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Apparent Digestibility, Nitrogen Retention, and Microbial Protein Yield in Goats Fed a Diet Based on Macadamia Oil Cake

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Simple Summary: A study with 16 mixed breed goats aged 18-24 months was conducted to assess the digestibility, nitrogen retention, and microbial protein production in a diet based on Macadamia oil cake. The four dietary treatments (0, 100, 150, and 200 g/kg) macadamia oil cake were allocated four goats, and each goat was considered an experimental unit in a complete randomised design (CRD) . The study lasted 21 days, with 14 days for acclimatization and 7 days for sample collection. The results showed that Macadamia oil cake inclusion did not affect dry matter, organic matter, or crude protein intake. Nitrogen retention and the apparent digestibility of dry matter, crude protein, neutral detergent fibre, and acid detergent fibre were not affected. Microbial protein yield increased with more than 10% macadamia oil cake supplementation. The findings support the use of Macadamia oil cake as an alternative protein source in goat diets. Further research is recommended to determine the optimal dietary inclusion.

Abstract: The study assessed the apparent digestibility, nitrogen retention, and microbial protein production in goats fed a Macadamia oil cake-based diet. Sixteen mixed breed bucks about 34 kg and age ranging from18 to 24 months old, were randomly allocated to four dietary treatments of 0, 100, 150, and 200 g/kg macadamia oil cake in a complete randomised design and each goat was considered an experimental unit. Faecal digestibility and nitrogen balance were assessed through total faecal and urine collection. Microbial protein production was estimated using urinary purine derivatives allantoin method. The study lasted 21 days, with 14 days for acclimatization and 7 days for sample collection. Data were analysed using one way analysis of variance, through General Linear Models procedures. Treatment means were compared using Tukey's post-hoc test. Macadamia oil cake inclusion did not affect dry matter, organic matter, or crude protein intake. There were no differences in nitrogen retention and the apparent digestibility of dry matter, crude protein, neutral detergent fibre, and acid detergent fibre. Positive nitrogen retention was observed across treatments. Microbial protein yield increased with more than 10% macadamia oil cake supplementation. The findings supported its use as an alternative protein source in goat diets. Further research is recommended to determine the optimal dietary inclusion.

Keywords: goats; rumen fermentation; rumen microbial protein; purine derivative technique

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1. Introduction

Feed quality and quantity for ruminants often fluctuate across seasons and years, with protein typically being the most limiting nutrient [1]. This is especially true during the dry season when ruminants rely heavily on natural pastures that are high in fibre but deficient in protein [2]. Adequate nutrient intake is essential for maintaining optimal production, health, and reproduction in ruminants [3]. In South Africa, during winter, limited fodder availability and low-quality forage lead to inadequate nutrient intake, particularly of crude protein (CP), which is necessary for rumen microbial protein synthesis[4]. Despite these challenges, ruminants continue to play a vital role in global food production, particularly for meat and milk. To address the issue of protein deficiency, there is a need to develop feeding strategies that incorporate cost-effective protein sources into ruminant diets [1]. These strategies can improve the utilization of low-quality roughages by supplying nitrogen to rumen microorganisms, thereby enhancing overall feed efficiency [5,6]. [6] reported that supplementing pasture-based diets with protein sources can boost feed intake, digestibility, and animal performance. One major challenge in South African ruminant production is the scarcity and high cost of protein feed sources, which can account for up to 60–70% of total production costs [7,8]. While soybean meal (SBM) is a common protein source in concentrates, with a crude protein content of about 46%[9], it is also expensive. Alternative protein sources like Macadamia oil cake (MOC), which is locally available, offer a potential solution to high protein costs [10] . MOC contains approximately 20% CP [11], and its high rumen degradability (33.75 g/kg DM) makes it a promising protein supplement for ruminants [9]. However, information on the efficiency of MOC utilization in goats is limited. The study hypothesised that MOC inclusion in goat diets would not affect DM and CP digestibility, nitrogen retention, or microbial protein yield.

2. Methods and Materials

2.1. Experimental Site

The study was conducted at the University of Venda Experimental Farm in Thohoyandou, Limpopo Province (22°58′32′′ S, 30°26′45′′ E). The region experiences summer temperatures ranging from 25°C to 40°C, while winter temperatures range from a minimum of 11°C to a maximum of 27°C [12].

