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Nutrient digestibility and nitrogen balance in growing pigs fed a 10% *Macadamia integrifolia* nut oil cake diet supplemented with exogenous enzymes

Q. Rambau, M. Mikasi, F. Fushai#

Department of Animal Science, Faculty of Science, Engineering and Agriculture, University of Venda, Thohoyandou, Limpopo, South Africa

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Abstract

The study tested a 10% dietary inclusion of Macadamia nut oil cake (MOC) fortified with exogenous enzymes on nutrient digestibility and nitrogen balance in growing pigs. The diets included a commercial maize–soybean pig grower control and a 10% MOC diluted iso-nutrient mix, each with a duplicate supplemented with 500 g/tonne of an enzyme cocktail containing 3000 FTU g^{-1} 6-phytase (IUB 3e1.3.26), 7270 U g^{-1} endo^{-1},4-beta-xylanase (EC-3.2.1.8), 300 U g^{-1} alpha amylase (EC-3.2.1.1), 6000 g^{-1} subtilisin protease (EC-3.4.21.62), and 532 U g^{-1} endo^{-1},4-beta-glucanase (IUB 3.2.1.6). Eight male Large White × Landrace 28-day-old weaned piglets were randomly assigned to the four diets in a 2 ×2 factorial experiment within two balanced 4 × 4 Latin squares for cross-over feeding over 8-day periods (3-d adaptation + 5-d feed intake) and total faecal and urine measurement. The digestibility of proximate and detergent fibre components, the nitrogen intake, faecal and urinary excretion; and the calculated total excretion, apparent digestibility, absorption, retention, utilization, and biological value of the dietary protein were evaluated. The 10% inclusion reduced dietary crude protein digestibility and the scaled live (g.kg^{-1} live weight) and metabolic (g/day/kg LW$^{0.75}$) nitrogen retention. Scaled on live weight, the 10% MOC diet reduced nitrogen retention only when the diets contained exogenous enzymes. Further research is recommended to test the enzymes at a higher dosage or evaluate different enzyme activities.

Key words: Enzymes, byproducts, climate-smart feeds, nitrogen utilisation

#Corresponding author: felix.fushai@univen.ac.za

Introduction

The livestock industry is experiencing feed shortages, with increasing costs (Acheampong-Boateng *et al*., 2017). Traditional plant protein sources such as soybean oil cake, which are commonly used in monogastric diets, have become increasingly scarce and expensive, particularly for small-scale farmers (Nkosi *et al*., 2011). Many agro-industrial by-products are readily-available locally and cheap, of which there are potential substitutes such as macadamia nut oil cake (MOC) (Acheampong-Boateng *et al*., 2017). South Africa boasts a sizeable and growing Macadamia nut industry, currently estimated at 65 516 hectares and producing 77 659 nuts in shell-tonnes (SAMAC., 2023). The oil-extracted byproduct, MOC, contains approximately 19.5% protein (Acheampong-Boateng *et al*., 2008). However, its potential as an alternative protein source for monogastric livestock has not been fully explored. The

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challenge is its high fibre content, which can be as much as 25%, partly due to the addition of soybean hulls for efficient expeller oil extraction (Acheampong-Boateng *et al*., 2008). Given the high fibre, high MOC dietary inclusion in monogastric diets may suppress feed intake and depress nutrient digestibility (Jha *et al*., 2019). There is, therefore, a need to determine the appropriate dietary inclusion and processing which improves its nutritive value for its efficient integration into high-precision growing pig feeding systems. Exogenous cocktails of amylase, non-starch polysaccharide carbohydrase, phytase, and protease enzymes may enhance pig digestion by hydrolysing otherwise indigestible polymers into bioavailable nutrients (O'Neill *et al*., 2014). However, for MOC-supplemented diets, the enzyme–substrate affinity (Apajalahti *et al*., 2001) may be limited, given a chemically-unique, more fibrous dietary matrix. Guided by the recommended maximum limits of dietary fibre in growing pig diets, the present study evaluated the effects of 10% dietary inclusion of MOC as a protein source in a maize-based diet for growing pigs and assessed the efficacy of a cocktail of amylolytic, fibrolytic, and proteolytic exogenous enzymes in enhancing the dietary utilization.

