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Effects of *Moringa oleifera* leave meal in the diet on layer performance, haematological and serum biochemical values

Einfluss von Mehl aus *Moringa oleifera* – Blättern im Futter auf die Leistung, Hämatologie und Serumbiochemie von Legehennen

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Abstract

Utilisation of plant and leaf extracts in animal production is a strategy to improve health status and animals performance. Medical plants for use in animal diets need to be safe for animal health, so the effects on blood parameters need to be investigated. In this study the effect of different levels of *Moringa oleifera* leave meal (MOLM) in the diet on blood parameters and production performance of laying-type chickens from 1-day-old to 55 weeks of age was investigated. A total of 450 day-old chicks were distributed into three dietary treatment groups with 0% (M0), 1% (M1) and 3% (M3) addition of MOLM, with 5 replicates of 30 birds each. During the experimental period, feed intake (FI), body weight, egg laying rate and feed conversion ratio (FCR) were recorded. Blood samples for analysis of total protein, albumin, total iron and haematology were taken in 15 birds (3/replicate) at the 5^{th}, 15^{th}, 25^{th}, 35^{th}, 45^{th} and 55^{th} weeks of age between 9.00 am and 10.00 am. An increase in MOLM during the growing phase did not affect FI of the birds significantly, but live weights and FCR were improved ($p < 0.05$). In the laying period, FI was reduced in M1 and M3. Birds fed 1% of MOLM had the lowest FCR. The albumin level increased significantly ($p < 0.05$) in birds fed 3% of MOLM. Layers in the M1 group had the highest average egg-laying rate ($p < 0.05$). Red blood cells, white blood cells (WBC), packed cell volume and lymphocytes decreased with the increase in the *Moringa oleifera* level ($p < 0.05$). In conclusion, WBC and lymphocyte decreased probably because of a reduction of intestinal microflora. This resulted in an improvement of albumin level which subsequently affected bird's growth and egg production.

Key words

Chicken; Moringa; health; medical plant; haematology; blood cells; growth; egg production

Zusammenfassung

Die Nutzung von Pflanzen- und Blattextrakten in der Tierproduktion ist eine Möglichkeit den Gesundheitsstatus und die Leistung der Tiere zu verbessern. Für die Akzeptanz des Einsatzes von Heilpflanzen im Tierfutter ist es notwendig nachzuweisen, dass diese kein Gesundheitsrisiko für die Tiere darstellen. Daher muss der Einfluss dieser Pflanzen auf Blutparameter getestet werden. In dieser Studie wurde der Einfluss unterschiedlicher Anteile an Mehl von *Moringa oleifera* Blättern (MOLM) im Futter auf Blutparameter und die Produktionsleistung von Legehennen im Alter von einem Tag bis zu 55 Wochen untersucht. Insgesamt wurden 450 Eintagsküken auf drei Gruppen verteilt, die mit unterschiedlichen Anteilen an MOLM im Futter gefüttert wurden [0% (M0), 1% (M1) und 3% (M3), je Gruppe 5 Wiederholungen, jede mit 30 Tieren]. Während der Versuchsperiode wurden die Futteraufnahme (FI), das

Körpergewicht, die Legerate und die Futterverwertung (FCR) ermittelt. Blutproben wurden für die Analyse von Gesamtprotein, Albumin, Gesamteisen und zur Hämatologie von 15 Tieren (3/Wiederholung) in der 5., 15., 25., 35., 45. und 55. Lebenswoche zwischen 9:00 und 10:00 Uhr genommen. Ein Anstieg im MOLM-Gehalt beeinflusste in der Aufzuchtphase die FI der Tiere nicht signifikant, aber das Lebendgewicht und die FCR wurden verbessert ($p < 0,05$). In der Legeperiode wurde die FI in der M1- und M3-Gruppe verringert. Hennen, die mit 1% MOLM gefüttert wurden, hatten die geringste FCR. Der Albumingehalt erhöhte sich signifikant ($p < 0,05$) wenn 3% MOLM mit dem Futter verabreicht wurden. Legehennen der M1-Gruppe erzielten die höchste mittlere Legerate ($p < 0,05$). Rote und weiße Blutkörperchen (WBC), Hämatokritwert und Lymphozyten verringerten sich mit Anstieg des Gehalts an *Moringa oleifera* im Futter ($p < 0,05$). Es kann geschlussfolgert werden, dass sich offensichtlich der Gehalt an WBC und Lymphozyten im Blut aufgrund der Reduzierung der Darmmikroflora verringert hat. Das führt zu einer Verbesserung des Albumingehalts, was sich wiederum auf das Wachstum und die Eiproduktion der Hennen auswirkt.

