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Effect of *Moringa oleifera* leaves on feed transit and morphometric parameters of the digestive tract of layer pullets and laying hens

Einfluss von *Moringa olifeira* Blättern auf die Futterpassage und morphologische Kennwerte des Verdauungstraktes bei Küken und Legehennen

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Introduction

The chicken digestive tract contains a lot of micro-organisms including bacteria (pathogens and non-pathogens) of about 10^{14} per g of content (Vilă et al., 2010). Feed undertakes a transit along the digestive tract where it is subjected to digestion and absorption processes. Feed transit time is closely linked to amount of ingested nutrients by the animal and influences, subsequently, the digestion efficiency (Hocking et al., 2004). It depends on genotype (Warner, 1981; Hocking et al., 2004), age, health status and, especially, digestive tract integrity as well as diet composition. Digestive tract integrity can be affected by pathogens (Choct, 2009). Hence, the incorporation of antibiotics in chicken feed as growth promoters decreases significantly the detrimental effects of pathogens. However, this practice was banned in 2006 (Gould, 2008) in the European Union resulting in a decrease of production and productivity. In order to improve poultry production without incorporating antibiotics, alternatives to reduce the detrimental effects of pathogens in digestive tract could be useful. Several strategies were developed including the use of different types of prebiotics such as carbohydrates. It was reported that this could alleviate *Camphylobacter* (Iji and Tivey, 1998) and *Salmonella* (Bailey et al., 1991) populations significantly in the digestive tract, maintain the integrity of the gut and therefore improve nutrient absorption. In addition, carbohydrates, especially oligosaccharides and non-starch polysaccharides, are known to influence feed transit and histological structure of liver, pancreas and gut. The consumption of high amounts of oligosaccharides (Hellendoorn, 1979) and insoluble fibres (Krogdahl, 1986) shortened transit time with the excretion of high amount of nutrients. Moreover, it is reported that pullets fed with wheat-based diets containing 10% of husk of oats (Hetland et al., 2003), 4% of wood shavings (Hetland et al., 2004), 10% of Alfalfa (Duke et al., 1984) or 1% of inulin (Rehman et al., 2007) had high feed transit time and high relative weights of gizzard and caeca. In addition, Shen et al. (2005) demonstrated that incorporation of fructo-oligosaccharides in leghorn layer diets resulted in a heavier and longer small intestine. These previous results suggest that amount and origin of carbohydrates are key factors affecting transit time.

Plants and plant products are natural sources of carbohydrates with variable characteristics according to different factors. Therefore, the use of plant or plant product in poultry feed requires studies about its effect on peristaltic movements. For instance, it was pointed out that incorporation of *Moringa oleifera* leaves in chicken diets improves health, growth and production performances (Suarez et al., 2005; Kakengi et al., 2007; Banjo, 2012; Portugaliza and Fernandez, 2012). This positive effect of these leaves is related to its composition in polysaccharides, phenols and nutrients (Makkar and Becker, 1996; Foidl et al., 2001 and Teteh et al., 2013a). However, the effects of these leaves on digestion and absorption mechanisms of macronutrients through the digestive tract were not clarified. Therefore, the purpose of this study was to evaluate the effects of *Moringa oleifera* leaves on feed transit time and morphometric measures of the digestive tract according to the age and the incorporation level of leaves in the feed, as an attempt to better understand the mechanisms of performance improvement and to determine the optimum level of their incorporation in poultry diets.

Material and Methods

Experimental design

The trial, lasting for 40 weeks, was conducted at the Centre d'Excellence Regional sur les Sciences Aviaires (CERSA) of University of Lomé (Togo). Day-old chickens Isa brown (layer type) were produced by CERSA and fed with three diets containing different levels of *Moringa oleifera* leaves. Leaves, collected in the rural area of Togo, were rinsed, disinfected with Virocid® and dried on washed and disinfected batches under an air conditioning system. At last, they are pulverised before incorporating into diets.

Chicken feeding treatment

A total of 600 day-old ISA Brown chickens (layer-type), were divided into three groups (M0, M1 and M2) with 4 replications of 50 chickens for each group. The three groups were respectively fed with Diet 1, Diet 2 and Diet 3 from day-old to d 280. Diet 1 was the basal diet with 0% of *Moringa* leaves, while Diet 2 and Diet 3 contained respectively 1% and 2% of *Moringa* leaves. Each diet was formulated to fit crude protein (CP) and metabolisable energy (ME) of birds according to the developmental stage (Table 1).

