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Growth performance and blood profiles of weaned New Zealand rabbits (*Oryctolagus cuniculus*) supplemented with *Moringa oleifera* leaf meal

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ABSTRACT. This study was conducted to assess the inclusion of varying levels of *Moringa oleifera* leaf meal (MOLM) on nutrients digestibility, feed intake, growth rate and serum metabolites of New Zealand white weaned rabbits (NZWR). Sixty-four (64) male NZWR (600 ± 8.58 g body weight, 28 days old) were raised on four isonitrogenous and isoenergetic diets formulated by adding MOLM at 0% (MOLM0), 5% (MOLM5), 10% (MOLM10) and 15% (MOLM15) in a factorial arrangement. There was a significant quadratic trend for fibre digestibility ($y = 21.5 (\pm 0.04) - 0.58 (\pm 0.01) x + 0.03 (\pm 0.0008) x^2$; $R^2 = 0.99$), and fat digestibility linearly increased ($y = 3.9 (\pm 0.34) - 0.07 (\pm 0.11) x$) with MOLM levels. The analysis of repeated measures showed a significant interaction effect of diet × week on weekly feed intake and growth performance. Feed intake demonstrated a linear trend, whilst weight gain and feed conversion ratio showed both linear and quadratic trend; however, the protein efficiency ratio exhibited a quadratic tendency. There were no significant linear and quadratic dependencies for serum biochemical components with the exception of aspartate aminotransferase and albumen. Therefore, it can be concluded that MOLM can be incorporated (up to 15%) in rabbit diets without any adverse effect on growth performance parameters.

Introduction

It is well known that the global population is growing, resulting in an increased demand for animal protein (Mengesha, 2012). Furthermore, this predicament poses a huge threat to global food security as it increases the number of people in need of food (Seleiman et al., 2020). Therefore, to meet the projected growth, the livestock sector will need to double or even triple its current production (FAO, 2012). This can be achieved if alternative and perhaps neglected sources of protein are explored (Mennani et al., 2021). One possible protein source that can assist in addressing these challenges, especially in Sub-Saharan Africa, is important but often overlooked rabbit meat.

Rabbit meat is one of the best tender and juicy white lean meats, rich in protein, B vitamins and low in calories, fat and cholesterol. Furthermore, rabbit farming is increasingly expanding due to its proliferous production and excellent meat attributes of these animals (Dorning and Harris, 2017). Currently, rabbit farming is practiced in countries such as China, Italy, Spain, France, Czech Republic and

Germany (FAO, 2012). Moreover, rabbits are attractive as they require little production space, are less labour intensive and emit fewer greenhouse gases. For these reasons, rabbits are gaining much attention as they can convert 20% protein from feed consumed into muscles than those with high growth rate and short gestation period (FAO, 2012). It is evident that rabbits have the potential to address the world's needs, which makes it necessary to promote their optimal growth performance through appropriate feeding strategies. Perhaps one of the solutions may include the utilisation of alternative and cost effective feed ingredients that could be partially or completely incorporated into rabbit diets to reduce production costs. One potential source of inexpensive protein is leaf meal of certain tropical browse legumes such as *Moringa oleifera* (MO), which is planted in Africa, Asia and the US because of its adaptability to various conditions (Saalu et al. 2011; Selim et al., 2021). Sarwatt et al. (2004) and Selim et al. (2021) indeed found that *M. oleifera* leaves (MOL) are a possible cheap feed source for animal feeding.

Moringa oleifera is recognized as a high-yielding nutritive browse plant, whose all portions are of food/feed importance (Duke, 1998). Its protein content ranges from 285.2 to 324.6 g/kg (Ahmed, 2017; Briones et al., 2017; Sebola et al., 2017), which is sufficient to meet the production requirements of various livestock. Additionally, MOL contains minerals, antioxidants, essential and sulphurcontaining amino acids, as well as beneficial bioactive compounds (El-Desoky et al., 2018). However, *M. oleifera* leaf meal (MOLM) contains secondary plant compounds, such as condensed tannins, which might hinder its utilisation in monogastric animals such as rabbits. Hence, the main objective of this research was to determine the influence of different levels of MOLM inclusion on growth performance and blood profile of New Zealand white weaned rabbits (NZWR). It was hypothesised that the addition of MOLM would have no effect on growth performance and blood profile of rabbits.