Stichworte

Huhn; Moringa; Gesundheit; Heilpflanze; Hämatologie; Blutzelle; Wachstum; Eiproduktion

Introduction

Antibiotic growth promoters, which are added to feed to improve feed efficiency, productivity and financial results, have been withdrawn from poultry feed (Fallah et al., 2013). Alternative solutions to improve health and production traits of poultry industry are needed. One of the alternatives is phytobiotic, which is defined by Windisch et al. (2008) as plant-derived products added to the feed in order to improve farm animal performances. Leaves of trees vary considerably in their nutrient contents and are good sources of vitamins, essential amino acids, proteins and minerals (Fasuyi, 2006). Plants also contain active components such as anthraquinones, flavonoids, glycosides, saponins, tannins, which possess medicinal properties (Chevalier, 2000; Doughari et al., 2009; D'Incalci et al., 2005). Although phytochemicals have been found to possess many useful effects on animal health, several reports have shown that some of them are potentially toxic and cause poisoning (Lorent et al., 2014; Khattab et al., 2010). Generally, blood components (both biochemical and haematological) are influenced by the quantity and quality of feed and act as a biomarker of the status of animals exposed to toxicants and other pathological conditions (Olafedehan et al., 2010). The haematopoietic system is considered as an important blood parameter of physiological and pathological status in animals since it is one of the most sensitive targets to toxic agents (Babatunde et al., 1992). For a feed to be considered safe for animal health, its effect on blood parameters needs to be investigated in order to understand the nutritional potential and safety of such feeds in a view of their acceptability. *Moringa oleifera* is a good source of phytonutrients such as protein, carbohydrate, minerals and vitamins (Oduro et al., 2008). It contains also other components like tannins, steroids, terpenoids, flavonoids, glycosides, saponins, alkaloids and polyphenols (Bennett et al., 2003; Teteh et al., 2013) which are considered as secondary metabolites. These secondary metabolites may act as antioxidants, antimicrobial (Dolara et al., 2005) or modulator of gene expression and signal transduction pathways (Doughari et al., 2009). They also possess lytic action on erythrocyte (Glauert et al., 1962) and inhibit digestive enzyme and vitamin absorption (Khattab et al., 2010). Emiola et al. (2013) reported that tannins decreased protein digestibility which in turn has negative effects on red blood cells and haemoglobin in chickens at high doses. Plants may not induce toxic effects at low concentration during short period feeding, but when consumed daily for a prolonged time, adverse effects could occur. To our knowledge, there are scarce scientific reports regarding the change of haematological profiles and biochemical parameters for long-term use of *Moringa* leaves as feed supplements. Therefore, this study was carried out to investigate the effect of *Moringa oleifera* on haematology and biochemical parameters as well as productive performance of layer chickens raised during 55 weeks.

Materials and Methods

Experimental design

Chicks were randomly assigned to three treatment groups according to the different levels of *Moringa oleifera* leave meal (MOLM) supplementation in the diets. The groups were: 1) Control (M0) diet without MOLM; 2) diet with1% of MOLM (M1) and diet with 3% of MOLM (M3). Harvested Moringa were dried under air conditioning and pulverised with a blender before using. All the diets had similar levels of crude protein and metabolisable energy and met the requirement of the bird according to their stage of development (Table 1). Within the groups, five replicates of 30 birds each were reared for 55 weeks of age in a completely randomised design. During the rearing period, the birds had access to water and feed *ad libitum*. At 5 weeks of age and every 10 weeks onwards, blood samples were collected from the wing vein of 15 birds per treatment (3 per replication) between 9.00 am and 10.00 am in order to avoid the effect of circadian rhythm. Blood was collected into a blood storage tube containing anticoagulant agent Ethylene Diamine Tetra Acetic Acid (EDTA) for the haematological assay and a second set of glass tubes without anticoagulant to determine selected biochemical parameters.

Table 1. Chemical analysis of the feed composition

Chemische Analyse der Futterbestandteile

	Feed composition according to age and group								
	Starter mash			Grower mash			Layer mash		
Feed stuff	M0	M1	M3	M0	M1	M3	M0	M1	M3
Maize	56	55.62	54.87	54	53.6	52.85	58	57.5	56.75
Wheat bran	11	10.74	10.23	24	23.75	23.25	8	7.9	7.35
Fish meal	8	8	7.8	7	7	6.8	8	7.6	7.6
Soya seed	20	19.64	19.1	11	10.65	10.1	17	17	16.3
Concentrate	4	4	4	2	2	2	2	2	2
Oyster shell	1	1	1	2	2	2	7	7	7
Moringa leaves (%)	0	1	3	0	1	3	0	1	3
Total	100	100	100	100	100	100	100	100	100
Analysis									
Metabolisable energy (MJ/kg)[1]	12.38	12.38	12.38	11.45	11.45	11.45	11.85	11.85	11.85
Crude protein (%)	20.11	20.11	20,11	16.80	16.80	16.80	17.67	17.68	17.69
Calcium (%)	0.97	0.97	0.96	1.14	1.14	1.13	2.58	2.56	2.56
Phosphorus (%)	0.74	0.73	0.72	0.73	0.73	0.72	0.62	0.61	0.60
Lysine total (%)	1.09	1.08	1.06	0.87	0.87	0.84	0.96	0.94	0.92
Methionine total (%)	0.48	0.48	0.47	0.38	0.38	0.37	0.40	0.39	0.39
Meth. + cysteine (%)	0.72	0.71	0.70	0.60	0.60	0.58	0.61	0.60	0.58

0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal.
[1] Metabolisable energy (ME) is calculated according to the method provided by BOURDILLON et al. (1990).