Methods and Materials

The experimental protocols for the management and care of animals were approved by the Animal Care and Use Committee of the University of Venda (Reference: SARDF/19/ANS/16/0612). The study was conducted at the University of Venda, which is in Vhembe district, Limpopo province, in South Africa at geo location 22°58'32'' S, 30°26'45'' E. The mean summer and winter daily temperature for the location are 25–40 °C and 12–26 °C, respectively (Mzezewa & Gwata, 2012).

The macadamia oil cake **(**MOC) was obtained from Royal Macadamia (Pty) Ltd in Levubu, Limpopo province, South Africa (Table 1).

Table 1 Analysed chemical composition (DM basis) of macadamia nut oil cake

Chemical Components	**Composition**
Ash (g kg^{-1})	35.1
Crude protein (g kg^{-1})	151.7
Ether extract (g kg^{-1})	236.2
Neutral detergent fibre (g kg^{-1})	526.3
Acid detergent fibre (g kg^{-1})	378.8
Ca (g kg^{-1})	2.7
P (g kg^{-1})	1.7
Amino acids (g/100g)	
Arginine	1.34
Alanine	0.42
Asparagine	0.52
Glutamine	0.37
Glycine	0.15
Histidine	0.97
Isoleucine	0.45
Leucine	0.64
Lysine	0.88
Methionine	2.09
Phenylalanine	0.54
Proline	0.89
Serine	0.67
Threonine	0.95
Tyrosine	0.42
Valine	0.54

The dietary exogenous enzyme cocktail contained 3000 FTU g^{-1} 6-phytase (IUB 3e1.3.26), 7270 U g^{-1} endo-1,4-beta-xylanase (EC-3.2.1.8), 300 U g^{-1} alpha amylase (EC-3.2.1.1), 6000 g^{-1} subtilisin protease (EC-3.4.21.62), 532 U g^{-1} endo-1,4-beta-glucanase (IUB 3.2.1.6)

Experimental diets (Table 2) were a standard maize–soybean diet as the control and a test diet in which MOC was included at 10%, each with (+) and without (-) 500 g/tonne of the enzyme cocktail.

The 10% MOC was the approximate dietetic upper limit imposed by its high fibre (80 g DM crude fibre, 526 g.kg^{-1} DM NDF, 379 g.kg^{-1} DM ADF) content. The four diets were formulated and manufactured by the Brennco Feed Company (Pty) Ltd in Louis Trichardt, Limpopo Province, South Africa, and complied to the physico-chemical properties of their standard commercial pig grower diet and the minimum nutrient standards recommended for growing pigs (National Research Council, NRC, 1998; Table 2)

Table 2 Ingredient and analysed nutrient composition of pig grower diets

Composition	**Diets**	
	Macadamia oil cake inclusion (%)	
	0	**10**
Ingredients (% as is)		
Maize	58.4	33.9
Corn gluten feed	20.0	18.5
Corn-hominy feed	7.5	25.0
Macadamia oil cake	0.0	10.0
Soybean Meal (>46.5 % CP)	9.5	8.0
Corn gluten meal (60% CP)	3.0	3.0
Limestone flour	0.4	0.4
Mineral & Vitamin mix	1.2	1.2
Total	100.0	100.0
Chemical components (g kg^{-1} DM)		
Dry matter	895.0	834.0
Ash	52.2	46.9
Crude protein	201.9	193.9
Starch	380.3	188.2
Ether extract	45.8	28.1
Crude fibre	80.0	80.0
Neutral detergent fibre	233.4	324.8
Acid detergent fibre	53.7	107.5
Acid detergent lignin	9.6	17.8
Ca	4.2	5.1
P	4.2	3.9
Amino acids (g/100g DM)		
Arginine	1.18	1.13
Alanine	1.23	1.10
Asparagine	1.50	1.43
Glutamine	3.52	3.30
Glycine	0.93	0.95
Histidine	0.73	0.72
Isoleucine	0.74	0.71
Leucine	2.28	2.12
Lysine	0.87	0.88
Methionine	0.57	0.43
Phenylalanine	1.24	1.13
Proline	1.60	1.46
Serine	1.00	1.00
Threonine	0.88	0.82
Tyrosine	0.97	0.88
Valine	0.93	0.94