Material and methods

Ethical statement

All procedures involving animal handling received the approval for care and use by the North West University (NWU-00402-18-A5).

Study area and feed components

The research was performed at the Kaffirstad farm (latitude: 26°34′S and longitude: 29°18′E) in the Mpumalanga Province of South Africa. During, this time, the ambient temperatures ranged from 25 to 35 °C. MOLM (Table 1) was acquired from the Patience Wellness Centre (latitude: 24°30′S and longitude: 29°56′E) in Limpopo Province of South Africa. Soybean meal, maize, molasses, lime and premix were obtained from Opti feeds (Lichtenburg, South Africa).

Table 1. Chemical composition of *Moringa oleifera* leaf meal (Sebola et al., 2017)

Component	Quantity
Dry matter (DM), g/kg	950.8
Moisture, g/kg	49.5
Organic matter, g/kg	870.5
Ash, g/kg	80.4
Ether extract, g/kg	53.9
Neutral detergent fiber, g/kg DM	761.7
Acid detergent fiber, g/kg DM	52.1
Fat, g/kg DM	42.3
Crude protein, g/kg DM	263.4
Crude fiber, g/kg DM	54.9
Condensed tannins, $AU_{550\ nm}$/10 mg	0.33
Total phenolic, $AU_{725\ nm}$/10 mg	0.989
Total carbohydrates, %	56.1
Energy value, kcal/g	367.4

Experimental design, treatment diets and proximate analyses

A total of 64 male NZWR (600 ± 8.58 g live weight) were randomly allocated to four experimental diets (Table 2) according to NRC, 1977. A completely randomised design with 4 diets was

Table 2. Ingredients and proximate analysis of diets fed to New Zealand white weaned rabbits

Composition	Diets			
	MOLM0	MOLM5	MOLM10	MOLM15
Ingredients, %				
corn	38	38	38	38
hay	42	42	42	42
soybean	17	12	7	2
molasses	1.5	1.5	1.5	1.5
limestone	1	1	1	1
premix	0.5	0.5	0.5	0.5
MOLM	0	5	10	15
total	100	100	100	100
Proximate analyses, %				
dry matter	90.81	89.42	90.35	89.29
protein	17.59	17.68	17.88	17.98
fat	3.62	3.79	3.81	3.93
crude fibre	21.87	21.23	20.38	21.89
ash	5.31	5.28	5.92	5.01

MOLM – *Moringa oleifera* leaf meal, MOLM0 – rabbit grower diet without MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% of MOLM as a substitute

used in this experiment. The experimental unit was a pen (0.07 m^2 per weaned rabbit) housing 4 NZWR, replicated 4 times, amounting to a total of 16 floor cages. The four following diets were constituted by diluting a commercial rabbit diet with ground air-dried MOLM: MOLM0 – rabbit grower diet without MOLM as a substitute (control), MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% MOLM as a substitute.

The diets were milled (Polymix PX-MFC 90 D, Kinamatica AG, Malters, Switzerland) to pass through a 1-mm sieve for chemical analyses. For laboratory dry matter determination (method no. 930.15; AOAC International, 2005), approx. 1 g of each sample was transferred into pre-weighed crucibles and placed in an oven at 105 °C for 12 h. Weight loss was measured as moisture content, and dry matter (DM) was calculated as the difference between the initial sample and moisture weight. Organic matter (OM) content (method no. 924.05; AOAC International, 2005) was determined by ashing the dried samples in a muffle furnace at 600 °C for 12 h. Weight loss was measured as OM content and the residue as ash. Total nitrogen content was determined using the standard macro-Kjeldahl method (method no. 984.13; AOAC International, 2005) and converted to crude protein by multiplying the percentage N content by a factor of 6.25. Crude fibre was determined using an ANKOM^{2000} Fibre Analyser (ANKOM Technology, New York, USA) with 0.255 N crude fibre acid solution, followed by 0.313 N crude fibre base solution.