2.2. Experimental Diets

Macadamia Oil Cake (MOC) was sourced from Green Farms (Pty) Ltd, Levubu, Limpopo, South Africa. Four dietary treatments were formulated with varying levels of MOC inclusion: 0% (Control), 10%, 15%, and 20%. The chemical composition of both the MOC and the experimental diets is shown in Table 1. The diets were formulated and prepared by Brennco Feeds Company (Pty) Ltd in Louis Trichardt, South Africa, with a crude protein (CP) content of approximately 180 g/kg dry matter (DM), meeting the nutritional requirements of growing goats [13].

Table 1. Chemical composition (g/kg dry matter) of Macadamia oil cake and experimental diets fed to goats.

		[1]Diets			
Nutrients	**MOC[a]**	**MOC0[b]**	**MOC10[c]**	**MOC15[d]**	**MOC20[e]**
Dry matter	926.1	917.1	918.2	919.0	920.8
Ash	42.8	91.2	81.7	81.8	85.1
Crude protein	137.0	33.5	42.4	50.4	54.6
Ether extracts	179.3	175.3	165.2	177.8	186.7
Neutral detergent fibre	500.0	342.9	321.7	309.5	280.9
Acid detergent fibre	364.5	148.2	159.0	174.2	151.3
Acid detergent Lignin		24.7	29.3	29.7	30.5
Calcium	3.0	8.8	8.0	7.3	6.9
Magnesium	1.9	3.5	3.1	3.0	2.9
Potassium	10.0	14.8	13.5	13.4	13.1
Sodium	0.7	3.3	2.9	2.6	2.5
Phosphorus	1.6	4.1	3.5	2.9	2.8
Zinc	0.0	0.1	0.1	0.1	0.1
Copper	0.1	0.0	0.0	0.0	0.0
Manganese	0.1	0.1	0.1	0.1	0.1
Iron	0.4	0.8	1.0	0.8	0.7

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[1]MOC - Macadamia oil cake; MOC0 = 0.0g/kg; MOC10 = 100g/kg: MOC15 = 150g/kg; MOC20[e] 200g/kg.

2.3. Determination of Apparent Digestibility

At the start of the trial, the goats had an average weight of 34 ± 8.39 kg. The goats were acclimatized to the diets over 14 days, followed by seven days during which daily feed intake, refusals, and faecal and urinary output were measured. Refusals were weighed each morning before feeding, and feed intake was calculated as the difference between the feed offered and refused. The amount of feed offered was based on 5% of the goats' body weight, adjusted as needed. The goats were also accustomed to faecal bags. Daily faecal output was weighed, and 10% of the total faeces was sampled and stored at -20°C for later chemical analysis.

2.4. Determination of Nitrogen Retention

Total daily urine was collected in plastic containers with 100 ml of sulfuric acid to prevent ammonia volatilization. The urine was filtered through a gauze cloth to remove any contaminants, and the total volume was recorded. A 10% sample of the total urine from each goat was collected and stored at -20°C for nitrogen analysis.

2.5. Chemical Analysis of Samples

The dry matter content of feed, refusals, and faeces was determined by drying 1 g of each sample in duplicate at 105°C until a constant weight was reached [14] (method 942:15; AOAC, 2010). Ash content was measured by combusting the samples in a muffle furnace at 550°C for 6 hours [14]. Nitrogen (N) content in feed, refusals, faeces, and urine was determined using the Kjeldahl method[14] and crude protein (CP) was calculated as N × 6.25. Crude fat was extracted using the Soxhlet method [14]. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined following the technique of [15]. The mineral content (calcium, magnesium, potassium, sodium, phosphorus, zinc, copper, manganese, and iron) was analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) according to Standard Operating Procedure [16].

2.6. Analysis of Purine Derivative (PD) Allantoin

The urinary excretion of purine derivatives, including allantoin, was measured to estimate microbial protein yield. Urine samples were frozen and diluted with distilled water to bring the allantoin concentration within the standard range (10-50 mg/L). Allantoin levels were determined using the colorimetric method with absorbance measured at 522 nm, following the procedure of [17]. The readings were taken using a digital spectrophotometer.

