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The appropriate time to improve day-old chick production and post-hatch growth through *Moringa oleifera* leaf extract inoculation into the hatching egg

Der richtige Zeitpunkt für *Moringa oleifera* Blattextrakt-Applikation in das Brutei zur Verbesserung der Leistung von Eintagsküken und des Wachstums nach dem Schlupf

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Abstract

Moringa oleifera in feed post-hatch improves growth in early postnatal life. One may question the effects of *Moringa oleifera* after *in ovo* feeding. This study was carried out to determine the effect of different time periods of *in ovo* inoculation of *Moringa oleifera* leaves (MOL) extract on hatchability and juvenile growth performance. For the experiment, a total of 868 fertile broiler eggs identified by candling were used. They were randomly allotted to 7 groups with 124 eggs per group. Three groups were used for MOL extract inoculation at d 16, 17 and 18 (M groups). Further three groups were used for saline solution inoculation at d 16, 17 and 18 as positive controls (T groups) for those used for MOL extract inoculation. A negative control (T0) received no inoculation at all. The results showed that *in ovo* inoculation of MOL extract on d 18 of incubation significantly increased hatchability and reduced late embryonic mortality rate and hatching curve. In addition, MOL extract injection improved the percentage of day-old chicks with high quality as well as the average chick quality score ($P < 0.05$). Furthermore, post-hatch growth up to 7 days of age showed that the chicks hatched from eggs inoculated with MOL extracts on d 18 of incubation had significantly higher body weight gain than those injected on other days. It can be concluded that *in ovo* inoculation of MOL extract on d 18 of incubation into the air chamber provided the best strategy to improve hatchability, chick quality and post hatch growth of broiler.

Key words

Hatchability rate; post hatch; growth; late embryonic mortality rate; *Moringa oleifera* extract; broiler; hatching egg; air chamber; *in ovo* inoculation

Zusammenfassung

Futtermittel mit *Moringa oleifera*-Zusatz verbessern das Wachstum in den ersten Tagen nach dem Schlupf. In dieser Studie wird die Fragestellung untersucht, inwiefern *in-ovo* Fütterung mit *Moringa oleifera* das Schlupfergebnis und das Wachstum juveniler Broiler beeinflussen kann. Zur Ermittlung des optimalen Zeitraums für die Injektionen erfolgten die *in-ovo* Applikationen von *Moringa oleifera* Blätter(MOL)-Extrakt an verschiedenen Bruttagen. Insgesamt 868 befruchtete Broilereier wurden nach dem Zufallsprinzip 7 Versuchsgruppen mit je 124 Eiern zugeordnet. In drei Versuchsgruppen wurde die *in-ovo* Applikation von MOL-Extrakt (M-Gruppen) an unterschiedlichen Bruttagen (E16, 17 oder 18) durchgeführt. Weitere drei Gruppen dienten als Positivkontrolle (T-Gruppen) mit *in-ovo* Applikation von Kochsalzlösung am E16, 17 oder 18. Die Negativkontrolle (T0) erhielt keine *in-ovo* Applikation. Eine *in-ovo* Applikation von MOL-Extrakt am E18 erhöhte signifikant die Schlupffähigkeit und reduziert die späte embryonale Mortalität und Schlupfdauer. Darüber hinaus erhöhte die MOL-Extrakt-Applikation den Anteil der Eintagsküken mit hoher Qualität und verbesserte die durchschnittliche Kükenqualität ($P < 0,05$). Am 7. Tag nach dem Schlupf waren die Körpermassezunahmen der Küken, die am E18 eine *in-ovo* Applikation mit MOL-Extrakt erhielten, signifikant höhere als die der Tiere aus den Eiern, die an den übrigen Bruttagen mit MOL-Extrakt behandelt wurden. Es kann geschlussfolgert werden, dass bei Masthühnern eine *in-ovo* Applikation von MOL-Extrakt in die Luftkammer des Bruteies am E18 zu den besten Ergebnissen in der Schlupffähigkeit, der Kükenqualität und des Wachstums nach dem Schlupf führt.