Preparation of the house, experimental rabbits and management

The rabbit house and cages were cleaned thoroughly with water and detergent and then disinfected with F10 (Health and Hygiene (Pty) Ltd, Roodepoort, South Africa). All drinking nipples and feeders were cleaned before use and the house was given a grace period of 2 weeks rest. Feed and water were provided *ad libitum* and constant lighting (24 h) was provided.

Feed intake (FI) and growth performance

Weekly feed intake (WFI), body weight gain (WWG) and feed conversion ratio (WFCR) were calculated (Manyeula et al., 2019). Protein intake (PI) was determined as the product of crude protein concentration in the diet and FI. The protein efficiency ratio (PER) was calculated as the ratio of body weight gain to PI (Manyeula et al., 2019).

Nutrient digestibility

New Zealand white weaned rabbits were randomly placed in 25 cages (0.51 × 0.49 × 0.36 m, 2 rabbits per cage) to which the four experimental diets were randomly allocated. Feed and water were provided between 07:00 and 08:00. The first 3 days were used to adapt the rabbits, while in the last 3 days, one sample (feed offered, refused and faeces) per pen per day were collected, pooled, weighed, oven-dried, milled and stored (−15 °C). At the end of each collection period, the samples were bulked for each pen for chemical analyses. The apparent nutrient digestibility was measured according to McDonald et al. (2002) using the following formula:

$$ND = \frac{ANI - ANE}{ANI} \times 100,$$

where: ND – nutrient digestibility, ANI – amount of nutrients ingested, ANE – amount of nutrients excreted.

Serum biochemical parameters

Blood was collected from 2 rabbits per cage from the neck during slaughter into 4 ml red top anticoagulant free tubes for serum biochemical analysis. Blood was stored for 45 min at room temperature to coagulate and then refrigerated at 4 °C (Washington and Van Hoosier, 2012). Serum biochemistry analysis was conducted within 48 h after collection. Subsequently, the clotted blood was centrifuged for 20 min at 1 500 rpm and the serum supernatant settled on top, while clotted blood deposited at the bottom. The serum was then poured into a 0.5-ml centrifuge tube and stored at −20 °C. Next, serum biochemical parameters were determined using an automated Idexx Vex Test Chemistry Analyser (IDEXX Laboratories, Inc, Westbrook, ME, USA).

Statistical analysis

Data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Moyo et al., 2011) was applied to describe the responses of white weaned rabbits to MOLM inclusion levels using the following quadratic model:

$$Y = ax^2 + bx + c,$$

where: Y – response variable, a and b – coefficients of the quadratic equation, c – intercept, and x – dietary MOLM levels (%). WFI, WWG and WFCR data were analysed utilising SAS procedures for the analysis of repeated measures (SAS, 2010). Apparent nutrient digestibility and serum metabolites data were analysed using the general linear model procedure (SAS, 2010) for

a completely randomized experimental design with pen as the experimental unit. For all statistical tests, the significance level was set at $P > 0.05$. The probability of difference (PDIFF) option in the least square means statement of SAS was used to separate the means.

Results

Apparent nutrient digestibility

There were no significant linear or quadratic trends for protein and ash digestibility in response to increasing MOLM levels (Table 3). However, there was a significant quadratic trend for fibre digestibility (y = 21.5 (± 0.04) − 0.58 (± 0.01) x + 0.03 (± 0.0008) x^2; $R^2 = 0.99$; $P = < 0001$) with incremental MOLM levels. Digestibility of crude fat linearly increased (y = 3.9 (± 0.34) − 0.07 (± 0.11) x; $R^2 = 0.54$; $P = 0.002$) with raising MOLM levels in the diet. The diets did not significantly influence digestibility of crude protein, fibre, ash and fats.

Table 4 shows no significant ($P > 0.05$) linear and quadratic trends on AWFI at week 5. Quadratic response was observed to MOLM levels at week 6. However, linear trends were observed at weeks 7, 8, 9, 10, 11 and for overall feed intake (OFI) with MOLM levels. Therefore, feed intake of rabbits decreased with increasing MOLM inclusion levels.