Data collection

Production performance. The amount of feed consumed was determined weekly as the difference between feed offered and remaining feed. Birds were weighed each week individually and the average in each pen was calculated. Body weight change was calculated as the difference between the final and initial body weight. Average daily body gain was calculated as body weight change divided by the number of experimental days. These data were used to determine feed conversion ratio (FCR) by dividing feed intake (FI) by body weight gain. During the laying period, egg number (EN) and egg weight (EW) were recorded daily and were used to calculate laying rate (%) and egg mass (EM).

$$\text{Layingrate}(\%) = \frac{\text{EN}\times 100}{\text{Period(days)}}$$

FRC was calculated via:

$$\text{EM} = \frac{\text{EN}\times\text{EW}}{\text{Period(days)}}$$

Haematology. In average, 1.5 ml of blood sample collected from each bird were used within 3 h to analyse red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), haemoglobin (Hb), lymphocytes and neutrophils by using ABX Micros 60, which is a fully automated haematology analyser from Sysmex Corporation International Company according to NAKUL et al. (2003).

Biochemical parameters. Total protein, albumin and total iron concentration of serum was determined by using an enzymatic colorimetric method on 200, 10 and 10 µl of serum respectively for iron, total protein and albumin according to the protocols provided by the reagent (BIOLABO). This method is based on the formation of a coloured product and the intensity of the coloration is proportional to concentration of the parameter. In order to validate the assay, a standard was assayed and the optical density measurements were made at 600, 546 and 630 nm respectively for iron, total protein and albumin by using BA-88A Mindray Auto-biochemical analyser.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5. One-way ANOVA was used to evaluate the effects of MOLM on blood chemistry values. Tukey multiple range test was used for testing difference of means.

Results

Effect of MOLM on the productive performance

Overall, feed intake values were comparable between M0, M1 and M3 during the rearing period and were reduced ($p<0.05$) in birds fed with MOLM during the egg laying period (Table 2). The results of the present study showed a significant increase ($p<0.05$) in growth performance of the birds fed with MOLM supplemented diets at 8 weeks of age ($p<0.05$) whereas, at 20 weeks of age values were comparable between M1 and M0 and significantly lower ($p<0.05$) as compared to M3 (Table 2). The same trend was observed with the daily weight gain (Table 2). At 8 weeks of age FCR were significantly different between the three groups and in the following order: M3 < M1 < M0 ($p<0.05$). At 20 weeks of age, chicks in group M3 had the lowest FCR value ($p<0.05$). During the laying period, birds of the M1 group showed the lowest FCR value ($p<0.05$). Between the treatments, birds in the M1 group reached statistically higher values ($P < 0.05$) in average egg laying rate during the period investigated as compared to M0 and M3 whose values were comparable.

Table 2. Effect of *Moringa oleifera* leaves meal (MOLM) on the productive performance

Einfluss von Mehl aus *Moringa oleifera* Blättern (MOLM) auf die Produktionsleistung

	Parameters	Groups		
		M0	M1	M3
0–8 weeks	Daily feed intake(g)	31.25 ± 7.39^a	31.18 ± 7.27^a	30.13 ± 7.97^a
	Initial live body weight (g)	33.27 ± 0.51	33.24 ± 0.89	33.28 ± 0.56
	Live body weight(g)	497.89 ± 6.22^c	543.20 ± 4.97^b	563.66 ± 5.88^a
	Daily weight gain (g)	8.28 ± 0.12^c	9.08 ± 0.24^b	9.45 ± 0.43^a
	Feed conversion	3.76 ± 0.18^a	3.41 ± 0.21^b	3.17 ± 0.14^c
8–20 weeks	Daily feed intake(g)	50.49 ± 3.39^a	49.93 ± 3.29^a	49.29 ± 3.48^a
	Live body weight (g)	1585.52 ± 12.74^a	1592.90 ± 11.26^a	1604.80 ± 12.12^b
	Daily weight gain (g)	13.37 ± 0.63^b	14.13 ± 0.40^b	14.93 ± 0.38^a
	Feed conversion	3.66 ± 0.15^a	3.53 ± 0.16^a	3.19 ± 0.09^b
20–55 weeks	Daily feed intake(g)	119.55 ± 4.12^a	108.13 ± 3.74^b	110.62 ± 4.37^b
	Laying rate (%)	66.71 ± 1.20^b	71.05 ± 1.13^a	68.03 ± 1.16^b
	Feed conversion	2.83 ± 0.08^a	2.59 ± 0.03^b	2.67 ± 0.07^b

[abc] Means in the same row with different superscripts are significantly different ($p < 0.05$).
0% (M0), 1% (M1) and 3% (M3) addition of MOLM in the diet.