Stichworte

Schlupfrate; Wachstum; späte embryonale Mortalität; *Moringa oleifera*-Extrakt; Masthähnchen; Brutei; Luftkammer; *in-ovo*-Applikation

Introduction

In poultry egg incubation, hatching occurs around d 21 with 95% of chicks hatched within a window of 36 to 48 h (KOP-BOZBAY and OCAK, 2015). In such practice, the first hatched chicks do not have access to feed and water until after 2 to 3 d and have to depend on using residual yolk as major source of energy. This delay to access feed often leads to low growth performance with potential effects on slaughter weight (BIGOT et al., 2003), health status (DIBNER et al., 1998) and mortality rate (WILLEMSEN et al., 2010). To overcome these problems, several strategies including *in ovo* inoculation, have been proposed in order to help chicks store more nutrients and set up enough nutritional stock for additional energy to overcome that critical period. *In ovo* inoculation methods have been described by TAKO et al. (2004), FOYE et al. (2006) and consisted of nutrient solution injection into the air chamber, the amniotic fluid or directly into the embryo a few days before pipping stage (UNI and FERKET, 2004; OLIVEIRA et al., 2008). Many nutrients have been used for *in ovo* feeding including amino acids, carbohydrates, fatty acids, vitamins and other modulators. MOLENAAR et al. (2010) improved hatchability after injecting physiological saline into the amniotic fluid at d 18 of incubation. Studies of UNI and FERKET (2004); UNI et al. (2005) have shown muscle development, immune system and carcass yield improvement by injecting carbohydrates and beta-hydroxy-beta-methylbutyrate into amniotic fluid on d 17.5 of incubation. Generally, plants are good sources of vitamins, essential amino acids, proteins, minerals as well as active component (FASUYI, 2006). These active substances, that have medicinal property can be extracted and used in different forms to prevent or treat some diseases (SOFOWORA, 1996). *In ovo* feeding research has shown that vegetal extracts from plants such as *Toussaintia patriciae*, *Eugenia jambolana* and *Guiera senegalensis*, improved chicken immune status against infectious bursal virus, avian influenza virus (H5N1) and fowl poxvirus (NYANDORO et al., 2014, SOOD et al., 2012, LAMIEN et al., 2005). *Moringa oleifera* is also another promising tree with a good profile of important trace elements, good sources of proteins, vitamins and can be used as animal feed (ANWAR et al., 2007; DOUGNON et al., 2012; SULTANA et al., 2015). It contains also secondary metabolites, which are bioactive molecules in the plant. These bioactive compounds have been known to modulate gene expression and signal transduction pathways (DOUGHARI et al., 2009). They include alkaloids, steroids, flavonoids, terpenoids, tannins, and saponins (BENNETT et al., 2003; TETEH et al., 2013), may also act as a good source of natural antimicrobial and antioxydants (DOLARA et al., 2005) and may be helpful for the hatching process. Several studies have shown *Moringa oleifera* effects on chick's growth (NKUKWANA et al., 2014; VOEMESSE et al., 2018) and egg production (TETEH et al., 2013). One may question the effects of *Moringa oleifera* after *in ovo* feeding. OHTA and KIDD (2001) demonstrated that, *in ovo* injection site and time affect hatchability. Studies of SALAHI et al. (2011) with saline solution suggested that the best time for *in ovo* injection of substances into the amniotic fluid of poultry egg should be at 421 and 453 h of incubation. Based on this result, N'NANLE et al. (2017) have injected *Moringa oleifera* leaves extract in egg air chamber at d 18 with no effect on feed intake and body weight gain up to 7 weeks of age. It is possible that the best time for *in ovo* inoculation depends on the nature of substance and the site of injection. In literature, the best time for *in ovo* inoculation in the air chamber to improve day-old chick production has not yet been clearly determined. Our hypothesis is that d 18 is the best time for substance inoculation into the egg air chamber. Therefore, this study was undertaken to determine the appropriate time period between d 16 and d 18 of incubation when *Moringa oleifera* extract can be inoculated *in ovo* into the air chamber of eggs to improve hatchability and post hatch growth of broilers chickens.