Lack of linear or quadratic trends was observed for AWWG at weeks 5, 6 and 10 (Table 5). However, data from Table 5 indicate the presence of quadratic trends at week 7 (y = 18.16 (± 0.71) + 1.22 (± 0.23) x − 0.07 (± 0.014) x^2; $R^2 = 0.58$; $P = < 0001$), 8 (y = 20.97 (± 0.37) + 0.78 (± 0.11) x − 0.04 (± 0.007) x^2; $R^2 = 0.68$; $P = < 0001$), 9 (y = 22.84 (± 0.76) + 1.03 (± 0.24) x − 0.07 (± 0.02) x^2; $R^2 = 0.46$; $P = 0.0004$), and overall week weight gain (y = 23.53 (± 0.36) – 0.71 (± 0.12) x − 0.03 (± 0.007) x^2; $R^2 = 0.69$; $P = < 0001$); linear trends were observed at weeks 11 (y = 26.4 (± 1.10) + 0.48 (± 0.35) x; $R^2 = 0.21$; $P = 0.038$) and 12 (y = 26.7 (± 1.22) + 0.99 (± 0.39) x; $R^2 = 0.33$; $P = 0.017$).

Table 3. Apparent nutrient digestibility (%) of New Zealand white weaned rabbits fed a diet containing *Moringa oleifera* leaf meal (MOLM)

Parameters	Diets				SEM	*P*-value	
	MOLM0	MOLM5	MOLM10	MOLM15		linear	quadratic
CPd	80.5	81.2	80.6	79.5	1.32	0.463	0.691
CFd[1]	21.6	18.6	25.4	27.6	2.83	0.104	<0001
Ash digestibility	6.9	6.5	6.2	5.9	0.60	0.221	0.912
CFd[2]	3.6	5.2	4.1	6.0	0.00	0.002	0.618

CPd – crude protein digestibility, CFd[1] – crude fibre digestibility, CFd[2] – crude fat digestibility, MOLM0 – rabbit grower diet with 0% MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% of MOLM as a substitute; SEM – standard error of the mean; $P < 0.05$

Table 4. Regression equation for average weekly feed intake of New Zealand white weaned rabbits in response to a diet containing incremental levels of *Moringa oleifera* leaf meal (MOLM)

Week	Regression equation	*P*-value	R^2
5	y = 38.2 (± 1.30) + 0.46 (± 0.41) x − 0.03 (± 0.03) x^2	0.23	0.06
6	y = 58.3 (± 1.09) − 3.57 (± 0.35) x + 0.22 (± 0.02) x^2	<0001	0.83
7	y = 67.66 (± 1.70) − 0.05 (± 0.55) x	<0001	0.54
8	y = 66.96 (± 1.03) − 0.65 (± 0.33) x	<0001	0.70
9	y = 74.05 (± 1.46) − 1.46 (± 0.47) x	<0001	0.56
10	y = 74.05 (± 1.47) − 1.45 (± 0.47) x	<0001	0.57
11	y = 73.98 (± 1.56) − 1.36 (± 0.50) x	<0001	0.56
OFI	y = 74.96 (± 0.91) − 1.09 (± 0.29) x	<0001	0.68

OFI – overall feed intake

Feed intake and growth performance

Repeated measures analysis revealed significant effects of diet × week interaction on average weekly feed intake (AWFI) (Table 4), average weekly weight gain (AWWG) (Table 5), feed conversion ratio (FCR) (Table 6), protein consumed (PC) (Table 7) and protein efficiency ratio (PER) (Table 8).

The diets did not affect AWWG at weeks 5 and 10. At weeks 6 and 12, rabbits in the control group (MOLM0) had lower ($P < 0.05$) AWWG compared to the MOLM15 group, but had the same ($P > 0.05$) AWWG as those in the MOLM5 and MOLM10

Table 5. Average weekly weight gain of New Zealand white weaned rabbits fed diets containing *Moringa oleifera* leaf meal (MOLM)