Effect of MOLM on total protein, albumin and total iron level

Plasma total iron levels increased between week 5 and 35 and were not affected by treatments (Figure 1). At 45 and 55 weeks of age, total iron levels of birds from M3 group were higher ($p < 0.05$) than those of birds in the control and

M1 groups which were similar. At 5 weeks of age the total protein (TP) concentration was higher ($P < 0.05$) in birds of the M1 and M3 group than in the control. At 15th to 25th weeks of age, in the M3 group TP is significantly lower ($P < 0.05$) compared with M1 and M0 (Figure 2). At 55 weeks of age, the birds of the M1 and M3 groups had the highest content of TP ($p < 0.05$). In general, average TP concentrations were comparable in all treatments. Their values were: 40.09 ± 4.72; 41.48 ± 4.09 and 40.64 ± 5.92 g/l respectively for M0, M1 and M3 (Figure 3). Contrary to TP, a linear increase in serum albumin was observed with regard to the treatment and in general, the highest value ($p < 0.01$) was recorded in M3 (16.45 ± 1.36 g/l) group as compared to M1 (12.92 ± 1.34 g/l) and M0 (11.74 ± 1.29 g/l) which were comparable (Figure 3).

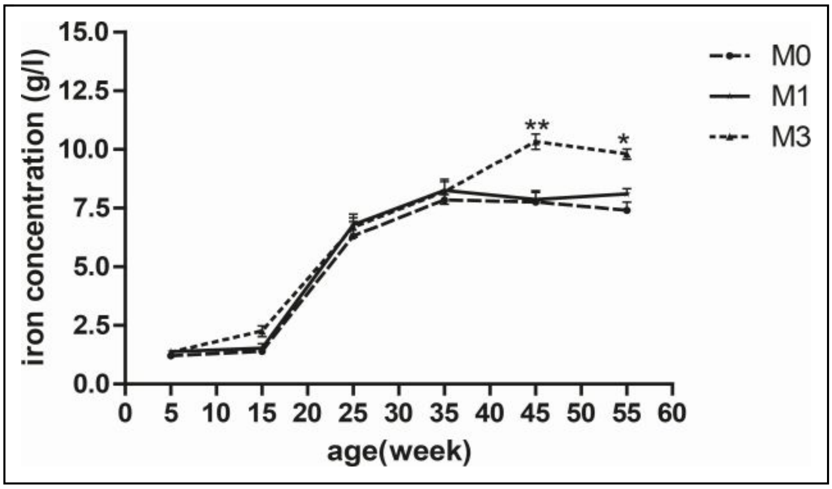

Figure 1. Iron concentration (g/l) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * ($p < 0.05$) and ** ($p < 0.01$).

Eisenkonzentration (g/l) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *($p < 0.05$) und ** ($p < 0.01$).

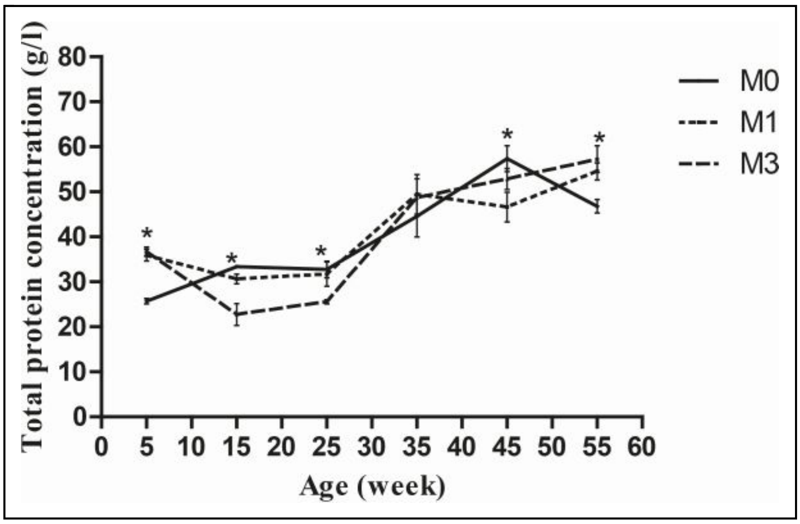

Figure 2. Total protein concentration (g/l) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * ($p < 0.05$).

Gesamtproteinkonzentration (g/l) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *($p < 0.05$).