Table 1. Gross composition of experimental diets (%)

Hauptbestandteile der Versuchsfutterrationen (%)

	Feed composition according to age and group								
	Starter mash			Grower mash			Layer mash		
Feed stuffs	M0	M1	M2	M0	M1	M2	M0	M1	M2
Maize	58.0	57.4	56.8	52.0	51.5	51.0	58.0	57.4	57.0
Wheat bran	9.30	9.70	9.00	25.0	25.0	25.0	9.00	9.00	9.00
Cotton cake	4.00	3.96	3.82	0	0	0	0	0	0
Fish meal	10	9.90	9.80	8	7.92	7.54	9.00	8.91	8.52
Soya seed	15	14.8	14.7	10	9.9	9.7	15	14.8	14.7
Concentrate	3	2.97	2.94	3.00	2.97	2.94	3.00	2.97	2.94
Oyster shell	0.750	0.740	0.740	1.75	1.73	1.72	5.75	5.69	5.64
Sodium chloride	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Moringa leaves (%)	0	1	2	0	1	2	0	1	2
Total	100	100	100	100	100	100	100	100	100
Calculated analysis									
CP (%)	19.7	19.7	19.8	17.1	17.2	17.2	17.9	17.9	17.9
ME (MJ/Kg)	12.2	12.2	12.2	11.3	11.3	11.4	11.9	11.9	11.9
CF (%)	4.89	5.69	5.85	5.60	5.75	5.91	4.58	4.74	4.91

CP = Crude Protein; ME = Metabolisable Energy; CF = Crude Fibre

Feed transit evaluation

Feed transit was evaluated using the method of Hocking et al. (2004). During 24 h after feeding, these authors collected and weighed, at 5 sampling times, digesta of different compartments of digestive tract of broiler breeders to evaluate feed transit.

About the present assay, at d 56 and d 280, 100 birds per group were isolated in three different cages. They had water *ad libitum* and were deprived of feed during 12 h. After this fasting period, birds had 2 h to feed again and had still water *ad libitum*. After this latter feeding period, the amount of feed ingested was evaluated. Birds were again deprived of feed for 24 h. During this latter period, at 1, 4, 8, 12 and 24 h after the end of the two h feeding, 20 birds of each cage were slaughtered. The digestive tract was removed and each segment (crop, gizzard, small intestine, caecum and rectum) isolated to collect its content. Wet digesta collected were immediately weighed.

Gut morphometric evaluation

After emptying, the 5 segments were suggested to morphometric measures. So, length and weight of small intestine, caeca and rectum were collected to determine relative weight (weight of segment × 100/body weight) and density (weight of segment/length of segment). About the gizzard, only its weight was registered to determine its relative weight.

Statistical analysis

Data obtained were expressed as mean ± standard error (SE) of mean and processed with statistical software package Systat 11. ANOVA model was used to analyse the effects of experimental diets on weights of different digest, relative gizzard weight, length and density of small intestine, caecum and rectum. If the overall *F*-value was statistically significant ($P < 0.05$), further comparisons among groups were made using Tuckey's test.

Results

Feed intake

Table 2 shows feed intake during the last feeding period. At 56 days old, feed intake (32.30 ± 0.23 for M0, 32.10 ± 0.33 for M1 and 31.90 ± 0.18 for M2) were similar for the three groups. This similarity was also observed at d 280 (98.3 ± 0.20 for M0, 97.5 ± 0.90 for M1, 97.3 ± 0.80 for M2).

Table 2. Feed intake during the two h of feeding (g)

Futteraufnahme während der 2 Fütterungsstunden (g)

	Groups		
Age	M0	M1	M2
D 56	32.3 ± 0.230	32.1 ± 0.330	31.9 ± 0.180
D 280	98.3 ± 0.200	97.5 ± 0.900	97.3 ± 0.800

Values are means ± SEM and are not significantly different.

Effects of Moringa oleifera leaves on feed transit at 56 days

Digesta weight. In general, weights of crop, gizzard, small intestine, caeca and rectum contents decreased from the beginning to the end of the transit (Figure 1). At 1 h, weight of every segment content was comparable while, from 4 to 24 h, significant differences appeared. Contents in crop (Figure 1A), gizzard (Figure 1B), small intestine (Figure 1C) and rectum (Figure 1E) were heavier ($P<0.05$) in M1 and M2 than M0 while their caeca content (Figure 1D) was the lowest.