The trial facility was an open structure which housed movable, individual (117.5 cm length × 57.8 cm width x 83.9 cm height) steel-framed metabolic pig cages. The cage design allowed for lateral and longitudinal adjustments to continuously accommodate pig growth, promoting pig welfare and the efficient separation of pig faeces from the urine. Each cage had a nipple drinker to provide free access to water, which was strategically positioned to exclude water splashing into a frontal feeding trough designed for free access to feed, and for minimal feed spillage. The base of the metabolic cage was fabricated with steel gauze plates padded with serviceable plastic sheeting, which was positioned to collect and funnel urine into a collection bucket, allowing free, tail-end fall of faeces to a clean floor. Eight F1 Large White × Landrace 28-day-old weaned piglets were dewormed using Virbamec® LA prior to transfer to the metabolic cages in the trial house. The pigs were acclimatised to the experimental setup for two weeks, before assignment to the dietary treatments in a 2 (diet) × 2 (enzyme) factorial arrangement within two balanced 4 (period) × 4 (diet) Latin squares for changeover feeding using 8-day feeding periods, each split into 3 d adaptation plus 5 d for intake measurement and total faecal and urine collection. Feed and water were provided *ad libitum.*

Piglets were weighed at each period interchange. At induction, they weighed in at 15.3 ± 1.91 kg and had grown to 28 ± 0.92 kg by the end of feeding period 4. The feed input and all leftovers and any spillage were fully accounted for on each day during each measurement period to estimate the period dry matter intake. Due to the fine feed texture, there was no evidence of selective feeding, such that the leftover and any spilled age were mixed into the fresh daily feed. The total faeces and urine were collected daily at 08:00–09:00. The cage setup largely ensured most faeces dropped directly to the floor, and any faeces pasted onto the cage base frames were scrapped by hand and mixed thoroughly with the floor faeces and weighed. Representative 10% faecal samples from the daily collections were retained and frozen at -20 °C. At the end of collection periods, frozen daily faecal samples from each pig were thawed and pooled, mixed by gloved-hand, and oven-dried at 60 °C before dry storage pending chemical analyses.

Daily total urine was collected into floor plastic buckets containing 100 ml of 1M H_2SO_4 to reduce nitrogen volatilisation and prevent microbial growth. The total urine was filtered through fine mutton cloth to remove solid contaminants, was weighed, and the volume measured. Aliquots (10% *v/v*) of the daily urine collection from each pig were frozen-stored at -20 °C pending N analyses of the pooled 5-day samples.

The dry matter content of feed and faeces was estimated by oven-drying 1 g samples to constant weight at 60 °C (method 930.15; AOAC, 1990). Ash was analysed by overnight combustion of 0.5 g at 500 °C (method 942.05; AOAC, 1990). Feed, faecal, and urinary N were determined using the Kjeldahl procedure (method 984.13; AOAC, 1990). Crude fat was extracted using the Soxhlet procedure (method 920.39; AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to the methods of van Soest *et al*. (1991).

Nitrogen balance parameters were computed using the equations listed in Table 3. The weight-dependent parameters (NI, FNO, UNO, TNE, NR, AN) were further scaled to the pig live ($g.kg^{-1}$ BW) or metabolic ($g.kg^{-1}$ $BW^{0.75}$) weight.