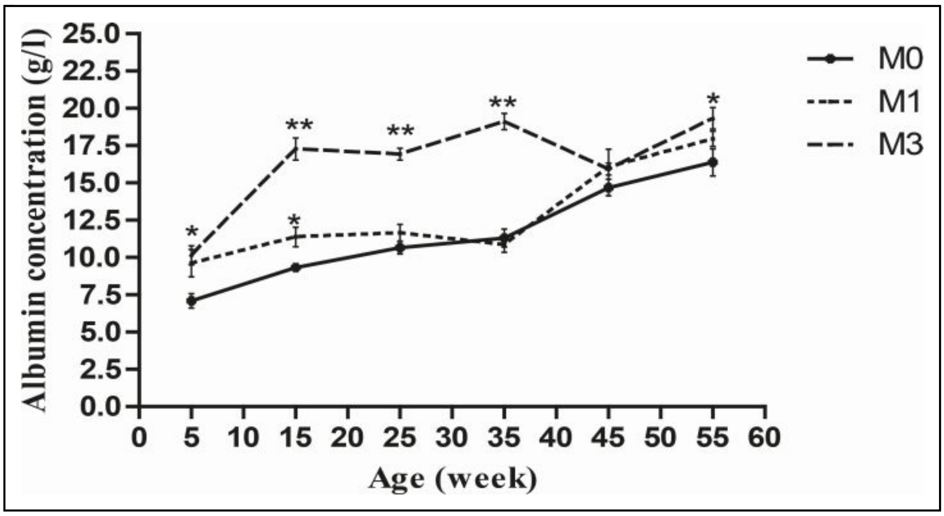

Figure 3. Albumin concentration (g/l) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * (p < 0.05) and ** (p < 0.01).

Albuminkonzentration (g/l) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *(p < 0.05) und ** (p < 0.01).

Effect of MOLM on red blood cell count (RBC), packed cell volume (PCV) and haemoglobin (Hb) concentration

Figure 4 shows RBC number according to the age of the birds. Overall, RBC number varied from 2.53 to 2.70; 2.29 to 2.54 and 2.28 to 2.45×10^6/µl, respectively for M0, M1 and M3. In general, RBC number decreased with the MOLM levels in the diet ($p < 0.05$). With regard to the age of the birds, the number of RBC was fluctuating between 5 and 35 weeks of age. However, from 35 weeks of age onward, the number of RBC increased with age ($p < 0.05$). Similar to RBC, the birds of the M0 group had the highest level of PCV ($p < 0.05$) (Figure 5) compared to those of M1 and M3 which were similar. MOLM in the diet reduced Hb concentration within a narrow range in group M1 and M3 when compared with the control group without significant difference between the treatments (Figure 6). With regard to the age of the birds, the highest level of Hb was obtained at 55 weeks of age.

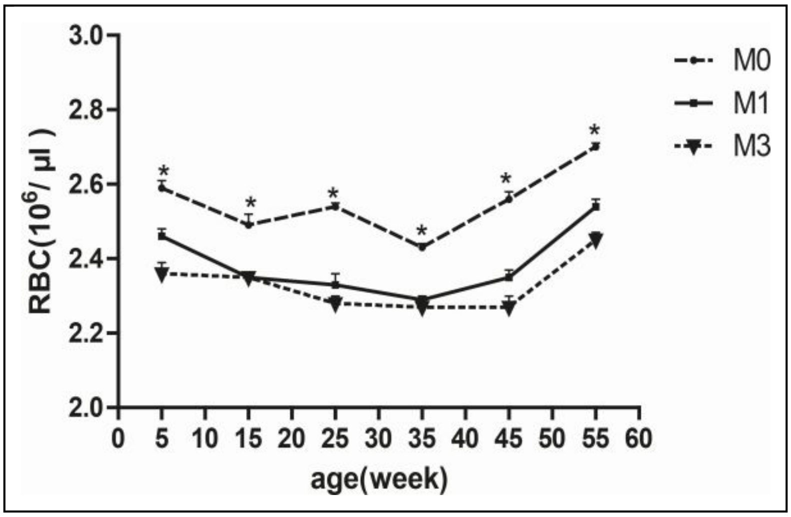

Figure 4. Red blood cell count (RBC, 10^6/µl) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * (p < 0.05).

Rote Blutkörperchen (RBC, 10^6/µl) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *(p < 0.05).

