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Effect of *in ovo* inoculation of *Moringa oleifera* leaves extract on hatchability and chicken growth performance

Einfluss der in ovo-Inokulation eines Extraktes aus *Moringa olifeira* Blättern auf die Brutfähigkeit und das Wachstum der Eintagsküken

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Introduction

Egg and poultry meat account for 35.56% of human animal protein requirements in the world (Nys et al., 2011). Considering the progress of animal product consumption in developing countries, a 3.9% increase in poultry product consumption is projected, while cattle and pigs will contribute respectively for 2.9% and 2.4% up to 2020 (Delgado et al., 2003). The supply of this demand requires a substantial production of day-old chickens. Hatchery objectives are to obtain high hatchability with day-old chickens of optimal quality in order to maximise profitability. According to Tona et al. (2004), chicken quality is correlated to hatching egg quality, which depends partly on its nutrient content. Klasing (1998) pointed out that from d 15 of incubation, the chicken embryo starts absorbing nutritive substances through the amniotic fluid and reported that besides incubation conditions, hatching egg nutrient content influences hatchability and chicken quality. Based on these results, studies were undertaken to improve hatching performance through exogenous nutrient administration into hatching eggs. Indeed, Bhanja et al. (2012) reported better hatchability with *in ovo* administration of amino acids. Contrary to this, Nouboukpo et al. (2010) showed that L-carnitine administration, at d 18 of incubation, in the air chamber of hatching eggs from *Hissex Brown* layer breeders did not improve hatchability, although the yolk sac content was better utilised by the embryo. In addition, *in ovo* administration of ghrelin (an acylated peptide) (Lotfi et al., 2013) and glucose (Salmanzadeh et al., 2012) resulted in an increased hatching weight. Also, *in ovo* inoculation of extracts of many plant products such as *Toussaintia patriciae, Eugenia jambolana* and *Guiera senegalensis,* have 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). Immune status reinforcement was also shown by Chollom et al. (2012) who fed embryos with *Moringa oleifera* seeds. *Moringa oleifera* leaves and seeds are widely consumed in Africa and Asia by humans as a dietary supplement and medicinal plant (Moyo et al., 2011; Tété-Bénissan et al., 2013). It is also used for water purification (Suarez et al., 2003). Several studies have shown that *Moringa oleifera* contains many nutrients (carbohydrates, proteins, amino acids, vitamins and mineral salts) and can be used as animal feed (Dougnon et al., 2012; Sultana et al., 2015). At certain rate of incorporation, *Moringa* leaves should be able to improve chicken growth (Teteh et al., 2013; Safa, 2014) and egg production (Abou-Elezz et al., 2012; Teteh et al., 2016). Although literature about *Moringa oleifera* leaves is rich in reports on poultry performance, effects of these leaves on chicken embryo development and hatching performance retained very little attention. Therefore, the current study was designed to investigate the effects of *Moringa oleifera* leaves extract on hatchability, chicken quality and post-hatch growth performance.

Material and methods

Experimental design

A total of 650 hatching eggs from ISA Brown layer-breeders provided by Belgabroed (Hoogestraten, Belgium) were incubated at standard incubation conditions including temperature of 37.6°C, relative humidity of 50 to 60% and turning once an hour until d 18 of incubation. The previous study (McGruder et al., 2011) showed that there was no difference between eggs injected with saline solution and untreated control group on hatchability and growth of broilers. Then at d 18 of incubation, incubated eggs were divided into 4 groups of 150 eggs each. These groups were (1) Control (MO): eggs without any treatment, (2) $MO_{0.5}$: eggs injected with 0.5 µg/ml, (3) MO_5: eggs injected with 5 µg/ml and (4) MO_{50}: eggs injected with 50 µg/ml. *Moringa oleifera* extract was injected into the air chamber of incubated eggs with evidence of a living embryo. At hatch, chicken weights were recorded and chicken quality was determined. Then, chickens were fed *ad libitum* with broiler standard diets during 7 weeks.

Moringa oleifera leaves extraction

Moringa oleifera leaves collected from rural areas of Togo were dried under an air conditioning system. Dried leaves were pulverised into powder and weighed. Four hundred and twenty-five grams of the powder was soaked in a liquid containing 2 l ethanol and 2 l distilled water during 72 h. The mixing obtained was homogenised and filtered. The pure solution obtained was evaporated with Rotavapor R-210 and Heating bath B-491 (Büchi, Switzerland) according to a manual for phytochemical screening of medicinal plants (Debale, 2002) to obtain 140 g of hydro-alcoholic extract.