Table 3 Equations for estimating nitrogen balance components

Parameter	[1]Formula
Nitrogen intake (NI)	(N feed/100) × daily feed intake
Faecal nitrogen output (FNO)	(N faeces/100) × daily faecal output
Urinary nitrogen output (UNO)	(N urine/100) × daily urine output
Total nitrogen excretion (TNE)	FNO + UNO
Nitrogen retention (NR)	NI - TNE
Absorbed nitrogen (AN)	NI - FNO
Apparent nitrogen digestibility (ND)	(AN/NI)
Nitrogen utilization (NU)	NR/NI × 100
The biological value of feed protein (BVFP)	NR/ND × 100

[1]Otto *et al.* (2003)

The generalised linear model (GLM) of MINITAB software (Version 19.0; 2020) was used to analyse the dry matter and nutrient digestibility coefficients; the N balance parameters were analysed using a mixed-model analysis of variance (ANOVA), with the diet and enzyme supplementation as fixed effects, and the Latin Square, feeding period, and pig as random effects in the model:

$$Y_{jklm} = \mu + D_i + E_j + S_k + P_l + A_m + (\alpha\beta)_{ij} + e_{ijklm} \tag{1}$$

where Y_{ijklmn} is the observed parameter value, μ the overall mean, D_i the fixed effect of the i^{th} diet, E_j the fixed effect of the j^{th} enzyme dosage, S_k the random effect of the k^{th} Latin square, P_l the random effect of the k^{th} period, A_m the random effect of the m^{th} animal within a Latin square, (αβ) the diet × enzyme interaction, and e_{ijklm} the residual error. Means were separated using Tukey's test, at the $P < 0.05$ level of significance.

Results

Table 4 shows the effects of a 10% dietary inclusion of MOC and supplementary enzymes on the dry matter intake and digestibility coefficients of DM, OM, CP, FAT, NDF, and ADF. The 10% MOC diet had low ($P < 0.05$) digestibility of crude protein, with no ($P > 0.05$) effect on the digestibility of other chemical components.

Tables 5 and 6 show the effects of 10% dietary inclusion of macadamia oil cake (MOC) and supplementary enzymes on N balance parameters on LW or $W^{0.75}$ basis, respectively. The 10% MOC dietary inclusion reduced ($P < 0.05$) the NR with an interaction ($P < 0.05$) on a LW basis; the reduction in NR was significant only when the diets contained exogenous enzymes.

Discussion

The 10% MOC was included in the diet at the approximate upper dietary limit imposed by its high fibre content (NRC, 1998), which matched the dietary limit previously estimated for broiler diets (van Ryssen *et al*., 2014). High levels of MOC dietary inclusion in monogastric diets risk incremental negative effects on dietary efficacy since dietary fibre has a negative effect on intake and nutrient digestion (Nortey *et al*., 2008). Such deleterious effects can theoretically be mitigated by potent exogenous enzymes (Zijlstra *et al*., 2010). In the current study, dietary inclusion of MOC at 10% marginally (0.73–0.72) depressed the CP digestibility, without an effect on the digestibility of DM, OM, fat, NDF, and ADF. Depressed CP digestibility was associated with increased quantitative faecal N excretion and total N excretion. Consequently, the 10% MOC dietary inclusion reduced the NR.

