant was lightest in 8 day egg storage chicks and heaviest in 16 day egg storage chicks.

Table 3: Effect of egg storage duration on chick weight and yield (mean±sem) in FUNAAB-alpha chickens

Parameter	Egg storage duration (days)					P
	0	4	8	12	16	
<i>n</i>	23	27	25	14	12	
Egg weight (g)	*53.6±1.01	52.4±0.93	53.0±0.97	52.0±1.30	51.4±1.40	0.733
Chick weight (g)	36.3±0.74	35.3±0.68	35.5±0.71	35.3±0.95	37.0±1.02	0.630
Chick yield (%)	68.1±1.98	68.1±1.83	67.8±1.90	68.7±2.53	72.6±2.74	0.663

n: number of observations

Table 4: Effect of egg storage duration on qualitative traits (mean±sem) in FUNAAB-alpha chicks

Parameter	Egg storage duration (days)					P
	0	4	8	12	16	
<i>n</i>	10	10	10	10	10	
Chick activity	6.0 ^a ±0.35	6.0 ^a ±0.35	6.0 ^a ±0.35	6.0 ^a ±0.35	4.5 ^b ±0.35	0.010
Downs and appearance	9.8±0.20	9.7±0.20	9.8±0.20	9.8±0.20	9.5±0.20	0.700
Retracted yolk	9.0±1.04	11.0±1.04	12.0±1.04	11.0±1.04	11.0±1.04	0.366
Eye	16.0±0.71	13.3±0.71	16.0±0.71	14.7±0.71	15.3±0.71	0.057
Leg	16.0±0.62	16.0±0.62	16.0±0.62	15.7±0.62	14.7±0.62	0.481
Navel	4.0 ^b ±0.98	7.5 ^{ab} ±0.98	8.0 ^a ±0.98	7.0 ^{ab} ±0.98	7.5 ^{ab} ±0.98	0.040
Remaining membrane	9.7±0.61	8.7±0.61	9.3±0.61	9.0±0.61	8.0±0.61	0.369
Remaining yolk	16.0±0.41	15.0±0.41	15.3±0.41	15.0±0.41	15.7±0.41	0.366
TONA chick quality (%)	86.5±2.33	87.2±2.33	92.5±2.33	88.2±2.33	86.2±2.33	0.313
Chick length (cm)	16.5 ^b ±0.13	16.9 ^{ab} ±0.11	17.0 ^a ±0.12	16.4 ^b ±0.16	15.7 ^c ±0.17	0.000

^{a,b} Means within the same row with different superscripts differ significantly ($P \leq 0.05$)

n: number of observations

Table 5: Effect of egg storage duration on relative weight (mean±sem) of some body organs in FUNAAB-alpha chicks

Parameter	Egg storage duration (days)					P
	0	4	8	12	16	
<i>N</i>	10	10	10	10	10	
Chick weight (g)	35.5±1.36	36.8±1.36	37.2±1.36	32.4±1.36	36.9±1.36	0.108
Heart (%)	0.73±0.057	0.70±0.057	0.82±0.057	0.92±0.057	0.74±0.057	0.084
Lungs (%)	0.62±0.073	0.76±0.073	0.78±0.073	0.72±0.073	0.61±0.073	0.397
Gizzard (%)	4.8 ^{ab} ±0.26	5.0 ^{ab} ±0.26	5.6 ^a ±0.26	5.5 ^a ±0.26	4.2 ^b ±0.26	0.006
Proventriculus (%)	0.85±0.068	0.84±0.068	0.86±0.068	0.86±0.068	0.78±0.068	0.920
Liver (%)	2.4±0.15	2.4±0.15	2.4±0.15	2.7±0.15	2.2±0.15	0.292
Intestine (%)	3.3±0.19	3.6±0.19	3.7±0.19	3.6±0.19	3.3±0.19	0.498
Yolk sac (%)	10.8 ^{ab} ±1.28	11.0 ^{ab} ±1.28	8.1 ^b ±1.28	11.3 ^{ab} ±1.28	14.4 ^a ±1.28	0.040