Moringa leaves extract administration

To 20 ml of saline solution (0.9% of NaCl), 10 mg of *Moringa* leaves extract were added. The mixture obtained was homogenised with vortex to obtained 500 µg/ml of solution. After successive dilutions, a series of injectable solutions ($MO_{0.5}$, MO_5 and MO_{50}) containing respectively 0.5 µg/ml, 5 µg/ml and 50 µg/ml were obtained. Injection of *Moringa* extract was made possible by candling each egg for evidence of a living embryo and air chamber localisation. Then, a needle of 18 G was used to drill two holes through the shell above the air chamber in order to decrease the pressure within and thereby facilitating the retention of the injected solution. The injection volume for all groups was 100 µl/egg. After injection into one of the holes, both holes were sealed with adhesive tape and the egg was placed in the hatching baskets.

Hatchability, chicken quality assessment and organs' weighing

At hatch, day-old chickens were recorded according to treatment, weighed and subjected to chicken quality assessing. All unhatched eggs were opened and classified as "unfertile eggs" and eggs with dead embryos. The numbers of hatched chickens and fertile eggs were used to determine hatchability as: 100 × number of hatched chickens/number of fertile eggs. Chicken quality assessment was done using the Tona scoring system (Tona et al., 2003). According to this method, physical parameters including reflex, down and appearance, eyes, conformation of legs, navel area, yolk sac, and remaining membranes and yolk were scored. The quality score for a chicken was defined as the sum of the scores assigned to each quality parameter.

After chicken quality assessment, samples of 20 chickens per treatment were weighed and slaughtered to collect heart, liver, yolk sac and hatching muscle. These data were used to calculate relative organ weight (organ weight × 100/chicken body weight).

Chicken rearing

According to each treatment, the remaining chickens (300) were divided into 3 replications of 25 birds and were assigned to a corresponding floor pen. Chickens were reared during 7 weeks. During this period, they were fed *ad libitum* with starter diet formulated to meet their requirements in metabolisable energy, crude protein, Ca, P, lysine and methionine (Table 1). Weekly, body weight and feed intake of each replication were recorded to determine body weight, feed intake and weight gain of each treatment.

Table 1. Composition of the experimental diet (%)

Zusammensetzung der Versuchsrationen

Ingredients	*Level*
Maize	56.6
Wheat bran	11.0
Soybean	17.0
Oyster shell	0.400
Broiler concentrate 5%	3.00
Fish meal 40%	12.0
Calculated analysis	
ME (MJ/kg)	12.43
Crude protein (%)	20.0
Calcium (%)	0.925
Phosphorus (%)	0.789
Lysine (%)	1.17
Methionine (%)	0.495
Crude fibre (%)	4.88

Statistical analysis

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

Results

Effect of Moringa extract on hatchability and chicken quality

Hatchability and day-old chicken quality were affected by *in ovo Moringa oleifera* administration as shown in Table 2. Indeed, hatchability of eggs subjected to 0.5 µg/ml (97.8%) was higher than those of the control and other *Moringa* extract treatments ($P<0.05$). Hatchability of control eggs and eggs subjected to 5 µg/ml and 50 µg/ml were comparable.

Table 2. Effect of *Moringa oleifera* leaves extract injection on hatchability and day-old chicken quality (data sharing no common letter are different; *P*<0.05)

Einfluss der Injektion des Extraktes aus Moringa olifeira Blättern auf die Schlupfrate und die Kükenqualität (Zahlen mit unterschiedlichen lateinischen Buchstaben sind signifikant verschieden, P < 0,05)

Parameters	Treatments			
	MO_0	$MO_{0.5}$	MO_5	MO_{50}
Hatchability (%)	68.8[b]	93.8[a]	71.1[b]	73.9[b]
Activity	5.64 ± 0.253	5.47 ± 0.257	4.31 ± 0.485	5.12 ± 0.370
Down and appearance	9.76 ± 0.242	10.0 ± 0.000	10.0 ± 0.000	10.0 ± 0.000
Retracted yolk	7.64 ± 1.02	8.00 ± 0.853	8.25 ± 0.999	7.41 ± 1.02
Eyes	15.8 ± 0.242	16.0 ± 0.000	15.5 ± 0.500	14.6 ± 0.790
Legs	16.0 ± 0.000	16.0 ± 0.000	15.5 ± 0.500	16.0 ± 0.000
Navel area	4.91 ± 0.713[c]	8.13 ± 0.608[a]	8.25 ± 0.750[a]	7.24 ± 0.609[b]
Remaining membrane	12.0 ± 0.000	11.7 ± 0.186	10.4 ± 0.544	11.8 ± 0.235
Remaining yolk	16.0 ± 0.000	15.4 ± 0.415	14.2 ± 0.774	15.5 ± 0.471
Chickens with score of 100 (%)	12.1	33.3	9.38	20.6
Chickens with score between 90 and 100 (%)	27.3	33.3	34.3	35.3
Average score for all chickens	87.7 ± 1.45	90.8 ± 1.42	86.4 ± 1.72	87.7 ± 1.93