When scaled to the pig live weight, depression of NR was more pronounced in the enzyme-fortified MOC diet, which suggests chemical modification of the MOC to produce antinutrient components which impaired protein digestion or amino acid absorption. The treatments did not influence the N excretion route, nor did they affect the N efficiency parameters. Relationships between the excretion parameters, FNO and UNO, and the efficiency indicators, NR, NU, and BVFP, can be complex. Their correlation is subject to both the pig and the gut microbial N metabolism. Imbalance in dietary amino acids leads to catabolism of excess amino acids, which increases urea excretion in the urine (Ball *et al*., 2013). Dietary crude protein that evades digestion in the upper tract offloads nitrogenous substrates that increase colonic microbial fermentation (Bindelle *et al*., 2009). If the diet contains the requisite colon fermentable energy, the energy is used to assimilate both the endogenous blood urea N and the indigestible dietary protein N, causing a shift in blood urea N excretion from urinary to faecal excretion as microbial protein (Bindelle *et al*., 2009). Such an effect was confirmed by Hlongwana *et al*. (2021), who observed high N excretion through faeces due to colon microbial assimilation of escaped dietary N and endogenous blood urea N. If the diet is deficient in fermentable energy that escapes to the colon, increased colon protein fermentation is undesirable since it may yield toxic nitrogenous metabolites (Tuśnio *et al*., 2017).

In the present study, when scaled to pig live weight, a more marked reduction of NR when both diets contained exogenous enzymes implied relatively more exogenous enzyme action on the 0%, compared to the 10%, MOC dietary protein. The non-phased feeding regime across feeding periods which spanned 32 d of pig feeding meant a constant profile of dietary amino acids, which did not meet the changing pig amino acid requirement. When expressed on a LW or W^{075} basis, a decrease in NR over time was therefore possible, consistent with declining efficiency of amino acid utilisation due to the mismatch in supply versus demand. Though less so for a lean pig genotype, with a decreasing metabolic requirement for muscle growth with pig age, pigs should incrementally metabolise surplus dietary amino acids to accrue body fat, with increased excretion of the waste N in urine (Remesar & Alemany, 2020). However, perhaps also due to the short feeding period, declining N or protein efficiency was not confirmed by the estimates of NU and BVFP. In multiphase-feeding systems, commercial growing pigs typically attain approximately 40% N utilisation efficiency (Rotz, 2004). In the current study, a comparatively high dietary protein efficiency was obtained in all diets, as indicated by the BVFP coefficient of 0.42–0.55 and NU 35–46%, consistent with genetic improvements for fast growth in the lean-type, Large White breed and given the pig age and early growth experimental phase.

Conclusion

A 10% MOC dietary inclusion in a standard maize–soybean growing pig diet marginally reduced the digestibility of CP and depressed the live- and metabolic weight-scaled NR, with an MOC–enzyme interaction for the live-weight scaled NR, which suggests a beneficial action in the control, compared to the MOC diet. Further research is recommended to test the same enzymes at higher dosages or test different enzymes.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Rambau, with the assistance and guidance of Mikasi and Fushai. The first draft of the manuscript was written by Rambau, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest declaration

Authors declare no conflict of interest and agreed to its submission for publication.

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Table 4 Effects of 10% dietary inclusion of macadamia oil cake and supplementary enzymes on dry matter intake and nutrient digestibility in growing Large White × Landrace pigs

Treatments		**Intake (g DM day $^{-1}$)**	**Digestibility coefficients**					
			Dry matter	**Organic matter**	**Crude protein**	**Crude fat (ether extract)**	**Neutral detergent fibre**	**Acid detergent fibre**
Diet	**[1]Enzyme**							
0%	**+**	670.95	0.86	0.84	0.72	0.54	0.66	0.52
	-	703.36	0.86	0.88	0.72	0.55	0.66	0.52
10%	**+**	649.03	0.83	0.88	0.73	0.66	0.70	0.62
	-	586.20	0.84	0.86	0.73	0.71	0.72	0.66
SEM		98.100	0.024	0.018	0.006	0.059	0.042	0.060
Diet		-						
0%		687.16	0.86	0.86	0.72^{a}	0.55	0.66	0.52
10%		617.61	0.84	0.87	0.73^{b}	0.69	0.71	0.64
SEM		69.400	0.013	0.013	0.004	0.042	0.029	0.043
[1]Enzymes								
-		644.78	0.85	0.87	0.72	0.63	0.69	0.59
+		659.99	0.85	0.86	0.73	0.60	0.68	0.57
SEM		69.400	0.013	0.013	0.004	0.042	0.029	0.043
P-Values								
Diet		0.499	0.160	0.598	0.045	0.051	0.297	0.084
Enzyme		0.881	0.750	0.520	0.935	0.614	0.807	0.798
Diet × Enzyme		0.640	0.655	0.200	0.923	0.737	0.876	0.690