Material and Methods

Experimental design

Hatching eggs, produced by Ross 308 broiler breeders, were used for the experiments carried out at the experimental unit of the Regional Excellence Center on Poultry Sciences (CERSA), University of Lomé (Togo). A total of 868 fertile Ross 308 broiler eggs (breeder age 40 weeks), provided by Incubel (Hogestraten, Belgium), were studied. Prior to incubation, eggs were weighed and numbered for individual identification. The eggs were then set for incubation in Petersime incubators at standard conditions (temperature: 37.8°C, relative humidity: 55%, turning: every 1 h until 18th d of incubation at an angle of 90°). At d 15 of incubation, all incubated eggs were candled to identify fertile eggs, which would be used for injection with *Moringa oleifera* leaf (MOL) extract (M groups) or 0.9% saline solution (T groups). All fertile eggs were allotted into seven groups (T0, M16, T16, M17, T17, M18 and T18). T0 group was the negative control group with non-injected eggs, M16 and T16 groups were injected at d 16 of incubation, groups M17 and T17 were injected at d 17 while M18 and T18 groups were injected at d 18 of incubation. The M groups were those injected with MOL extract dissolved in a saline solution vehicle. The T groups (positive controls) were injected with the vehicle (saline solution only). From 454 to 512 h of incubation, hatching events were monitored. Samples of embryos at internal pipping stage were used to record embryo, yolk sac, heart and liver weights. At the end of incubation, samples of day-old chicks were reared up to 7 days of age.

Moringa oleifera leaf extraction

Fresh leaves of *Moringa oleifera* collected in rural area of Togo were dried under air room condition for 72 h. They were grounded using an automatic miller. The powder (300 g) obtained was mixed gradually with alcoholic solution (3000 ml of ethanol + 600 ml of distilled water) and kept for 72 h in a bottle at ambient temperature. During this period, the supernatant was collected daily and the bottle was refilled with water-alcohol solution. At the third day, the macerate was filtered successively through a sieve, a thick layer of cotton and a filter type pump MW63/4. The filtrate and the supernatant obtained were then evaporated in vacuo using the Rotavapor (Debala, 2002) to obtain 64.70 g of extract representing a yield of 21.56%.

Injectable solutions preparation and in ovo inoculation

MOL extract injectable solution was obtained by mixing 50 µg of extract and 100 µl of 0.9% saline solution. The saline solution was purchased from a local drug pharmacy and used as injectable solution. Eggs of positive control and treated eggs were respectively injected with saline solution and MOL extract solution into the egg air chamber. A negative control was not injected at all. Handle egg injection method was used to inject the solution. To prevent cross contamination between individual eggs, complete disinfection was done after each injection by alcohol 95%. For injection, a 23-gauge manual needle was used to drill two holes at a depth of about 15 mm through the eggshell above the air chamber in order to decrease the pressure within and thereby facilitating the retention of the injected solution. After injection into one of the holes, both holes were sealed with adhesive tape and the egg was replaced in the incubator without turning until the time of transfer to the hatcher.

Hatching events, hatchability and chick quality

From 454 to 512 h of incubation, the transferred eggs were checked individually every 2 h for *pipping* and hatching. During this period, the time of the occurrence of *external pipping* (EP) and chick hatching for individual eggs were recorded. These data were used to calculate the duration of EP (duration between EP and hatch) and total incubation duration (time between setting and hatching). Also, the numbers of the hatched chicks were recorded according to treatments and injection day to determine hatchability and spread of hatch as the time spent between the hatching of the first and the last chick. A total of 15 day old chicks per treatment were used to assign chick quality using Tona scoring method (Tona et al., 2003). According to this method, physical parameters including reflex, down and appearance, eyes, conformation of legs, navel area, yolk sac, remaining membranes and yolk were scored. The chick quality score was defined as the sum of the scores assigned to each quality parameter.