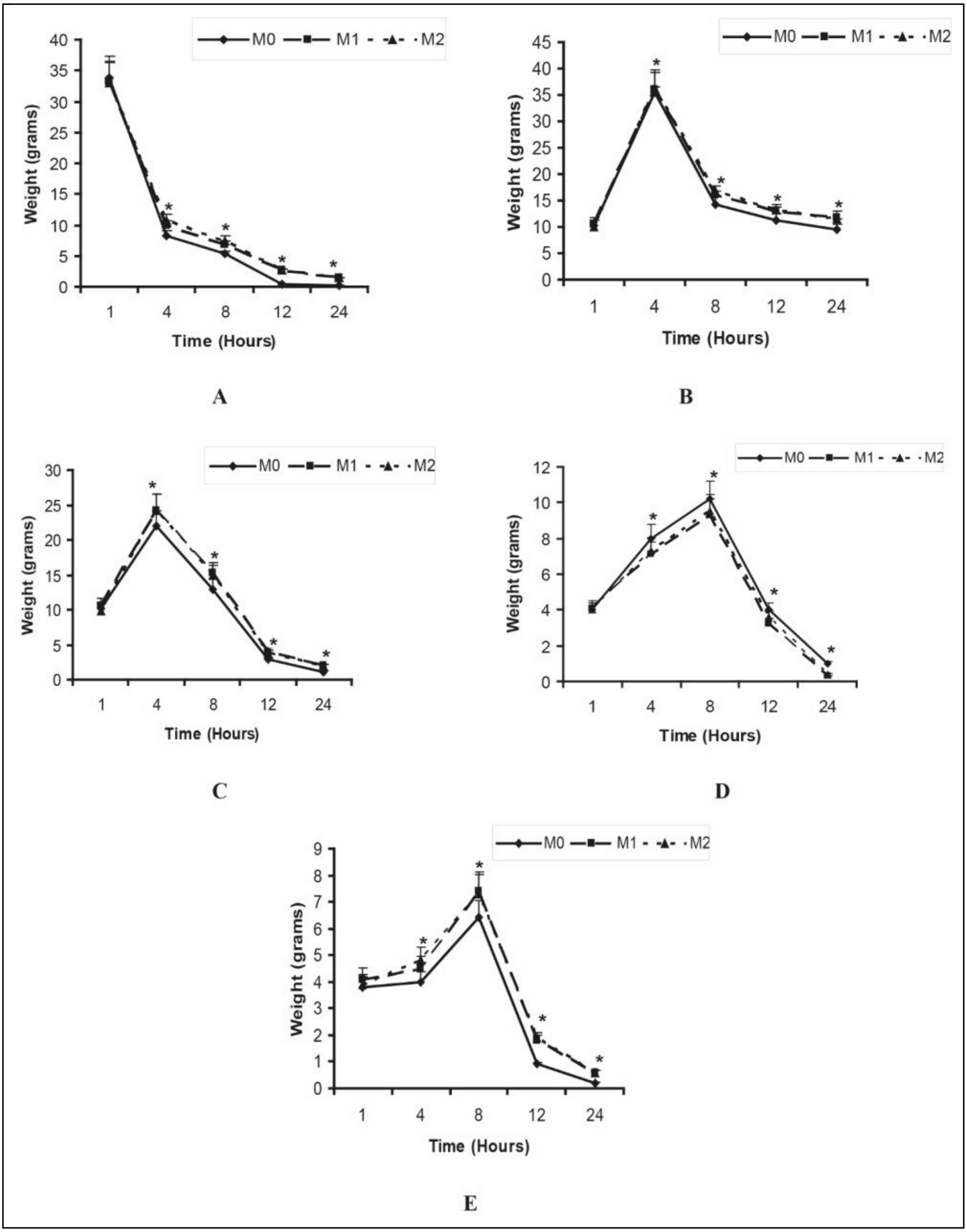

Figure 1. Digesta weight in the different segments during 24 h at d 56. Crop (A), Gizzard (B), Small intestine (C), Caecum (D) and Rectum (E). Significant differences indicated by * (*P*< 0.05)