2.7. Mathematical Calculations and Statistical Analyses

The apparent digestibility coefficient for each parameter was calculated using the following equation[18]:

$$Apparent\ digestibility\ coefficient\ =\ (Nutrient\ in\ feed\ -\ Nutrient\ in\ faeces)\ /\ Nutrient\ in\ feed\ \ (1)$$

Nitrogen retention was calculated using the formula:

$$Nitrogen\ retention\ (g/day)\ =\ NI - (FN\ +\ UN)$$

Where NI = Nitrogen intake; FN = Faecal nitrogen; UN = Urinary nitrogen.

Digestible organic matter intake (DOMI) was calculated as follows:

$$DOMI\ (g/day)\ =\ Organic\ matter\ intake\ -\ Faecal\ organic\ matter$$

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Metabolizable energy (ME) intake and digestible organic matter in the rumen (DOMR) were calculated based on DOMI content [19]:

$$ME\ intake\ (MJ/day) = 0.0157 \times DOMI$$

$$\mathrm{DOMR\ (g/day)} = 0.65 \times \mathrm{DOMI}$$

2.8. Microbial Protein Yield

The microbial protein yield was calculated from equations as described by [17]:

$$Y = 0.84X + 0.15W^{0.75}e^{-0.25X})$$

Where Y= purine derivative excreted in the urine (mMol/d), X=concentration of microbial purines absorbed after duodenal and intestinal digestion (mMol/d), The calculation of X will be performed by means of Newton-Raphson iterative process until it approaches a constant value [17], $W^{0.75}$= the metabolic body weight of the animal (kg).

2.9. Microbial Nitrogen Yield Was Calculated as Follows:

$$MNY\ (g/d) = \frac{X * 70}{0.83 * 0.116 * 1000} = 0.727X$$

Where; 70 is the N content of purines (mg N/mMol), 0.83 is the average digestibility of mixed microbial purines based on observations of [17], 0.116 is the proportion of purine N in the total microbial nitrogen of mixed rumen microbes, 1/1000 is used to convert the estimate from mg to g

Microbial true protein (MTP), digestible microbial true protein (DMTP), and efficiency of microbial nitrogen supply (E_{mns}) were calculated according to the [19]

$$MTP\ (g/d) = 0.80 \times MNY \times 6.25$$

$$DMTP\ (g/d) = 0.85 \times MTP$$

$$Emns\ (g/kg\ DOMR) = MNY/DOMR$$

Data on voluntary nutrient intake, apparent digestibility, nitrogen retention, and microbial protein yield were analysed using the General Linear Model (GLM) procedure for a one-way ANOVA in a completely randomized design (CRD) using SPSS [20] . Treatment means were compared using Tukey's test, with initial animal weight included as a covariate in the analysis.

3. Results

Macadamia oil cake (MOC) inclusion significantly affected (P < 0.05) neutral detergent fibre intake (NDFI), acid detergent fibre intake (ADFI), and ether extract intake (EEI) (Table 2). An increase in MOC supplementation led to a significant reduction (P < 0.05) in digestible organic matter intake (DOMI) and metabolizable energy intake (MEI). MOC0 showed the highest DOMI and MEI, comparable to MOC10 and MOC15. However, no significant differences (P > 0.05) were found for dry matter intake (DMI), organic matter intake (OMI), and crude protein intake (CPI) between the treatments.

Table 2. Voluntary intake of nutrients (g d^{-1}) and energy (MJ d-1) by goats fed Macadamia Oil Cake (MOC) based diets.

		[1]Diets					
Parameter	**N**	**MOC0**	**MOC10**	**MOC15**	**MOC20**	**SEM**	**P value**
Dry matter	28	1186.24	974.49	1108.76	1120.34	57.00	0.07
Organic matter	28	1078.55	887.79	987.25	1024.81	51.20	0.07
Crude protein	28	207.96	173.19	183.07	189.02	9.88	0.09

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Ether extract	28	39.68[b]	41.26[b]	56.00[a]	61.11[a]	2.41	0.00
Neutral detergent Fiber	28	409.91[a]	313.65[ab]	342.94[b]	314.89[b]	18.3	0.00
Acid detergent Fiber	28	175.73[ab]	154.87[b]	193.36[a]	169.52[ab]	8.78	0.02
Digestible organic matter	28	1023.68[a]	912.22[ab]	909.34[ab]	823.15[b]	49.90	0.05
Metabolizable energy	28	16.07[a]	14.32[ab]	14.28[ab]	12.92[b]	0.78	0.05

[a,b] Values in the same row with different superscripts are significantly different at P<0.05; [1]MOC - Macadamia oil cake; MOC0 = 0.0g/kg; MOC10 = 100g/kg: MOC ; MOC15 = 150g/kg; MOC20[e] 200g/kg; SEM=Standard error of the mean.