Organ and embryo body weights during incubation

At *internal pipping* and hatching stages, samples of 15 eggs from each group were broken to collect and weigh yolk sac, heart, liver and embryo. At the end of incubation, unhatched eggs were counted and broken to identify eggs containing dead embryos while day-old chicks were collected, counted and weighed. These data were used to determine chick body weight, relative weight of the embryo, heart, liver and the yolk sac and late embryonic mortality rate.

Post hatch juvenile growth performance

At the end of hatch, a total of 672 day-old chicks, representing the 7 experimental groups (T0, M16, T16, M17, T17, M18 and T18), were reared for 7 days. Each group was divided into 3 replicates of 32 chicks each. The chicks were offered feed (broiler starter diet) (Table 1) and water *ad libitum* during 7 days. Then, the birds were weighed to determine the 7 day old body weight.

Table 1. Starter diet formulation and calculated values of crude proteins and metabolisable energy

Zusammensetzung der Futtermischung (Starter) und kalkulierte Werte für Rohprotein und umsetzbare Energie

Feed ingredients (%)	Starter diet
Maize	53
Wheat bran	5.7
Fish meal	8.5
Soybean	25.8
Broiler concentrate	5
Methionine	0,6
Lysine	0,4
Oyster shell	1
Total	100
Calculated values	
Crude Proteins (%)	22,50
Metabolisable energy (MJ/Kg)1	12.73
Crude fibre	4.70
Available Phosphorus (%)	0.05

[1] Metabolisable energy (ME) is calculated according to the method provided by BOURDILLON et al. (1990)

Statistical analysis

Data were processed using GraphPad Prism 5.0 software. Hatchability was considered as binomial in distribution. A 2-tailed test for comparison of variances was used to analyse the influence of injection time on hatchability. The generalised linear regression model was used to analyse the effect of injection time on organ relative weight, body weight and incubation duration. If the overall p-value was statistically significant ($P < 0.05$), further comparisons among groups were made according to Tukey's test. The results are expressed as mean ± standard error of mean.

Results

Effect of injection time on total incubation and external pipping durations, late embryonic death rate, hatchability and chick quality.

The effects of *in ovo* inoculation of MOL extract at different time periods during incubation on EP duration, total duration of incubation, late embryonic death rate and hatchability are presented in Table 2. EP duration was not affected. The injection of saline solution at d 16 of incubation significantly prolonged the total incubation duration compared to other groups ($p < 0.05$). The day of MOL extract injection did not affect the total incubation duration in M16, M17 and M18. However, the duration was significantly lower than the values of T0 and all saline injected groups ($p<0.05$). The lowest hatchability in T16 was followed by increasing values in T17 and T18. Hatchability of eggs injected with MOL was lowest at d 16, followed by an increase at d 17 and d 18. Eggs of the T0 group had the same hatchability as those injected with MOL extract at d 18. Mortalities were higher in the eggs injected with saline compared with their counterparts injected with MOL extract. The highest effect of saline was at d 16 and d 17 with 48 and 46% embryo mortality, respectively. MOL extract injection also caused significant mortality at d 16 and d 17 compared to the uninjected eggs (T0). But the results were significantly lower in the T groups. With exception of the T18 group, the percentage of day-old chicks with high quality (higher average chick quality score) ($p < 0.05$) was better in the M groups than in the T groups. However, no difference between M groups and T0 group was observed. The highest chick quality was observed in the M18 groups and the lowest in the T16 groups.