Chymusgewichte in den verschiedenen Abschnitten des Verdauungstraktes über 24 Stunden am 56. Lebenstag. Kropf (A), Muskelmagen (B), Dünndarm (C), Blinddärme (D), Dickdarm (E). * zeigt signifikante Unterschiede zwischen den Werten an (*P*< 0,05)

Morphometric parameters. Table 3 indicates that gizzard of M0 was lighter than those of M1 and M2 ($P<0.05$). About length and density, values of small intestine and caeca were significantly lower in M0 than M1 ($P<0.01$) and M2 ($P<0.05$), while lengths and densities of rectum were similar between the three groups.

Table 3. Morphometric measures of different segments of digestive tract at 56 days of age

Morphologische Parameter der verschiedenen Abschnitte des Verdauungstraktes am 56. Lebenstag

	Groups		
Digestive tract segments	M0	M1	M2
Gizzard			
Relative weight	0.030 ± 0.001[b]	0.100 ± 0.002[a]	0.120 ± 0.004[a]
Small intestine			
Length (cm)	117 ± 4.93[c]	136 ± 3.96[a]	126 ± 3.21[b]
Density (g/cm)	0.120 ± 0.012[b]	0.270 ± 0.010[a]	0.210 ± 0.009[a]
Caecum			
Length (cm)	19 ± 1.52[c]	24.0 ± 2.00[a]	21.7 ± 1.34[b]
Density (g/cm)	0.110 ± 0.010[b]	0.180 ± 0.010[a]	0.190 ± 0.020[a]
Rectum			
Length (cm)	5.66 ± 0.160[a]	5.79 ± 0.120[a]	5.83 ± 0.440[a]
Density (g/cm)	0.320 ± 0.030[a]	0.330 ± 0.010[a]	0.310 ± 0.020[a]

Values are means ± SEM. [abc] Within rows, values sharing the same letter are not significantly different.

Effects of Moringa oleifera leaves on feed transit at 280 days

Digesta weight. Digesta weights were comparable between the three groups during 24 h of transit in crop (Figure 2A) and gizzard (Figure 2B). Contents of small intestine (Figure 2C), caeca (Figure 2D) and rectum (Figure 2E) were significantly different. So, from 4 to 24 h, digest of M2 were lower than those of M1 ($P<0.01$) and M0 ($P<0.05$) in small intestine and rectum while, M0 had the highest caeca content as compared to those of M1 and M2 ($P<0.01$).