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at $P < 0.05$. [1]Cocktail containing 3000 FTU g^{-1} 6-phytase (IUB 3e1.3.26), 7270 U g^{-1} endo-1,4-beta-xylanase (EC-3.2.1.8), 300 U g^{-1} alpha amylase (EC-3.2.1.1), 6000 g^{-1} subtilisin protease (EC-3.4.21.62), 532 U g^{-1} endo-1,4-beta-glucanase (IUB 3.2.1.6).

(-): diet without enzyme; (+): diet plus 500 g tonne^{-1} enzyme; SEM: standard error of mean

Table 5 Effects of 10% dietary inclusion of macadamia oil cake (MOC) and supplementary enzymes on scaled (g/day/kg live weight (LW)) N balance in growing Large White pigs

Treatments		**N Utilisation**								
		NI	**UNO**	**FNO**	**TNE**	**AN**	**NR**	**NU**	**Coefficient of ND**	**Coefficient of BVFP**
Diet	**^{1}Enzyme**	**g.kg-1 LW**	**g.kg^{-1} LW**	**g kg^{-1} LW**	**g kg^{-1} LW**	**g kg^{-1} LW**	**g kg^{-1} LW**	**%**		
0%	**+**	8.36	0.09	1.05	1.15	8.26	3.18^{a}	39.68	0.88	0.45
	-	7.99	0.09	0.87	0.96	7.77	2.94^{ab}	37.59	0.89	0.42
10%	**+**	6.60	0.05	0.99	1.39	6.55	2.69^{b}	46.52	0.85	0.55
	-	8.25	0.09	1.3	1.05	8.16	2.9^{ab}	35.85	0.84	0.43
SEM		0.577	0.024	0.171	0.178	0.578	0.195	4.820	0.021	0.055
Diet	**0%**	8.18	0.09	0.96	1.06	8.01	3.06^{a}	38.63	0.88	0.44
	10%	7.43	0.07	1.15	1.22	7.35	2.79^{b}	41.19	0.84	0.49
SEM		0.408	0.017	0.121	0.126	0.408	0.138	3.410	0.015	0.039
^{1}Enzymes	**-**	8.12	0.09	1.08	1.18	7.96	2.29	36.72	0.87	0.43
	+	7.48	0.08	1.02	0.10	7.40	2.93	43.09	0.86	0.50
SEM		0.408	0.017	0.121	0.126	0.408	0.138	3.410	0.015	0.039
P Values										
Diet		0.237	0.232	0.200	0.250	0.318	0.023	0.598	0.155	0.400
Enzymes		0.303	0.390	0.668	0.571	0.391	0.934	0.207	0.829	0.214
Period		0.174	0.558	0.521	0.477	0.207	0.0010	0.520	0.770	0.627
Diet × Enzyme		0.124	0.360	0.117	0.087	0.128	0.044	0.382	0.715	0.463

^{ab} Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at (*P* <0.05). **: *P* <0.01; (NS) not significant: (*P* >0.05); NI: Nitrogen intake; UNO: Urinary nitrogen output; FNO: Faecal nitrogen output; TNE: Total nitrogen excretion; AN: Absorbed nitrogen; NR: Nitrogen retention; NU: Nitrogen utilisation; BVFP: Biological value feed protein; ND: Apparent N digestibility; ^{1}Cocktail containing 3000 FTU g^{-1} 6-phytase (IUB 3e1.3.26), 7270 U g^{-1} endo-1,4-beta-xylanase (EC-3.2.1.8), 300 U g^{-1} alpha amylase (EC-3.2.1.1), 6000 g^{-1} subtilisin protease (EC-3.4.21.62), 532 U g^{-1} endo-1,4-beta-glucanase (IUB 3.2.1.6).