Table 2. Duration of external pipping, total incubation duration, hatchability and late embryonic death according to treatment

Dauer des „external pipping“, Gesamtbrutdauer, Schlupffähigkeit und späte embryonale Mortalität in den unterschiedlichen Versuchsgruppen

	Treatments						
Parameters	**T0**	**M16**	**T16**	**M17**	**T17**	**M18**	**T18**
External pipping duration (h)	9.76 ± 2.96	13.00 ± 0.68	11.7 ± 1.75	12.3 ± 1.82	12.7 ± 1.23	10.3 ± 2.73	11.0 ± 1.94
Total incubation duration (h)	490 ± 0.59^{b}	487 ± 0.79^{c}	495 ± 0.58^{a}	486 ± 1.71^{c}	490 ± 0.74^{b}	487 ± 0.88^{c}	490 ± 0.76^{b}
Hatchability (%)	81.7 ± 0.50^{a}	64.9 ± 0.50^{e}	52.7 ± 0.50^{f}	74.12 ± 0.50^{c}	67.2 ± 0.50^{d}	81.4 ± 0.50^{a}	77.7 ± 0.50^{b}
Chicks of higher quality (%)	85.79 ± 0.58^{a}	87.09 ± 0.55^{a}	78.34 ± 0.53^{b}	84.69 ± 0.52^{a}	81.90 ± 0.6^{c}	88.15 ± 0.65^{a}	86,79 ± 0,66^{a}
Late embryonic mortality (%)	8.00 ± 2.00^{d}	12.20 ± 2.00^{c}	48.00 ± 2.00^{a}	15.00 ± 1.90^{b}	46.0 ± 2.00^{a}	10.0 ± 0.01^{d}	12.5 ± 2.00^{c}

$^{a-f}$ Within rows, means sharing no common superscript letter are significantly different *($P < 0.05$).* T0 = Control; M16 = injection of MOL at d16; T16 = injection of saline at d16; M17 = injection of MOL at d 17; T17 = injection of saline at d 17; M18 = injection of MOL at d 18; T18 = injection of saline at d 18.

Effect of injection time on relative organ weight, relative embryo weight and day-old chick weight

Table 3 shows the relative weights of liver, heart, yolk sac, embryo body weight and day-old chick weights. At *internal pipping* stage, there was no significant difference between the treatments in the relative weight of the heart of the embryo. Similarly, the relative yolk sac weights or the relative embryo weights were not affected by the different treatments. However, the relative weights of liver of the embryos injected with saline or MOL extract at d 16 were significantly lower compared to those injected on d 17, on d 18 and the control. Those injected on d 17 or d 18 had similar relative liver weights compared to the uninjected ones (T0).

Table 3. Relative weight of organs and the embryo at internal pipping and relative weights of organs and body weights of newly hatched chicks according to treatments

Relative Gewichte von Organen und der Embryonen zum Zeitpunkt des „internal pipping“ bzw. der geschlüpften Küken in den unterschiedlichen Versuchsgruppen

Parameters	Treatments						
	T0	M16	T16	M17	T17	M18	T18
Internal pipping stage							
Liver (%)	2.20 ± 0.12[a]	2.00 ± 0.07[b]	1.96 ± 0.07[b]	2.14 ± 0.10[a]	2.16 ± 0.13[a]	2.21 ± 0.11[a]	2.12 ± 0.11[a]
Heart (%)	0.620 ± 0.03	0.570 ± 0.02	0.570 ± 0.03	0.560 ± 0.03	0.680 ± 0.11	0.610 ± 0.03	0.570 ± 0.04
Yolk sac (%)	24.7 ± 1.81	26.12 ± 0.06	25.7 ± 1.19	24.2 ± 0.87	23.2 ± 1.96	24.1 ± 1.12	25.9 ± 0.45
Relative embryo weight (%)	82.5 ± 0.62	82.8 ± 1.66	82.7 ± 0.57	82.8 ± 1.94	82.7 ± 1.16	83.9 ± 0.86	83.3 ± 1.98
Hatching stage							
Liver (%)	2.42 ± 0.08[a]	2.19 ± 0.05[b]	2.15 ± 0.05[b]	2.40 ± 0.07[a]	2.37 ± 0.08[a]	2.50 ± 0.07[a]	2.36 ± 0.08[a]
Heart (%)	0.790 ± 0.04	0.69 ± 0.07	0.700 ± 0.02	0.750 ± 0.04	0.750 ± 0.04	0.780 ± 0.04	0.730 ± 0.03
Yolk sac (%)	9.44 ± 0.75[b]	11.6 ± 0.50[a]	12.2 ± 0.68[a]	12.9 ± 1.38[a]	12.2 ± 0.78[a]	9.75 ± 0.47[b]	12.5 ± 0.83[a]
Chick weight (g)	43.4 ± 2.96	43.7 ± 1.57	44.8 ± 0.14	44.7 ± 2.30	40.7 ± 3.62	42.3 ± 1.96	42.2 ± 2.44