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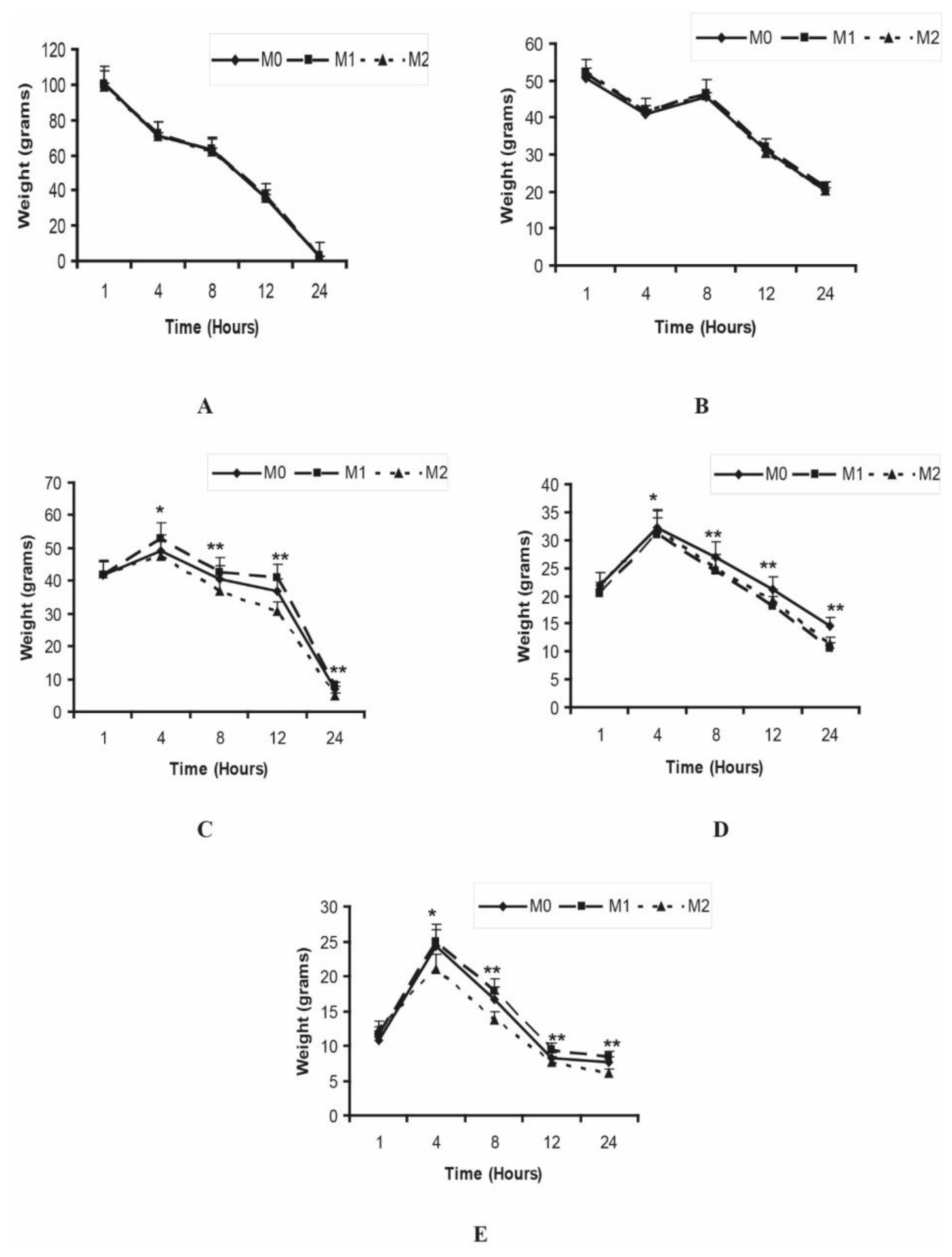

Figure 2. Digesta weight in the different segments during 24 h at d 28o. Crop (A), Gizzard (B), Small intestine (C), Caecum (D) and Rectum (E). Significant differences indicated by * (*P*<0.05) and ** (*P*< 0.01)

Chymusgewichte in den verschiedenen Abschnitten des Verdauungstraktes über 24 Stunden am 28o. Lebenstag. Kropf (A), Muskelmagen (B), Dünndarm (C), Blinddärme (D), Dickdarm (E). * zeigt signifikante Unterschiede zwischen den Werten an (*P*< 0,05)

Morphometric parameter. In Table 4, relative weight of gizzard, lengths of small intestine, caeca and rectum were comparable between M0, M1 and M2. In opposite, density of small intestine was lower in M2 than M0 (P<0.05) and M1

($P<0.01$) while M1 was heavier than M0 ($P<0.05$). On the other hand caeca of M0 were lighter than those of M1 and M2 ($P<0.05$).

Table 4. Morphometric measures of different segments of the digestive tract at 280 days of age

Morphologische Parameter der verschiedenen Abschnitte des Verdauungstraktes am 280. Lebenstag

	Groups		
Digestive tract segment	M0	M1	M2
Gizzard			
Relative weight	1.97 ± 0.080[a]	2.03 ± 0.130[a]	2.01 ± 0.090[a]
Small intestine			
Length (cm)	170 ± 0.040[a]	170 ± 0.010[a]	170 ± 0.020[a]
Density (g/cm)	0.380 ± 0.001[b]	0.480 ± 0.020[a]	0.210 ± 0.001[c]
Caecum			
Length (cm)	32.8 ± 0.010[a]	33.2 ± 0.010[a]	32 ± 0.090[a]
Density (g/cm)	0.210 ± 0.020[b]	0.32 ± 0.020[a]	0.30 ± 0.010[a]
Rectum			
Length (cm)	12.1 ± 0.050[a]	12 ± 0.020[a]	11.9 ± 0.040[a]
Density (g/cm)	0.510 ± 0.020[a]	0.530 ± 0.010[a]	0.520 ± 0.030[a]

Values are means ± SEM. [abc] Within rows values sharing the same letter are not significantly different.