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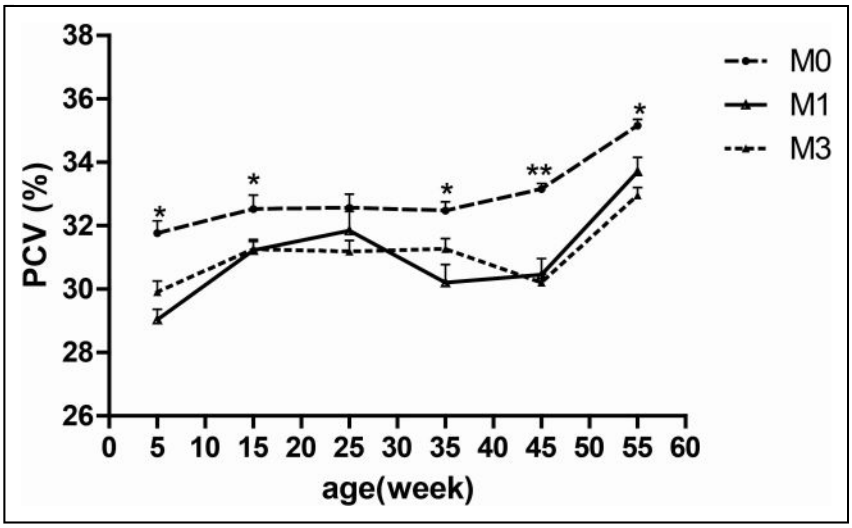

Figure 5. Packed cell volume (PCV, %) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * (p < 0.05) and ** (p < 0.01).

Hämatokrit (PCV, %) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *(p < 0.05) und ** (p < 0.01).

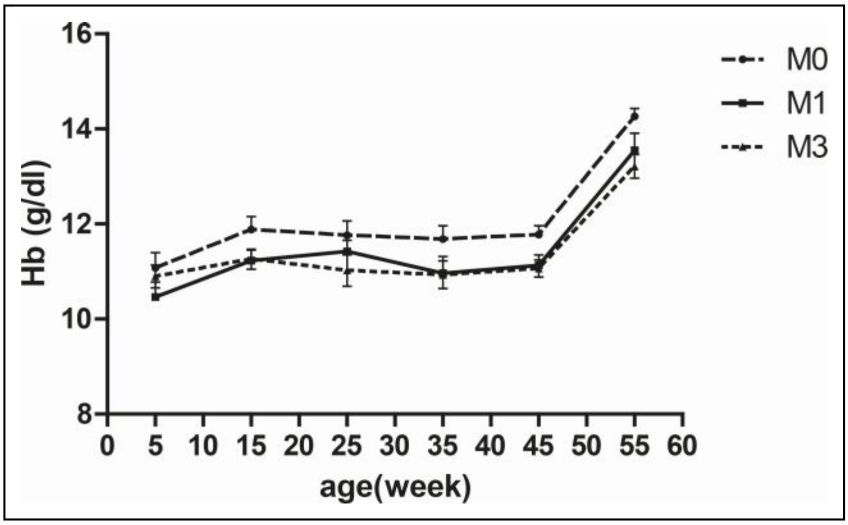

Figure 6. Haemoglobin (Hb, g/dl) concentration according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal].

Hämoglobinkonzentration (Hb, g/dl) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern].

Effect of MOLM on white blood cell (WBC) count, lymphocytes and neutrophils

According to the age of the birds, WBC number decreased in all treatments from 5th to 35th weeks of age and afterwards increased between the 45th and 55th weeks of age (Figure 7). With regard to the treatment, WBC decreased with the MOLM level in the diet ($p < 0.05$). Similarly, the percentage of lymphocytes decreased (Figure 8) with the level of MOLM in the diet ($p < 0.05$). With regard to the age of the birds, lymphocytes decreased slightly between 5 and 45 weeks of age. It is worth noting that the decrease of blood lymphocytes was less pronounced in birds of the control group. At week 55 of age, there was a slight increase in the lymphocyte proportion. The number of neutrophils (Figure 9) increased between 5 and 25 week without any significant differences ($p > 0.05$). From week 25 onward, the neutrophil proportions were comparable.

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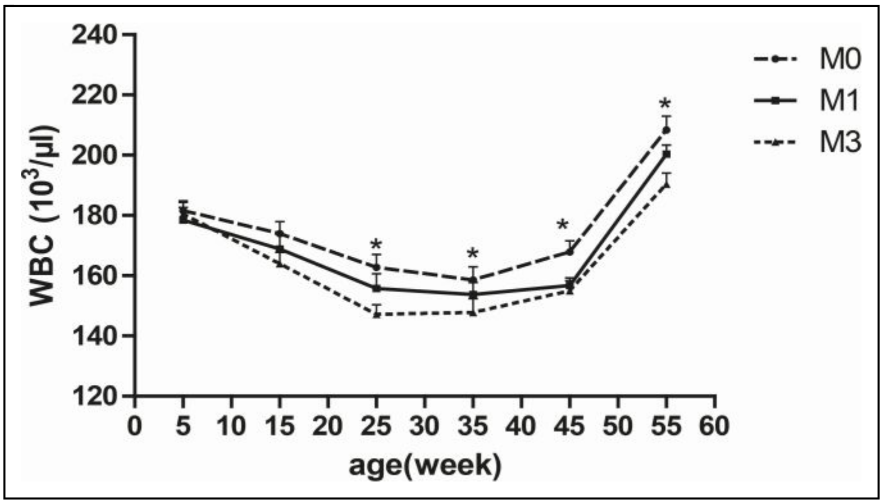

Figure 7. White blood cells (WBC, 10^3/µl) count according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * ($p < 0.05$).