[a-c] Within rows, means sharing no common superscript letter are significantly different ($P < 0.05$). T0 = Control; M16 = injection of MOL at d16; T16 = injection of saline at d16; M17 = injection of MOL at d 17; T17 = injection of saline at d 17; M18 = injection of MOL18; T18 = injection of saline at d 18.

At hatch, the heart and the liver maintained the trend in relative weights observed at the pipping stage. The relative yolk sac weight of the embryos injected with saline at d 16, d 17 and d 18 or MOL extract at d 16 and d 17 were significantly higher than that of the T0 and M18 groups. There was no significant difference between T0 and M18 groups. Chick body weights were not significantly different between treatments.

Effect of injection time on hatching curve

Figure 1 shows the effect of the injection time period of MOL extract and saline solutions on hatching curve. The eggs from M groups hatched better than those of the T groups. About 80% of the chicks of M and T0 groups hatched before 492 h of incubation whereas such percentage was not obtained in T group until after 502 h. In the T16 group, 60% of chicks hatched after 502 h. The first hatch occurred before 478 h in eggs from the M groups while the first hatch took place at 480 h in eggs from the T groups.

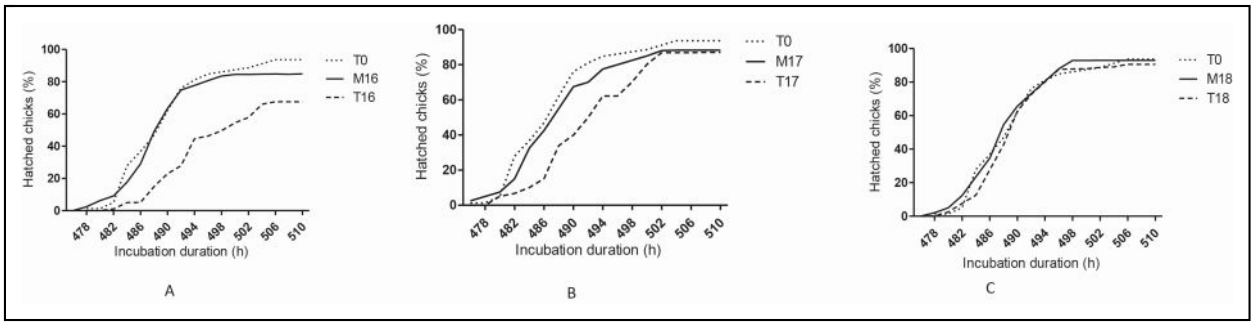

Figure 1. Hatching curves in relation to the incubation duration and injection time. A = injection at day 16; B = injection at day 17, C = injection at day 18

To = Control; M16 = injection of *Moringa oleifera* leaves (MOL) extract at d16; T16 = injection of saline at d16; M17 = injection of MOL at d 17; T17 = injection of saline at d 17; M18 = injection of MOL at d 18; T18 = injection of saline at d 18.

Schlupfkurven in Abhängigkeit von der Inkubationsdauer und des Zeitpunktes der Injektionen. A = Injektion am Bruttag 16; B = Injektion am Bruttag 17, C = Injektion am Bruttag 18

To = Kontrollgruppe; M16 = Injektion von *Moringa oleifera* Blätter(MOL)Extrakt am Bruttag 16; T16 = Injektion von Salzlösung am Bruttag 16; M17 = Injektion von MOL am Bruttag 17; T17 = Injektion von Salzlösung am Bruttag 17; M18 = Injektion von MOL am Bruttag 18; T18 = Injektion von Salzlösung am Bruttag 18.