Overall, total scores were similar between the 4 groups. Considering each chicken quality parameter, scores were similar between the 4 groups except for the navel area. Chickens from eggs subjected to *in ovo* administration of 50 µg/ml had the lowest score (7.24 ± 0.61) compared to groups $MO_{0.5}$ (8.13 ± 0.61) and MO_5 (8.25 ± 0.75) which had similar scores. Moreover, *Moringa* extract improved significantly the navel area score compared to the control group ($P<0.01$ and $P<0.05$).

Effect of extract on organ relative weight

Table 3 indicates relative weight of hatching muscle, heart and liver according to the treatments. Relative weights of heart and liver were not affected by treatments. But, relative hatching muscles of chickens from eggs subjected to *in ovo* administration of *Moringa* extract were similar but lower than that of the control group ($P<0.05$). Also, yolk relative weight was affected by treatment and was in the following order: $MO_{50} < MO_5 < M_0 < MO_{0.5}$ ($P<0.05$).

Table 3. Effect of *Moringa oleifera* extract injection on organs relative weight at hatch (data sharing no common letter are different; *P*<0.05)

Einfluss der Injektion des Extraktes aus Moringa olifeira Blättern auf das relative Organgewicht beim Schlupf (Zahlen mit unterschiedlichen lateinischen Buchstaben sind signifikant verschieden, P < 0,05)

Organs	*Treatments*			
	MO_0	$MO_{0.5}$	MO_5	MO_{50}
Hatching muscle	1.06 ± 0.204[a]	0.928 ± 0.151[b]	0.928 ± 0.074[b]	0.966 ± 0.107[b]
Yolk	12.5 ± 0.854[c]	10.8 ± 0.257[d]	13.2 ± 1.25[b]	14.1 ± 1.49[a]
Heart	0.796 ± 0.119	0.759 ± 0.186	0.708 ± 0.137	0.788 ± 0.091
Liver	2.39 ± 0.091	2.42 ± 0.055	2.61 ± 0.129	2.43 ± 0.153

Effect of the extract on chicken body weight feed intake and weight gain

Chicken weight at hatch, feed intake and daily weight gain are shown in Table 4 according to treatments. At hatch, chickens of group MO_{50} were significantly heavier (39.8 ± 0.39 g) than those of the control group (38.0 ± 0.37 g) ($P<0.05$), while the weights of chickens of both groups $MO_{0.5}$ and MO_5 were comparable to those of the control and MO_{50} groups. However, feed intake and body weight gain up to 7 weeks of age were not affected by *Moringa oleifera* leaves extract though increased body weight was noted with increased extract concentration. Irrespective of treatment, The Figure 1 shows that body weight increased with increasing age of broilers. From week 5 onward, chickens from the control group had the lowest body weight ($P<0.05$ at week 6 and $P<0.01$ at week 6) while chickens from eggs subjected to *in ovo* administration of *Moringa* extract had comparable weights during rearing period.

Table 4. Effect of *Moringa oleifera* extract injection on day-old chicken weight, daily feed intake and daily weight gain (data sharing no common letter are different (*P*<0.05)

Einfluss der Injektion des Extraktes aus Moringa olifeira Blättern auf das Gewicht der Eintagsküken, die Futteraufnahme und die Gewichtszunahme (Zahlen mit unterschiedlichen lateinischen Buchstaben sind signifikant verschieden, P < 0,05)

Parameters	*Treatments*			
	MO_0	$MO_{0.5}$	MO_5	MO_{50}
Day-old chicken weight (g)	38.0 ± 0.374^{b}	38.4 ± 0.323^{ab}	38.8 ± 0.607^{ab}	39.8 ± 0.387^{a}
Daily feed intake (g)	23.4 ± 3.77	26.2 ± 4.39	28.5 ± 4.81	33.3 ± 5.41
Daily weight gain (g)	4.69 ± 0.704	5.38 ± 0.799	5.68 ± 0.658	6.07 ± 0.862