Weiße Blutkörperchen (WBC, 10^3/µl) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *($p < 0.05$).

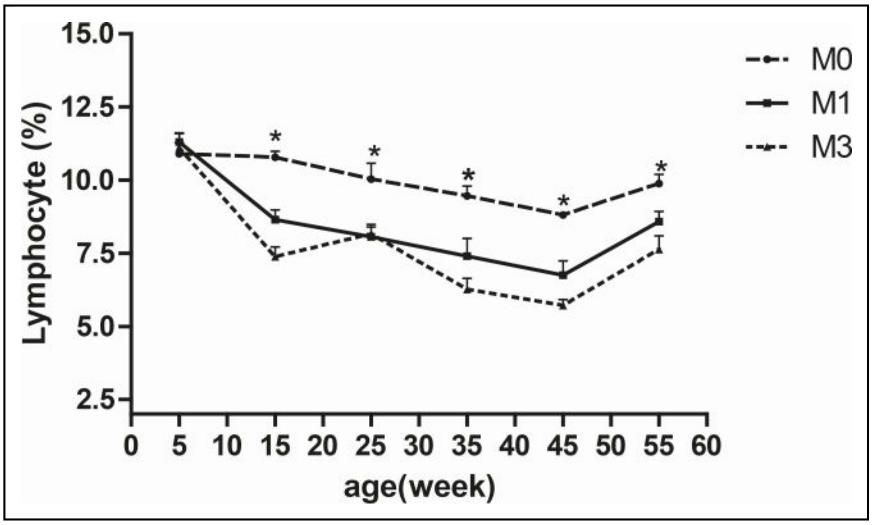

Figure 8. Lymphocyte (%) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal]. Significant differences are indicated by * ($p < 0.05$).

Lymphozyten (%) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern]. Signifikante Unterschiede sind gekennzeichnet durch *($p < 0.05$).

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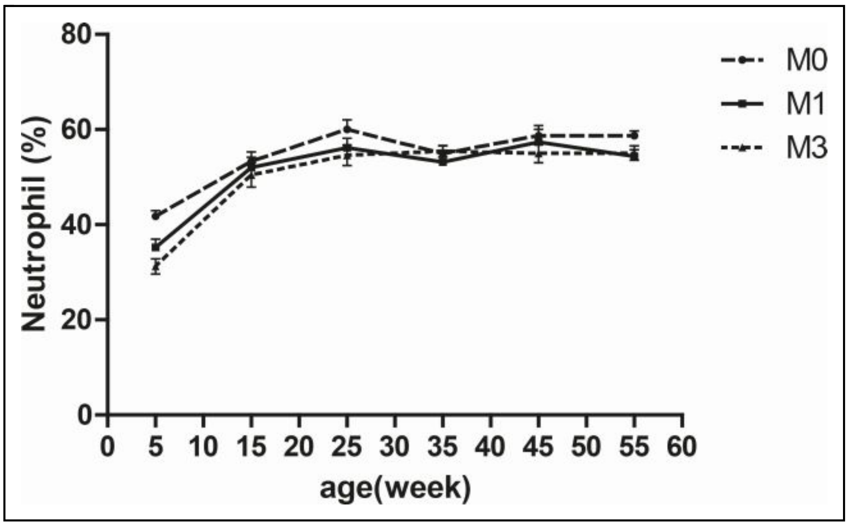

Figure 9. Neutrophils (%) according to age and treatment [0% (M0), 1% (M1) and 3% (M3) addition of *Moringa oleifera* leaves meal].

Anteil neutrophiler Granulozyten (%) in Abhängigkeit vom Alter und Fütterungsregime [0% (M0), 1% (M1) und 3% (M3) Zusatz von Mehl aus *Moringa oleifera* Blättern].