^{a,b} Means within the same row with different superscripts differ significantly ($P \leq 0.05$)

n: number of observations

Discussion

Effect of egg storage

Obtaining chicks of high quality is the aim of hatchery operations. Onagbesan et al. (2007) stated that excellent embryonic development is a dynamic process that requires regulation of factors around the eggs in pre-oviposition period, laying, storage and incubation to the time of hatching. Such conditions include breeder factors (genetic quality and age of breeders), egg factors (weight, internal qualities and shell), environmental factors (temperature, humidity, gaseous exchange, duration) and conditions of storage. The tradition in hatchery operation is to keep chicken eggs for about 7 to 8 days before incubation. However, extending the storage beyond this period is known to impact egg quality (Miles and Henry 2004), embryonic development (Rocha et al. 2013), hatchability (Rejrink et al. 2008), chick quality (Pawlowska and Sosnówka-Czajka 2019) and post hatch growth performance (Tona et al. 2003; Onbaşlılar et al. 2007; Senbeta 2016).

Hatchability

In the present study, storage length extension to 12 and 16 days negatively affected

hatchability, reducing it to 35 and 30%, respectively in FUNAAB-alpha chickens. This agrees with the findings of Addo et al. (2018) in naked neck chickens that egg storage up to 10 or 14 days reduced hatchability to 38 and 25% respectively. Similarly, Khan et al. (2014) reported a decline in hatchability in Rhode Island Red chickens as the days of egg storage increased from zero to nine. This might be adduced to early embryonic mortality in eggs stored for a long time. Once an egg is laid, any increment in environmental temperature triggers the continuation of blastoderm development (Bergoug et al. 2013). Though embryo development commences in the oviduct before egg laying, the development is arrested after oviposition until the appropriate temperature is attained. The arrest is essential because premature resumption will eventually result in mortality. The longer an egg is stored, the higher the chance of premature resumption of the embryo development when the surrounding temperature is high enough. Such embryo may die off when the temperature is lowered again, resulting in increased number of eggs to be discarded after candling. Eggs in store must be brought to physiological zero, a temperature at which incubation is further arrested until eggs are set at an appropriate temperature in an incubator. Beside this, deterioration of the albumin also contributes

immensely to loss of hatchability due to prolonged storage. The thick albumen thins out as a result of continuous copious amount of CO₂ passing through the shell, thereby making the albumen watery and alkaline. Hatchability seems to improve slightly when eggs are stored for 3 to 4 days compared to fresh eggs. Decline in egg hatchability commences after 5 days in storage (Schmidt et al. 2009).

Spread of hatch

Eggs of 4 day storage hatched earlier than others in the present study. This corroborates with the report of Tona et al. (2003), that eggs stored for 3 days hatched before those stored for 18 days. This may result from the loss of sources of energy during storage and subsequent incubation. The chicks at perinatal period require a lot of energy from carbohydrate sources. Embryo from prolonged storage time may not have the luxury of this. Turkey embryo from 14 day egg storage duration is said to rely more on gluconeogenesis during pipping and hatching, than those from 4 day egg storage (Fasenko 1996). It is advantageous for growth and survival if an embryo possesses the capacity to metabolize adequate carbohydrate reserves at the time of hatching. Another reason for possible delayed hatching in extensively long stored eggs is the delay in initiation of embryogenesis (Fasenko 2007). The present finding showed that eggs commenced hatching after 466 hours incubation time in the 4 day storage group, but did not start until 478 hours after 8 and 16 days storage. To achieve 95% hatchability, the delay from hatching of 4 day stored eggs was 60, 30, 7.5 and 65 minutes/day for 0, 8, 12 and 16 day storage durations respectively. Extended periods of egg storage increase the spread of hatch over time (Hassan et al. 2005).