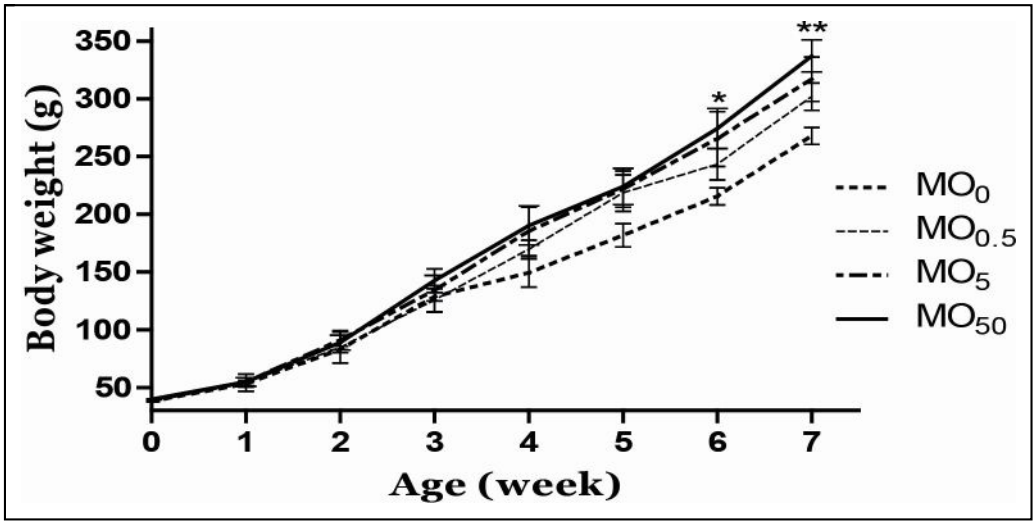

Figure 1. Effect of *Moringa oleifera* injection on chicken weight until 7th week. * *P*< 0.05 and ** *P*< 0.01 The comparison is done between treated and control groups.

Einfluss der Injektion des Extraktes aus Moringa olifeira Blättern auf die Gewichtsentwicklung bis zur siebten Lebenswoche (* P < 0,05, **< 0,01; es erfolgt ein Vergleich der Behandlungsgruppen mit der Kontrolle).

Discussion

In ovo administration of *Moringa oleifera* leaves extract to chicken embryos diversely affected embryo development and post hatch growth performance. At hatch, only chickens from eggs subjected to 50 µg/ml of *Moringa* extract were significantly heavier than those of the control group. In addition, the chickens of the MO_{50} group had the highest yolk relative weight suggesting that chicken body weight was associated with increased yolk sac mass as reported by De Oliveira (2007). The effect of *Moringa* extract on chicken weight and yolk relative weight may be due to the fact that increasing of *Moringa* extract concentration could reduce yolk utilisation, as energy source, by the embryo. Moreover, lower relative hatching muscle of chickens from eggs that received *Moringa* extract compared to the control group suggests that administration of *Moringa* extract at d 18 of incubation affects negatively energy utilisation to support the hatching process. In addition, Oluduro (2012) showed that *Moringa oleifera* leaves contain high levels of saponines, which could inhibit lipids metabolism by animals resulting in low use of the yolk sac and its high relative weight (Francis et al., 2002). The positive correlation between the yolk sac weight and the extract concentration is in the line with results of Richter et al. (2003), Moyo et al. (2011) and Teteh et al. (2016). The highest hatchability obtained in group $MO_{0.5}$ can be due to high availability of energy during the hatching process. The lowest relative yolk sac weight of chickens from $MO_{0.5}$ group together with the highest hatchability can be explained by an increase in lipid metabolism resulting in high energy production for the hatching process. It can be hypothesised that *in ovo* administration of *Moringa* extract inhibits or stimulates lipid metabolism in a dose-dependent manner. Moreover, Moran (2007) showed the glycogen utilisation during the hatchability process. Indeed, once the choriallantoide membrane is detached during pipping, oxygen supply and lipid catabolism are limited. From this stage onward, the hatching muscle uses exclusively glucose provided from glycogen reserves (Freeman, 1969), increases its size prior to internal pipping and loses its weight during hatch (John et al., 1987; Moran, 2007). *Moringa* extract contains substances, which, according to De Oliveira (2007), favour glycogen stocking in liver more than in hatching muscle when injected *in ovo*. In this study, the reduced hatching muscle weight confirms the finding of De Oliveira (2007) who reported decreased hatching muscle weight when turkey eggs were fed *in ovo* with exogenous carbohydrates.