The digestibility coefficients for DM, OM, CP, EE, NDF, and ADF in goats fed MOC-based diets are shown in Table 3. Significant differences (P < 0.05) were observed in OM and EE digestibility. Goats fed MOC0, MOC10, and MOC15 had significantly higher OM digestibility than those fed MOC20. MOC10 showed the highest (P < 0.05) EE digestibility compared to MOC20, while MOC0 and MOC15 showed similar digestibility.

Table 3. Apparent digestibility co-efficient of nutrients in goats fed diets supplemented with different Macadamia Oil Cake (MOC) inclusions.

Diets							
Component	**n**	**MOC0**	**MOC10**	**MOC15**	**MOC20**	**SEM**	**P-value**
Dry matter	28	0.76	0.74	0.75	0.66	0.04	0.27
Organic matter	28	0.94[a]	0.92[a]	0.92[a]	0.87[b]	0.01	0.00
Crude protein	28	0.77	0.75	0.75	0.65	0.43	0.20
Ether extract	28	0.88[ab]	0.91[a]	0.89[ab]	0.79[b]	0.03	0.01
Neutral detergent Fiber	28	0.53	0.45	0.45	0.23	0.08	0.07
Acid detergent Fiber	28	0.40	0.37	0.40	0.11	0.09	0.06

[a,b] Values in the same row with different superscripts are significantly different at P<0.05; [1]Macadamia oil cake; MOC0 = 0.0g/kg; MOC10 = 100g/kg: MOC; MOC15 = 150g/kg; MOC20 200g/kg: SEM=Standard error of the mean.

Nitrogen (N) intake and retention data (Table 4) for goats fed MOC-based diets revealed that MOC inclusion significantly influenced (P < 0.05) faecal-N, urinary-N, and total nitrogen excretion (TNE). Goats fed MOC0 had higher urinary-N and TNE compared to MOC15. However, no significant differences (P > 0.05) were observed in nitrogen retention, with all goats showing positive values.

The excretion of purine derivatives (PD) in the urine, particularly allantoin, increased significantly (P < 0.05) with MOC supplementation. Goats fed MOC20 had the highest allantoin excretion, microbial nitrogen yield (MNY), and microbial protein yield (MTP), although these values were like those in MOC10 and MOC15. There were no significant differences (P > 0.05) in digestible organic matter in the rumen (DOMR) or the efficiency of microbial nitrogen synthesis ((E_{mns})) across the diets.

Table 4. Nitrogen intake and retention in goats fed on Macadamia Oil Cake (MOC) based diets.

		[1]**Diets**					
Measurement (g/d)	**n**	**MOC0**	**MOC10**	**MOC15**	**MOC20**	**SEM**	**P value**
Nitrogen intake	28	33.50	27.76	29.36	30.30	1.58	0.09

Faecal Nitrogen	28	7.26[ab]	6.30[b]	7.16[ab]	8.61[a]	0.49	0.01
Urinary Nitrogen	28	17.04[a]	15.49[ab]	12.78[b]	16.87[a]	0.97	0.01
Total nitrogen excretion	28	24.49[a]	21.79[ab]	19.94[b]	25.49[a]	1.13	0.00
Nitrogen retention	28	9.02	5.97	9.40	4.08	1.70	0.16

[a,b] Values in the same row with different superscripts are significantly different at P<0.05; [1]MOC - Macadamia oil cake; MOC0 = 0.0g/kg; MOC10 = 100g/kg: MOC15 = 150g/kg; MOC20e 200g/kg: SEM=Standard error of the mean.

Table 5. Microbial protein yield based on urinary allantoin method in goats fed Macadamia Oil Cake (MOC) based diets.