Weeks	Diets				SEM	*P*-value	
	MOLM0	MOLM5	MOLM10	MOLM15		linear	quadratic
5	11.69	11.72	11.89	11.54	0.64	0.928	0.762
6	13.04^{b}	14.33^{ab}	15.08^{ab}	15.65^{a}	0.71	0.111	0.605
7	18.45^{c}	21.56^{b}	23.89^{a}	19.64^{c}	0.69	0.084	<0.001
8	21.95^{c}	23.82^{b}	24.19^{a}	22.50^{c}	0.34	0.073	<0.001
9	22.80^{b}	26.50^{a}	26.50^{a}	23.57^{b}	0.80	0.522	<0.001
10	24.88	24.43	26.19	27.98	1.15	0.089	0.919
11	26.64^{b}	27.54^{ab}	30.27^{a}	29.47^{ab}	1.13	0.038	0.461
12	26.83^{b}	29.98^{ab}	32.45^{a}	30.89^{a}	1.28	0.017	0.076
OWG	23.61^{c}	25.98^{b}	27.21^{a}	25.89^{b}	0.38	<0.001	<0.001

OWG – overall weight gain diets, MOLM0 – rabbit grower diet with 0% MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% of MOLM as a substitute; SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 6. Weekly feed conversion ratio of New Zealand white weaned rabbits fed diets containing *Moringa oleifera* leaf meal (MOLM)

Weeks	Diets				SEM	*P*-value	
	MOLM0	MOLM5	MOLM10	MOLM15		linear	quadratic
5	3.38	3.32	3.49	3.37	0.22	0.881	0.895
6	4.46^{a}	3.30^{c}	3.00^{cd}	3.52^{bc}	0.20	0.002	0.0004
7	3.64^{a}	3.21^{b}	2.52^{d}	2.93^{bcd}	0.14	0.0004	0.0110
8	3.20^{a}	2.70^{b}	2.43^{d}	2.54^{bcd}	0.07	<0001	0.0003
9	3.26^{a}	2.60^{bc}	2.45^{c}	2.73^{b}	0.09	0.0003	<0001
10	2.99^{a}	2.62^{ab}	2.52^{b}	2.30^{b}	0.14	0.003	0.065
11	2.79^{a}	2.52^{ab}	2.16^{b}	2.14^{b}	0.13	0.0006	0.344
12	2.69^{a}	2.40^{ab}	2.34^{b}	2.25^{b}	0.11	0.011	0.380
OFCR	3.17^{a}	2.73^{bc}	2.49^{d}	2.59^{cd}	0.06	<0001	0.0002

OFCR – overall feed conversion ratio, MOLM0 – rabbit grower diet with 0% MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% of MOLM as a substitute; SEM – standard error of the mean; $^{a–d}$ – means within a row with different superscripts are significantly different at $P < 0.05$

groups. At weeks 7 and 8, rabbits fed the MOLM 10 diet showed higher ($P < 0.05$) gain than those on the MOLM0 and MOLM15 diets. At week 9, rabbits receiving the MOLM5 and MOLM10 diets had higher ($P < 0.05$) gain than those fed the MOLM0 and MOLM15 diets. At week 11, rabbits from the MOLM0 group had lower ($P < 0.05$) AWWG than those from the MOLM10 group; however, the same ($P > 0.05$) AWWG was recorded for the MOLM0 group as for rabbits fed the MOLM5 and MOLM15 diets. The overall weight gain demonstrated that rabbits fed diets containing MOLM had higher ($P < 0.05$) weight gain than those on the control diet. However, weight gain of rabbits fed the MOLM10 diet was higher ($P < 0.05$) than those fed the MOLM5 and MOLM15 diets.