Chicken quality was not affected by *Moringa oleifera* extract administration. The first 18 d of incubation are mainly devoted to organogenesis and embryo growth while d 19 to 21 are used mainly by the embryo for the hatching process (Pearson et al., 1996, De Smit et al., 2006). Hence, the extract injected at d 18 and nutrients provided were not yet rationally used to perform their physical purpose except for the navel area that depends on the yolk absorption during the last three d of incubation (Hamburger and Hamilton, 1951). However, chickens of group $MO_{0.5}$ showed high total scores probably to a better nutrient metabolism and absorption through amniotic fluid (Klasing, 1998; Tona et al., 2004) induced by 0.5 µg/ml of extract concentration confirming the optimum feed transit and nutrient absorption at low *Moringa* leave concentrations in bird feed (Teteh et al., 2016 and 2017).

During the rearing period, although chickens were not fed with *Moringa* leaves, the extract injected since 18^{th} d of incubation affected significantly the body weight at 6 and 7 weeks of age. This supports results of Bhanja et al. (2006) and Shafey et al. (2014) who pointed out body weight improvement of meat- type chickens fed *in ovo* with vitamins and amino acids. This growth performance of chickens from eggs that received *Moringa* extract, especially in the group MO_{50} suggests that embryos stocked nutrients provided by the extract of *Moringa oleifera* leaves (Makkar and Becker, 1996). During the first days of rearing, chickens mobilise progressively the nutrients as an additional substance to the starter diet given that feed intake was not affected.

It can be concluded that *Moringa oleifera* leaves extract administration has differential effects on embryo and post-hatch performance in a dose-dependent manner. *In ovo* administration of 0.5 µg/ml at d 18 of incubation improves hatchability rate, day-old chicken weight and navel area aspects through optimising the yolk components.

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Summary

After the ban of antibiotic growth promoters, plants such as *Moringa oleifera* are subjected to studies to investigate their effect on poultry production. Following the same goal, this study was carried out to evaluate the effect of *Moringa oleifera* leaves extracts on hatchability and chicken growth performance. So, 450 g of *Moringa oleifera* leaves were subjected to extraction. The extract obtained was used to prepare injectable solutions administrated at d 18 of incubation into 580 fertile eggs obtained from *Isa Brown* layer breeders. Eggs were divided into 4 groups: MO_0, $MO_{0.5}$, MO_5 and MO_{50}, and injected respectively with 0 μg/ml, 0.5 μg/ml, 5 μg/ml and 50 μg/ml.

Results at hatch showed that 0.5 μg/ml had the highest hatchability and the most reduced yolk sac while the day-old chicken weight, chicken quality score, the relative weight of hatching muscle, heart and liver were similar between treated groups. During the rearing period, chickens of the treated groups were heavier than the control group with the best growth rate of group MO_{50}. These results can be explained by optimum utilisation of nutrients during incubation and the rearing period.

Key words

Broiler, incubation, *Moringa oleifera* leaves extract, hatchability, chicken quality, growth

Zusammenfassung

Einfluss der in ovo-Inokulation eines Extraktes aus *Moringa olifeira* Blättern auf die Brutfähigkeit und das Wachstum der Eintagsküken

Nach dem Verbot des Einsatzes von Antibiotika als Leistungsförderer in Futterrationen werden unter anderem die Verwendungsmöglichkeiten von Phytobiotika, wie *Moringa olifeira* Blätter, zur Verbesserung der Leistung in der Geflügelfütterung geprüft. In der vorliegenden Studie wurde daher die Auswirkung eines Extraktes aus *Moringa olifeira* Blättern auf den Bruterfolg und das anschließende Wachstum der Küken untersucht. Hierzu wurde ein Extrakt aus 450 g *Moringa olifeira* Blättern verwendet, aus dem eine injizierbare Lösung hergestellt wurde. Am 18. Bruttag wurde die Lösung mit einer Konzentration von 0,0 (MO_0), 0,5 ($MO_{0.5}$), 5.0 (MO_5) bzw. 50.0 (MO_{50}) μg *Moringa olifeira* Extrakt/ml in 580 befruchtete Bruteier einer Isa Brown Elterntierherde injiziert.

Die Injektion der Lösung mit 0,5 μg/ml führte zur besten Schlupfrate und zur deutlichsten Verminderung des Dottersacks. Dagegen wurden das Gewicht, die Qualitätsnote und die relativen Gewichte der Muskeln, des Herzens und der Leber des Eintagskükens nicht durch die Behandlungen beeinflusst. In der anschließenden Mastphase nahmen die Küken der Behandlungsgruppen besser zu als die der Kontrolle. Die höchste Zunahme erreichten Küken der Behandlung MO_{50}. Offensichtlich führte die Injektion des Extraktes zu einer besseren Nährstoffverwertung währen der Brut und in der Aufzucht.

Stichworte

Broiler, Brut, Extrakt, *Moringa oliveira* Blätter, Bruterfolg, Kükenqualität, Wachstum

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