Discussion

Moringa oleifera leaves influenced feed transit, affecting weight of digesta and the anatomical structure of different segments of the digestive tract although feed intakes during the two h of feeding were comparable. At 56 and 280 days, the similarity between weights of crop contents at the first h, on the one hand, and the significant difference between rectum digesta weights at 24 h of transit, on the other hand, showed that feed transit time changes according to feed treatment. At d 56, rectum contents of M0 at 24 h account for 0.60% of the crop contents at 1 h, while this value is 1.80% for M1 and M2. This delay in the progression of feed in M1 and M2 can be linked especially to the high length of their small intestine and caeca. Also, the increase of the density of those two segments is the line of reports of SAMANYA and YAMAUCHI (2002) and SORAYA et al. (2009) who attributed results to the presence of high numbers of villi increasing the absorptive surface of the intestinal mucosa. As a hypothesis, it could be claimed that feed should be more digested and nutrients more absorbed by small intestine and caeca of M1 and M2 in opposite to M0. In addition, little digesta of caeca of M1 and M2 could be linked to the inhibiting effect of *Moringa* leaves against pathogen germ proliferation and to the enhancing effect on the non-pathogens. OLUDURO et al. (2012) reported that the increasing of non-pathogen germs initiates a very important microbial digestion process of fibres. Also, the use of more effort to grind the high amount of fibres in diets 2 and 3 can justify the increased weight of their gizzard as showed by CARRE (2000). In addition, although small intestine and caecum of M1 were longer than those of M2, the similarity of their feed transit time can be related to the important amount of fibres in Diet 3 resulting in slow feed transit of M2 as claimed by BANJO (2012) feeding broilers with a diet containing *Moringa* leaves. Contrarily to d 56, at d 280, although hens have the capacity to digest quite an amount of fibres at this age, the fibre content in the three types of layer diets fits to the hen requirements in such a way that their grounding did not significantly affect gizzard weight. Moreover, their small intestine and caeca lengths were similar confirming results of UNI et al. (1999) and IJI et al. (2001) who showed that the optimum nutrient composition of diets might stimulate intestinal weight and length increasing during the growth period, while only weight might change during the laying period. Also, hen digesta weights along the digestive tract in the three groups, when comparing 56 to 280 days, were generally reduced leading to significant difference between transit times. So at 24 h, rectum contents in M0, M1 and M2 were respectively 7.60%, 8.90% and 6.10% of crop contents at 1 h in the 280 day birds. Relying on that percentages of digesta amount in the rectum, feed transit time was lower in young birds (d 56) than in the older one (d 280) as reported by NAU et al. (2010). Hens of M2 have the fastest feed transit due to the relative high amount of anti-nutritive factors in Diet 3. These results confirm those of GUPTA et al. (1989), FOIDL et al. (2001) and TETEH et al. (2013a) who pointed out that *Moringa oleifera* leaves contain different types of anti-nutritive substances such as tannins, non-starch polysaccharides and saponins which have the capacity to form complexes with macronutrients and make them insoluble and undigestable in the luminal intestine. As feed was taken away from luminal and mucosal digestion (RUCKEBUSH et al., 1991), its transit can be accelerated contrarily to M0 and M1 (ANNISON and CHOCT, 1991). So, slow feed transit observed in M1 and M0 can improve feed digestion and increase nutrients absorption resulting in high density of the small intestine with a lot of villi and microvilli in its mucosa if compared to M2 (SAMANYA and YAMAUCHI, 2002; SORAYA et al., 2009). Moreover, the accentuation of the significant differences between M1 and M2 about intestinal content weights from d 56 to d 280 can be due to possible anti-nutritional cumulative effects of *Moringa oleifera* leaves when consumption lasts for a long time as demonstrated by HETLAND et al. (2003) and TETEH et al. (2013b). Relying on previous results, HETLAND et al. (2003) found a higher sensitivity of birds to anti-nutritional factors when they become older, while studies conducted by TETEH et al. (2013b) on layer-type Isa brown growth during 280 days, pointed to the cumulative effects of anti-nutritive substances contained in *Moringa oleifera* leaves.

In conclusion, Group M1 (1% of *Moringa* leaves) is the one who shows the slowest feed transit speed, hence the highest transit time, with suitable intestinal anatomical structure leading to the best feed digestion and nutrients absorption.