Table 6 indicates that quadratic trends for FCR were observed at weeks 6 ($y = 4.46 (\pm 0.19) - 0.31 (\pm 0.06) x + 0.02 (\pm 0.004) x^2$; $R^2 = 0.59$; $P = 0004$), 7 ($y = 3.71 (\pm 0.15) - 0.18 (\pm 0.0047 x + 0.008 (\pm 0.003) x^2$; $R^2 = 0.55$; $P = 0.011$), 8 ($y = 3.20 (\pm 0.06) - 0.13 (\pm 0.02) x + 0.006 (\pm 0.001) x^2$; $R^2 = 0.78$; $P = 0.0003$), 9 ($y = 3.25 (\pm 0.09) - 0.18 (\pm 0.03) x + 0.009 (\pm 0.002) x^2$; $R^2 = 0.68$; $P = < 0001$), as well as for OFCR ($y = 3.18 (\pm 0.06) - 0.12 (\pm 0.02) x + 0.005 (\pm 0.001) x^2$; $R^2 = 0.78$; $P = 0.0002$). There were linear trends recorded in response to incremental levels of MOLM at weeks 10 ($y = 2.97 (\pm 0.14) - 0.06 (\pm 0.04) x$; $R^2 = 0.36$; $P = 0.003$), 11 ($y = 2.81 (\pm 0.13) - 0.08 (\pm 0.04) x$; $R^2 = 0.45$; $P = 0.0006$) and 12 ($y = 2.68 (\pm 0.11) - 0.06 (\pm 0.04) x$; $R^2 = 0.29$; $P = 0.01$). Significant dietary effects were observed for OFCR and FCR at weeks 6, 7, 8, 9, 10, 11 and 12. At week 5, rabbits fed the control diet showed higher ($P < 0.05$) FCR than those on the MOLM diets. The MOLM5 diet (3.30) promoted ($P < 0.05$) FCR the least compared to other diets. At weeks 7 and 8, the control rabbits had the highest ($P < 0.05$) FCR compared to those on the MOLM10 diets. However, MOLM5 intake resulted in the same ($P < 0.05$) FCR as for the MOLM15 diet. At weeks 9, 10, 11 and 12, rabbits fed the control diet had the highest FCR

compared to those on the MOLM5, MOLM10 and MOLM15 diets, which did not differ from each other ($P < 0.05$). The overall FCR was significantly higher in rabbits fed the control diet (3.17), while significantly lower for rabbits on the MOLM10 diet (2.49); however, no significant difference was noted in rabbits fed the MOLM5 and MOLM15 diets.

There were no linear and quadratic effects of PC in response to incremental MOLM levels (Table 7).

Table 8 shows that OPER and PER at weeks 6, 7, 8, 9 and 10 quadratically increased ($P < 0.05$) with MOLM levels, but a linear trend was observed at week 11 ($y = 0.03 (\pm 0.0.002) - 0.002 (\pm 0.0007) x$; $R^2 = 0.34$; $P = 0.016$) with increasing MOLM concentration.

Table 7. Protein consumed (g/rabbit) by New Zealand white weaned rabbits fed diets containing incremental levels of *Moringa oleifera* leaf meal (MOLM)

Week	Diets				SEM	*P*-value	
	MOLM0	MOLM5	MOLM10	MOLM15		linear	quadratic
5	524.19	511.44	546.22	521.96	17.03	0.226	0.136
6	787.90	626.05	578.63	749.96	14.66	0.883	0.165
7	907.62	914.57	795.22	783.34	20.9	0.479	0.162
8	907.62	856.92	795.22	783.34	14.36	0.315	0.440
9	1004.68	909.04	855.30	878.62	20.49	0.315	0.440
10	1006.69	909.05	856.35	879.70	20.70	0.340	0.439
11	1009.7	910.99	900.3	870.89	22.06	0.571	0.50
12	999.75	946.95	981.70	950.19	23.17	0.483	0.519
OPC	1017.13	939.86	894.69	915.70	12.76	0.061	0.743

OPC – overall protein consumed, MOLM0 – rabbit grower diet with 0% MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute and MOLM15 – rabbit grower diet with 15% of MOLM as a substitute; SEM – standard error of the mean; $P > 0.05$

Table 8. Regression equation for protein efficiency ratio of New Zealand white weaned rabbits in response to diet containing incremental levels of *Moringa oleifera* leaf meal

Week	Regression equation	*P*-value	R^2
6	$y = 0.02 (\pm 0.001) + 0.002 (\pm 0.0005) x - 0.0001 (\pm 0.00003) x^2$	0.003	0.36
7	$y = 0.02 (\pm 0.002) + 0.002 (\pm 0.0006) x - 0.0001 (\pm 0.00004) x^2$	0.024	0.33
8	$y = 0.02 (\pm 0.0009) + 0.002 (\pm 0.003) x - 0.00008 (\pm 0.00002) x^2$	0.0002	0.74
9	$y = 0.02 (\pm 0.0002) + 0.003 (\pm 0.0005) x - 0.0001 (\pm 0.00003) x^2$	0.004	0.44
10	$y = 0.02 (\pm 0.002) + 0.001 (\pm 0.00004) x - 0.00005 (\pm 0.00004) x^2$	0.023	0.21
11	$y = 0.03 (\pm 0.0.002) - 0.002 (\pm 0.0007) x$	0.016	0.34
OPER	$y = 0.02 (\pm 0.0005) + 0.002 (\pm 0.0002) x - 0.0001 (\pm 0.000001) x^2$	<0001	0.93