Effect of injection time on seven-day-old chick's growth performance

Growth performance of chicks that hatched from eggs in the different experimental treatments are summarised in Figure 2. In general, chicks from eggs inoculated with MOL extract solution were significantly heavier than chicks from eggs that were not injected (T0) ($p < 0.05$). The body weights of chicks from eggs injected with saline (T16, T17 and T18) were not different from those from the T0 group. Among eggs injected with MOL extract, the weight of chicks of the M18 group was significantly higher than those of groups M16 and M17 ($p<0.05$).

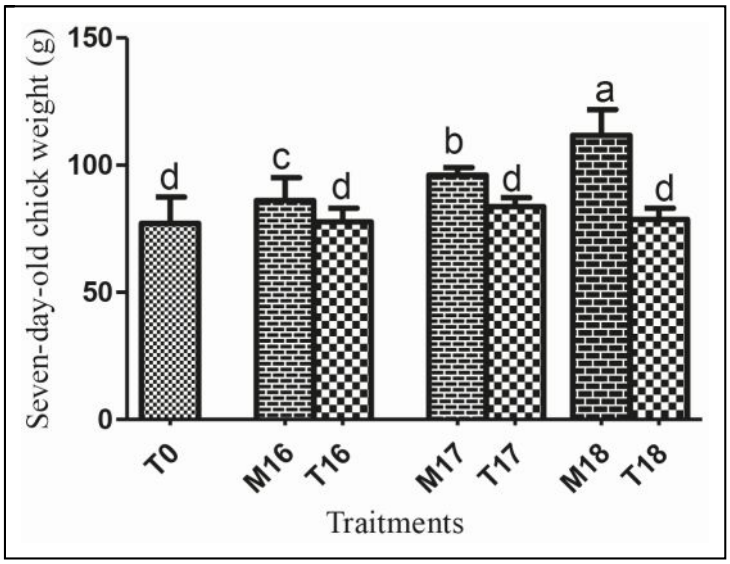

Figure 2. Seven-day-old chick weight (g) in broiler chickens experienced with different *in-ovo* feeding treatments

[a-d] Means sharing no common superscript letter are significantly different *(P < 0.05)*. To = Control; M16 = injection of *Moringa oleifera* leaves (MOL) extract at d16; T16 = injection of saline at d16; M17 = injection of MOL at d 17; T17 = injection of saline at d 17; M18 = injection of MOL at d 18; T18 = injection of saline at d 18.

Körpermassen sieben Tage alter Broilerküken (g) mit unterschiedlicher *in-ovo* Fütterung

To = Kontrollgruppe; M16 = Injektion von *Moringa oleifera* Blätter(MOL)Extrakt am Bruttag 16; T16 = Injektion von Salzlösung am Bruttag 16; M17 = Injektion von MOL am Bruttag 17; T17 = Injektion von Salzlösung am Bruttag 17; M18 = Injektion von MOL am Bruttag 18; T18 = Injektion von Salzlösung am Bruttag 18

Discussion

The results from this study showed that 1) injecting phytochemical materials in hatching eggs during incubation affects embryo development; 2) the response of the embryo depends on the timing of the injection during the last days of incubation and 3) the response depends on the nature of the material injected.

The positive effect of the injection of MOL extract dissolved in saline compared to the saline injected group on all parameters may be due to the active components that are present in *Moringa oleifera* leaves, which improved embryo development and viability. However, its effects compared with the non-injected group were masked by the injection itself. Thus, the effect of MOL extract on the parameters was less or just equal to those found in the uninjected eggs.