(-): diet without enzyme; (+): diet plus 500 g tonne^{-1} enzyme; SEM: standard error of mean

Table 6 Effects of 10% dietary inclusion of macadamia oil cake (MOC) and of supplementary enzymes on scaled (g/day/kg metabolic weight ($LW^{0.75}$) N balance in growing Large White pigs.

		N Utilisation								
		NI	**UNO**	**FNO**	**TNE**	**AN**	**NR**	**NU**	**Coefficient of ND**	**Coefficient of BVFP**
Diet	**[1]Enzyme**	**g/day/kg $LW^{0.75}$**	**g/day/kg $LW^{0.75}$**	**g/day/kg $LW^{0.75}$**	**g/day/kg $LW^{0.75}$**	**g/day/kg $LW^{0.75}$**	**g/day/kg $LW^{0.75}$**	**%**		
0%	**+**	19.01	0.238	2.37	2.59	16.41	7.23	39.68	0.88	0.45
	-	18.79	0.23	2.04	2.27	16.51	6.86	37.59	0.89	0.42
10%	**+**	19.11	0.13	2.36	2.49	13.02	6.33	46.52	0.85	0.55
	-	15.50	0.21	2.99	3.20	15.9	6.69	35.85	0.84	0.43
SEM		1.440	0.049	0.313	0.327	1.350	0.176	4.520	0.020	0.052
Diet	**0%**	18.89	0.23	2.21	2.44	16.46	7.05^{a}	38.63	0.88	0.44
	10%	17.30	0.17	2.67	2.84	14.46	6.51^{b}	41.19	0.84	0.49
SEM		1.010	0.035	0.222	0.231	0.958	0.125	3.190	0.014	0.038
[1]Enzymes	**-**	18.95	0.22	2.52	2.74	16.21	6.78	36.72	0.87	0.43
	+	17.25	0.18	2.36	2.54	14.71	6.78	43.09	0.86	0.50
SEM		1.010	0.035	0.222	0.231	0.958	0.125	3.190	0.014	0.038
P Values										
Diet		0.292	0.251	1.168	0.211	0.201	0.031	0.598	0.155	0.400
Enzymes		0.265	0.396	0.628	0.529	0.329	0.999	0.207	0.829	0.214
Period		0.287	0.523	0.485	0.429	0.356	0.017	0.520	0.770	0.627
Diet × Enzyme		0.213	0.414	0.159	0.123	0.361	0.111	0.382	0.715	0.463

[ab] Within each factor or factor interaction, for each parameter, means with different superscripts differ significantly at ($P < 0.05$). **: $P<0.01$; (NS) not significant: ($P > 0.05$); NI: Nitrogen intake; UNO: Urine Nitrogen Output; FNO: Faecal Nitrogen Output; TNE: Total Nitrogen Excretion; AN: Absorbed Nitrogen; NR: nitrogen retention; NU: Nitrogen utilisation; BVFP: Biological Value Feed Protein; ND: Apparent N digestibility; [1]Cocktail containing 3000 FTU g^{-1} 6-phytase (IUB 3e1.3.26), 7270 U g^{-1} endo-1,4-beta-xylanase (EC-3.2.1.8), 300 U g^{-1} alpha amylase (EC-3.2.1.1), 6000 g^{-1} subtilisin protease (EC-3.4.21.62), 532 U g^{-1} endo-1,4-beta-glucanase (IUB 3.2.1.6).

(-): diet without enzyme; (+): diet plus 500 g $tonne^{-1}$ enzyme; SEM: standard error of mean