OPER – overall protein efficiency ratio

Table 9. Serum metabolites of weaned rabbits fed diets containing *Moringa oleifera* leaf meal (MOLM)

Parameters	Diets				SEM	*P*-value	
	MOLM0	MOLM5	MOLM10	MOLM15		linear	quadratic
AST, IU/l	42.3^{ac}	41.0^{c}	32.5^{b}	45.0^{a}	2.1	0.984	0.024
ALT, IU/l	60.8	50.8	57.8	75.0	7.6	0.257	0.133
Glucose, mmol/l	5.0	5.3	6.3	5.5	0.4	0.187	0.182
Protein, g/l	55.3	54.8	54.3	50.0	2.0	0.086	0.355
Sodium, mmol/l	136.5	139.5	140.0	140.0	1.2	0.058	0.227
Potassium, mmol/l	5.6	5.8	5.4	5.0	0.3	0.065	0.208
Magnesium, mmol/l	1.6	1.5	1.6	1.3	0.1	0.102	0.326
Albumen, g/l	37.8^{b}	47.0^{a}	46.5^{a}	33.5^{b}	4.8	0.530	0.031
Creatinine, umol/l	58.0	55.8	56.5	49.9	2.6	0.052	0.375
Calcium, mmol/l	3.1	3.2	3.2	2.9	0.1	0.462	0.384
Cholesterol, mmol/l	1.4	1.5	1.7	1.8	1.0	0.097	0.969
Urea, mmol/l	0.03	0.03	0.03	0.03	0.002	0.815	0.134
Triglycerite, mmol/l	0.8	0.8	0.8	0.8	0.1	0.465	0.617

AST – aspartate aminotransferase, ALT – alanine aminotransferase, MOLM0 – rabbit grower diet with 0% MOLM as a substitute, MOLM5 – rabbit grower diet with 5% MOLM as a substitute, MOLM10 – rabbit grower diet with 10% MOLM as a substitute, MOLM15 – rabbit grower diet with 15% of MOLM as a substitute; SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Biomarkers and serum biochemical parameters

There were no significant ($P > 0.05$) linear and quadratic trends for any serum metabolic parameters, with the exception of aspartate aminotransferase (AST) and albumen (Table 9). However, AST increased quadratically ($y = 43.67 (\pm 2.62) - 2.07 (\pm 0.84) x + 0.14 (\pm 0.05) x^2$; $R^2 = 0.34$; $P = 0.024$), while albumen decreased quadratically ($y = 37.6 (\pm 4.49) + 3.07 (\pm 1.44) x - 0.22 (\pm 0.09) x^2$; $R^2 = 0.33$; $P = 0.03$) with increasing MOLM levels. Rabbits fed the MOLM10 diet had the lowest AST (32.5 IU/l) compared to those on the MOLM15 diet. Rabbits from the MOLM10 group showed the highest ($P < 0.05$) blood glucose levels as compared to animals from the MOLM0 group. No significant differences in blood potassium levels were observed between MOLM0 and other experimental diets. Rabbits fed MOLM15 had the lowest ($P < 0.05$) magnesium, creatinine and calcium levels in the blood. However, no dietary effects on alanine aminotransferase (ALT), protein, sodium, albumin, cholesterol, urea and triglyceride concentrations were detected.

Discussion

Moring oleifera leaf meal is an excellent source of minerals, crude protein and phenolic compounds, and these constituents have proven MOLM to be a good nutritional source for enhancing growth performance and the welfare of chickens (Sebola et al., 2017). Feed intake was shown to be reduced when the inclusion level of MOLM increased. This could be attributed to the presence of tannins and saponins in MOLM, which can affect taste, palatability and functional properties of diets, thereby altering feed intake (Stevens et al., 2015; Disetlhe et al., 2018). It is known that increasing MOLM levels in diets elevate the concentration of anti-nutritional factors. This could also explain why the rabbits had a decreased overall FI and linear FI at weeks 7, 8, 9, 10 and 11.