Our observation on the influence of injection of saline or MOL extract on organs in the present study suggests that the effect depends not only on the substance injected but also on the time of injection. The relative liver and heart weight was not affected when injected at d 17 or d 18, both at the pipping and hatching stage. These results are in line with the reports of N'NANLE et al. (2017) who pointed out that *Moringa oleifera* injection in the egg air chamber did not affect relative weight of liver and heart. The reduction, however, of relative liver weight of embryos of eggs injected at d 16 in both the MOL and saline treatment groups at the pipping and hatching stages, compared to those injected at the later days (d 17 and d 18) of incubation in this study suggest that the effect of injection on liver weight was time dependent. Except the injection of MOL extract at d 18, yolk sac relative weight was increased beyond the control when saline or MOL extract were injected at all time period. The similarity in the yolk sac weights of the eggs injected at d 18 across the treatments is in agreement with the finding of SALAHI et al. (2011) who stated that there was no difference in the yolk sac weight of eggs injected at d 18. However, our observation on the variation in the yolk sac weights at day 16 and 17 is at variance with the finding of SALAHI et al. (2011) who did not report any difference in the yolk sac weights at these days. This difference may be due to the strain of chickens. The similarity of the embryos and chicks weights during pipping and hatching, respectively, is in agreement with CHEN et al. (2009) who did not observe any difference on embryos and day old chick weight after glutamine injection.

Even though liver weight was reduced by both saline and *Moringa* injections, this did not affect the duration of external pipping of the embryos in all treatments. Surprisingly, the total incubation duration was reduced significantly by *Moringa* injection. Consequently, the hatching window was shorter in the M groups compared to the T groups. This result suggests that *Moringa oleifera* inoculation may improve hatching conditions and, therefore, may be reflected in chick quality. In fact, M groups recorded higher chick quality score than T groups. According to ARAÙJO et al. (2016), the shorter the hatch window is, the better the physical quality of broiler chicks. Thus, there was no consistent relationship between effects on organs and hatching duration.

The reduction of hatchability in the eggs injected at d 16 and 17 in this study may be due to injection stress, which the embryos at this stage could not withstand. The *in ovo* procedure may have caused an allergic reaction under the air cell thus causing the death of the embryos by asphyxie (SALMANZADEH et al., 2016). These finding is consistent with PEDROSO et al. (2006) and TONA et al. (2006) who reported that the injection at d 16 of dexamethasone lowered hatchability due to stress. The improvement in the hatchability in the M groups may be due to a potential role of bioactive substances in *Moringa* extract. *Moringa* has been reported to contain antioxidants, such as phenols and flavonoids (TETEH et al., 2013) and vitamins A, C and E (GOWRISHANKAR et al., 2010) which have antioxidant and free-radical-scavenging ability (ROBAK and GRYGLEWSKI, 1988) that could reduce oxidative stress. According to SURAI et al. (2016), if high numbers of reactive oxygen species are formed in bird cells, these need to be combated with antioxidants. Thus, it can be inferred that antioxidant elements present in *Moringa oleifera* extract acted by minimising the oxidative stress of bird, thereby improving hatchability and chick quality. The decrease in total incubation duration observed in eggs injected with *Moringa* might have impacted on the higher hatchability and chicks' quality observed at d 16, d 17 and d 18 compared to saline injections.

The growth promoting potential of *Moringa* was evident at d 7 post-hatch. Growth rate was enhanced significantly in the chicks of M group. Moreover, the effect was more pronounced in the birds hatched from the eggs injected with *Moringa* extract at d 18 followed by d 17 and d 16. It has been shown that improved growth performance was dependent upon the development of the gastrointestinal tract and *in ovo* feeding of the nutrient used (SALMANZADEH et al., 2016). The better post-hatch performance of the chicks from M groups can be associated to the effect of *Moringa* on intestinal morphology and digestive function (NKUKWANA, 2012).

It could be concluded that, *in ovo* inoculation into the egg air chamber should be done at d 18 for the enhancement of hatchability, reduction of late embryo mortality and the promotion of early juvenile post-hatch growth of day-old chicks.

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