Chick weight and yield

Chick weight was similar among the egg storage duration groups in the present study. On the contrary, a decrease in hatchling weight

was reported in eggs stored between 4 and 16 days (Goliomytis et al. 2015). Khan et al. (2014) also reported a significant decline in day-old chick weight hatched from eggs of varying storage durations. However, in line with the finding in this study, Senbeta (2016) reported no difference in mean chick weight from eggs stored at different lengths of time. Irrespective of the breeder's age, Tona et al. (2004) found no difference in day-old chick weight. Storage had no effect on chick weight at day-old in naked neck chickens (Addo et al. 2018). In other poultry species such as duck (Onbaşlılar et al. 2007) and quail (Petek et al. 2003), hatchling weight was not affected by length of egg storage. The ratio of hatchling weight to egg weight was similar in the present study. It is of note that Addo et al. (2018) found a significant interaction of storage length with storage temperature. It shows that hatchling weight is dependent on environmental temperature surrounding the eggs in storage. This study found that chick yield (ratio of day-old chick weight to egg weight in percentage) was not affected by length of storage in FUNAAB-alpha chickens. Goliomytis et al. (2015) showed similar results for broiler chickens. However, Alsobayel et al. (2013) reported a decline in chick yield (1.0 and 1.7%) as the length of egg storage increased from 0 to 7 and 14, days respectively. There are indications in the literature that there are breed by storage length interactions on chick yield (Tona et al. 2004). Different breeds may respond in diverse ways to the length of egg storage.

Chick quality

Chick quality is an important factor in determining the performance at post-hatch age in chickens (van den Ven et al. 2012). It is however, very difficult to determine because of its subjectivity. Chicks from 16-day stored eggs in this experiment were less active than other groups stored for shorter periods. Chick activity has been recognised as an indication of

the quality of chicks (Tona et al. 2003). This may suggest that the chicks are tired from stress during hatching or inability to assess nutrient reserves in the yolk sac. The current study shows that the degree of navel closeness is higher in chicks hatched from eggs stored for 8 days prior to incubation than those from fresh eggs. The presence of small navel buttons at hatching is indicative of impaired absorption of the remnant yolk sac content and decreased intestinal villi growth (Kawalilak et al. 2010). This affects the survival and post-hatch growth performance of chicks. Length of chicks is another indicator of chick quality found to be of importance in this study. The shortest chicks were obtained from eggs stored for 16 days. Chicks from extended egg storage (16 days) in this study had smaller gizzards. The stomach of chicken is grossly divided into two: glandular proventriculus and muscular ventriculus. Digestive juice containing enzymes is secreted in the proventriculus while the ventriculus also known as gizzard is to mechanically break down the ingested materials using grits like millstone. Important functions of gizzard include particle size reduction, chemical degradation of nutrients, digesta flow regulation and rapid response to feed particle size (Svihus 2011). The size of gizzard may have implications for nutrient digestibility and efficiency. Literature is scarce on the influence that the size of gizzard in day-old chicks may have on post-hatch life. The gizzard, when fully developed, allows the chicken to take full benefits of fibre-rich feed materials. This increases the energy utilization efficiency (Rodgers et al. 2012). The size of retained yolk sac was relatively big in chicks from eggs stored for 16 days. This suggests that extended egg storage may reduce yolk nutrient utilization in day-old chicks. Khan et al. (2004), in a review, stated that yolk retention is considered as an important cause of death in chicken especially if the retained yolk sac is infected (omphalitis). Stress during the peri-hatch period has also been implicated in increase remnant yolk sac (Mikec et al. 2006).

Conclusion

Egg storage for 4 days yielded better and earlier hatchability. To obtain chicks with good quality, egg storage should not be extended beyond 8 days. Storing eggs for 16 days yielded lowest hatchability, longest time to initiate hatching, least active chicks with shortest body length, smallest gizzard and heaviest yolk sac remnant. Setting eggs immediately after oviposition is not practically possible and advisable.

Animal welfare statement

The experimental procedure has been approved by the Animal Experimental Board of the Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria. Also, the Guideline for Animal Research of Nigeria Institute of Animal Science (NIAS), Abuja, Nigeria was followed.

Conflict of interest

There is no conflict of interest of any sort in this work.

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