In this study, repeated analysis showed a significant interaction of diet × week on WFI, WWG, WFCR, PER, PC and PER, suggesting that the capacity of rabbits to utilise MOLM-containing diets depended on their age.

Positive quadratic effects were observed for OWG and AWWG at weeks 7, 8, 9 with increasing MOLM levels, indicating that the highest levels of MOLM did not supress body weight gain of rabbits. Extensive literature (Omara et al., 2018; Jiwuba and Ogbuewu, 2019; Selim et al., 2021) reported similar findings when 10, 20 and 30% MOLM was included in rabbit diets. In addition, Dougnon et al. (2012) also reported similar findings when 15% MOLM was included in rabbit diets. These findings support claims that MOLM has the potential to improve the performance of livestock, and this may be due to the substantial amount of bioactive compounds, minerals (calcium, magnesium and phosphorus), amino acids (lysine and methionine) and vitamins (A, B and C) that are known to increase animal growth (Ahmed, 2017; Briones et al., 2017; Sebola et al., 2017; El-Desoky et al., 2018; Mahfuz and Piao, 2019). The improved protein and fibre digestibility, as well as increased body weight explain higher FCR observed in the current study, which is in line with the studies of Omara et al. (2017) and Jiwuba and Ogbuewu (2019). Furthermore, MOLM has the potential to improve qualitative and quantitative nutrient utilisation due to its fibrous nature. Sun et al. (2018) attributed better average daily weight gain and FCR of rabbits to protein and amino acid contents of MOLM.

The inclusion of MOLM into the rabbit diet did not affect any serum biochemical parameters, reflecting the lack of post-ingestive feedback. Similar findings were reported in other works concerning rabbits fed diets containing MOLM (Maina et al., 2007; Olatunji et al., 2013). Interestingly, ALT and AST biomarker levels were reported within a normal range of 45–80 and 35–130 U/l, respectively (Melillo, 2007), suggesting that rabbits were able to tolerate anti-nutritional factors present in MOLM like tannins, phenols, saponins, oxalate, phytate and alkaloids, which may cause potential toxic effects when ingested in large quantities. Biomarkers are indicators of the integrity of certain vital organs and are released into the blood when liver cells are damaged. ALT levels are known to raise above the normal range in cases of liver duct damage, congestive heart failure and hepatitis (Bona et al., 2018). Rabbits fed the control diets had similar serum glucose levels compared to the MOLM15 group, which indicated that the highest MOLM inclusion levels in the current study did not negatively affect fat metabolism. High blood magnesium concentrations in rabbits fed MOLM-containing diets can be explained by the presence of magnesium in this additive. In addition, MOLM is a good source of minerals (Foidl et al., 2001; Moyo et al., 2011). Bovera et al. (2007) reported that blood protein levels were not affected by partial changes in dietary protein.

For this reason, similar blood protein and albumin levels were observed in rabbits fed different dietary treatments, indicating that MOLM could be incorporated into rabbit diets without compromising protein nutrition. Blood albumin is known to be related to total protein concentration in the diet (Omidi and Ansari nik, 2013). Phytate content of 0.43 mg/g in MOLM was shown to reduce mineral availability (Oladeji et al., 2017), and this could be the reason why rabbits fed MOLM15 had low serum potassium and calcium levels. Similarities in serum sodium and cholesterol, urea and triglyceride concentrations across the experimental diets indicate that the bioavailability of this nutrients is not negatively affected by the inclusion of MOLM into rabbit diets. The results, therefore, imply that the addition of MOLM into diets do not affect nutrient utilisation and health status of rabbits.

Conclusions

The study demonstrated that up to 30% MOLM can be safely included in rabbit diets without adversely affecting growth parameters and health status of the animals. Positive results regarding weight gain, feed conversion ratio, as well as serum protein, AST and ALP levels indicate that MOLM can be used to replace soybean ingredients in rabbit diets without compromising performance.

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Conflict of interest declaration

The Authors declare that there is no conflict of interest.

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