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Summary

The variability of carbohydrate (oligosaccharides, insoluble fibres etc.) content in leaves depends on species and age of the plant as well as the collection season resulting in the need for a determination of the optimum incorporation rate in poultry diets. Important improvement of chicken productivity induced by the use of *Moringa oleifera* leaves in poultry farming led to an evaluation of its effects on the feed transit process. A total of 600 day-old ISA Brown chickens (layer-type) were divided into three groups (M0, M1 and M2). The three groups were fed respectively with diet M0 (0% of *Moringa* leaves), diet M1 (1% of *Moringa* leaves) and diet M2 (2% of *Moringa* leaves). At d 56 and d 280, 100 birds per group were isolated. They were deprived of feed and had 2 h to feed again. At 1, 4, 8, 12 and 24 h after the latter feeding period, 20 birds from each cage were slaughtered and their digestive tract removed. Each segment (crop, gizzard, small intestine, caecum and rectum) was isolated. Wet digesta collected was weighed and emptied segments were weighed and/or measured.

Results at 56 days indicate that birds fed with diets containing *Moringa oleifera* leaves had higher feed transit time and larger intestines compared to birds of M0. At 280 days, in spite of the similarity of small intestine and caeca sizes, M2 had the fastest feed transit and the lightest small intestine and caeca compared to M1. These results can be explained by anti-nutritive cumulative effects of these leaves. It can be concluded that *Moringa oleifera* leaves incorporated in poultry diets at 1% delay feed transit and improve anatomical structure of the gut.

Key words

Chick, hen, nutrition, *Moringa oleifera* leaf, digestive tract, feed transit, digesta, morphometric parameter.

Zusammenfassung

Einfluss von *Moringa olifeira* Blättern auf die Futterpassage und morphologische Kennwerte des Verdauungstraktes bei Küken und Legehennen

Der Gehalt an Kohlenhydraten (Oligosaccharide, unlösliche Faser usw.) variiert in den Blättern von *Moringa olifeira* in Abhängigkeit von den Arten, dem Alter der Pflanze und dem Erntezeitpunkt. Dies ist für die Festlegung der optimalen Einsatzmenge in Geflügelrationen zu beachten. Zur Optimierung der Leistungsfähigkeit der Tiere beim Einsatz von *Moringa olifeira* Blättern wurden in der vorliegenden Studie die Effekte auf die Futterpassagezeit im Verdauungstrakt untersucht. Hierzu wurden 600 Eintagsküken der Herkunft ISA Brown (Legetyp) auf drei Behandlungsgruppen verteilt (M0, M1, M2). Die drei Versuchsrationen enthielten 0% (M0), 1% (M1) bzw. 2% (M2) *Moringa olifeira* Blätter. Am 56. und am 280. Lebenstag wurden von jeder Behandlung 100 Tiere isoliert, über 2 Stunden genüchtert und danach wieder angefüttert. Jeweils 1, 4, 8, 12 und 24 h nach Beginn der Wieder-Anfütterung wurden von jeder Gruppe 20 Tiere geschlachtet und der Verdauungstrakt entnommen. Das Innereienpaket wurde in Kropf, Muskelmagen, Dünndarm, Blinddärme und Rektum separiert. Die Gewichte sowie die Dimensionen der gefüllten und der entleerten Abschnitte wurden erfasst.

Die Tiere, die mit *Moringa olifeira* Blättern gefüttert wurden, wiesen am 56. Lebenstag eine längere Futterpassagezeit und einen insgesamt größeren Verdauungstrakt im Vergleich zur Gruppe M0 auf. Bei den 280 Tage alten Tieren wiesen die Tiere der Gruppe M2, trotz ähnlicher Dimensionen des Dünndarms und der Blinddärme, gegenüber der Behandlung M1 eine schnellere Futterpassage, den leichtesten Dünndarm und Blinddarm auf. Die Unterschiede können mit kumulativen Effekten der anti-nutritiven Inhaltsstoffe der *Moringa olifeira* Blätter erklärt werden. Es wurde daher der Schluss gezogen, dass eine Zulage von 1% *Moringa olifeira* Blättern zur Futterration die Futterpassagezeit reduziert und die anatomische Struktur des Verdauungstraktes verbessert.

Stichworte

Küken, Legehenne, Fütterung, *Moringa olifeira* Blätter, Verdauungstrakt, Futterpassage, Chymus, morphomterische Parameter

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