Discussion

In this study, it was clearly demonstrated that inclusion of *Moringa oleifera* leaves in the layer diet affected haematological as well as biochemical values and productive performance. In the M1 and M3 groups FI was similar and did not differ significantly between the treatments during the rearing period. Furthermore, body weight and daily weight gain of the birds fed with MOLM supplemented diet increased. This implies that there is no adverse effect of *Moringa oleifera* leaves on the palatability of the layer diets. These results are consistent with the study of TETEH et al. (2016) who did not observe any significant differences regarding the effects of *Moringa oleifera* leaves on FI in layers. The increase in body weight and daily weight gain of birds during the rearing period may be due to the active components, which are present in *Moringa oleifera* leaves. These components improve animal health and nutrients uptake. Further, the results show a significant decrease ($P < 0.05$) in WBC counts and lymphocytes with the MOLM levels in the diet. It is well documented that a high WBC count is associated with a recent infection, usually with bacteria (AHAMEFULE et al., 2008), and the presence of foreign bodies or antigens in the circulating system. The reduction of WBCs and lymphocytes in MOLM treated birds may be due to the antimicrobial activity of phytochemicals in the *Moringa* leaves. It has been established that *Moringa oleifera* inhibits the growth of *Staphylococcus aureus* in the feed and animal intestines (DJAKALIA et al., 2011). Alternatively, it reduces the gut microflora population (DIVYA-MANDAL et al., 2014) while gut microflora and epithelial cells have to compete for nutrients (LAN et al., 2005). In addition, increased levels of intestinal microflora reduces nutrient uptake by inducing intestinal cell turnover and thereby increasing intestinal requirements for nutrients to maintain tissue integrity. By reducing intestinal microflora, leaves of *Moringa oleifera* moderate cell turnover, thereby reducing the intestinal competition of available nutrients. In the M1 and M3 groups such mechanism could contribute to the higher concentration of albumin, which is known as one of the main serum proteins serving as an amino acid source for the synthesis of tissue proteins in the period of somatic growth in birds (YAMAN et al., 2000). Moreover, in the M1 and M3 groups, decreased FI and increased average egg laying during the laying period are also indications of the statements for MOLM activity. In the present study, RBC and PCV decreased with increasing level of MOLM in diet from 5 to 55 weeks of age. This indicates an immediate effect of *Moringa oleifera* leave on RBC independently of the age. However, this reduction did not induce deleterious effect on bird's productivity. According to GULLAND and HAWKEY (1990), the normal values of RBC for domestic fowl (*Gallus domesticus*) range from 2.5–3.9 $x10^{12}$/l. The depressive effect of the MOLM supplemented diet in the M1 and M2 groups on RBC (M1: 2.29–2.54 $\times 10^{6}$/µl; M3: 2.28–2.45 $\times 10^{6}$/µl) confirms the report of TIJANI et al. (2016) who pointed out a decrease of RBC count in broilers fed with 20% of *Moringa oleifera* after 8 weeks of feeding. The adverse effect of *Moringa oleifera* on RBC may be due to the cumulative effect of a high level of tannins and saponins in the leaves (TETEH et al., 2013). Indeed, tannins have been found to reduce the absorption of vitamin B12 (LIENER, 1994; DOSS et al., 2011) which is important in the erythropoiesis. It has also been reported that saponins possess lytic action on the erythrocyte cell membrane. The haemolytic action of saponins is due to their affinity for the membrane cholesterol whereby they form non-dissolvable complexes leading to their destruction (Lorent et al., 2014). In addition, it could also be argued that tannins, saponins or other active components in *Moringa oleifera* leaves would have inhibited the release of erythropoietin, which is the humoral regulator of RBC production. Indeed, erythropoietin increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell and subsequently to mature erythrocytes (Sanchez-Elsner et al., 2004; Ganong, 2005). It is well documented that the iron level, prior to egg laying, will increase 2 to 3 times (Hochleithner, 1994) to respond to many physiological functions such as energy metabolism, gene regulation, cell proliferation and differentiation and synthesis of both neurotransmitters and proteins (Beard, 2001). This could explain the increase of total iron concentration in serum of birds in all treatments from 15 weeks of age onwards. However, the higher total iron concentration observed in chickens fed with 3% of *Moringa oleifera* at the end of the trial (45–55 weeks) could be explained by iron intake from *Moringa oleifera*, which is rich in iron (Moyo et al., 2011) or by haemolytic action of saponins and tannins which release iron. All haemoglobin values obtained in our study were within the reference intervals provided by Bounous and Stedman (2000) and Gylstorff (1983). Although iron is an important component of Hb, there was a trend of low concentration of Hb in the *Moringa oleifera* treatment groups. This low concentration of Hb in the *Moringa oleifera* groups corresponds with their low RBC numbers. This is also in line with the work done by Adedapo et al. (2009) who reported that *Moringa oleifera* extract could produce anaemic effect in rats exposed for a long period of time. Olugbemi et al. (2010) reported that Hb was not significantly affected when broiler chickens were fed with *Moringa oleifera*. According to Olugbemi et al. (2010), the blood-building properties of *Moringa oleifera* leaves are not well-studied.

Conclusion

In conclusion, the results of this study indicate that MOLM had a positive influence on the growth and egg production in laying hens, probably through reduction of the intestinal microflora, as shown by the decrease in the number of white blood cells and lymphocytes and improved albumin levels. The decrease in RBC did not affect the productivity. From the point of view of egg production, the use of 1% *Moringa oleifera* leaves in the diet of laying hens should be encouraged.

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