	Diets					
Parameter	**MOC0**	**MOC10**	**MOC15**	**MOC20**	**SEM**	**P-value**
Allantoin (mMol/d)	14.2[b]	15.7[ab]	15.5[ab]	16.3[a]	0.40	0.00
Microbial N yield (g d^{-1})	12.3[b]	13.5[a]	13.4[ab]	14.0[a]	0.34	0.00
True Microbial protein (g d^{-1})	61.3[b]	67.9[a]	67.9[ab]	70.0[a]	1.72	0.00
Digestible organic matter in the rumen (DOMR)	660.6	534.0	589.8	591.8	32.3	0.06
[2]E_{mns} (g/kg DOMR)	0.02	0.03	0.02	0.03	0.01	0.31

[a,b] Means in the same row with different superscripts are significantly different: [1]MOC - Macadamia oil cake; MOC0 = 0.0g/kg; MOC10 = 100g/kg: MOC15 = 150g/kg; MOC20 200g/kg; N=Nitrogen; [2]E_{mns}=Efficiency of microbial nitrogen synthesis (AFRC,1993); SEM=Standard error of the mean.

4. Discussion

The crude protein (CP) content of MOC in this study was 137 g/kg DM, lower than the 260 g/kg DM reported by [21]. However, this value aligns with [9,22], who reported 130 and 147 g/kg DM, respectively. The variation in CP content may be due to differences in processing methods, hull content, extraction methods, or climate influences on MOC.

High fibre fractions in diets can adversely affect DM intake, particularly when they exceed 15% DM [23]. The high NDF (500 g/kg DM) and ADF (364.5 g/kg DM) in MOC explain the decreased feed intake at higher MOC inclusion levels. These findings are consistent with [9] but contrast with the results of [21], who reported lower NDF and ADF contents in MOC.

Despite the high fibre content, the absence of significant differences (P > 0.05) in DMI, OMI, and CPI between the treatments indicates that MOC did not negatively affect palatability. These findings are consistent with [12] and [9] who reported no significant differences in DMI in cattle and sheep fed MOC-based diets.

The non-significant differences in CPI among treatments suggest that increasing MOC inclusion up to 20% does not affect CP intake, a finding supported by[9] This is important for determining the threshold level of MOC inclusion in ruminant diets.

The ME intake ranged from 12.92 to 16.07 MJ d-1, with the lowest MEI for MOC20 and the highest for MOC0, which was like MOC10 and MOC15. These findings suggest that MOC-based diets can meet the energy needs of ruminants, even for high-producing animals such as lactating cows [13].

Digestibility coefficients for DM, CP, NDF, and ADF did not differ significantly (P > 0.05) between the treatments. This aligns with the results of [9] , suggesting that MOC inclusion does not negatively affect the digestibility of these nutrients. However, [24] reported significant differences in NDF and ADF digestibility with Amarula and sunflower oil cakes, suggesting that differences in fibre content may explain the discrepancy.

Nitrogen intake did not differ between the treatments, but MOC inclusion significantly affected faecal-N, urinary-N, and TNE (P < 0.05). Higher faecal-N excretion was recorded in goats fed 20%

MOC, while urinary-N was higher in goats fed MOC0. Positive nitrogen retention in all treatments indicates efficient nitrogen utilization by the goats, consistent with previous studies [9,25]

The increase in PD allantoin excretion with higher MOC inclusion suggests enhanced ruminal microbial activity. This finding aligns with[26] but contrasts with [9], who found no significant differences in allantoin excretion. Differences in animal species and diet composition may explain these discrepancies.

The CP content of the diets (~18%) supports microbial activity in the rumen [27]. Significant differences in microbial nitrogen yield ($P < 0.05$) between treatments suggest that MOC supplementation promotes microbial protein synthesis. This finding is consistent with [28], who reported similar results in goats fed soybean and cottonseed meals.

The non-significant differences in the efficiency of microbial nitrogen synthesis (1Emns) may be due to the high NDF content in MOC, which limits the supply of metabolizable protein to the small intestine [29].[30] reported similar (E_{mns}) values, while [31] found higher ranges for forage legume diets.

5. Conclusion

The results of this study suggest that MOC-based diets can meet the protein and energy needs of growing goats, making MOC a viable protein supplement in ruminant diets, particularly during dry seasons. The urinary excretion of allantoin as a PD can serve as a useful tool for estimating microbial protein yield and nitrogen utilization efficiency in goats.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data used in the current study are not publicly available due to privacy restrictions of the patient data and the patient owners.

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