Urea-ammoniated cowpea husk in goat diet: Feed utilisation, ruminal fermentation, nutrient digestibility, nitrogen utilisation, blood metabolites and growth performance

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Abstract

The study investigated the effect of urea-ammoniated cowpea husk (UCH) as a substitute for dried brewers' grains (DBG) on voluntary intake, ruminal fermentation, apparent digestibility, nitrogen retention, serum metabolites and growth performance of eighteen growing Red Sokoto goats. Goats were stratified into three groups: no UCH (control), 350 g/kg UCH and 700 g/kg UCH replacing 0, 500 and 1000 g/kg of DBG respectively. Average daily gain, organic matter (OM) and acid detergent fibre digestibility, digestible OM, metabolisable energy, nitrogen balance and retention, ruminal volatile fatty acid, digestible OM fermented in the rumen, microbial protein synthesis, and serum glucose and aspartate transaminase were increased quadratically ($P < 0.05$) in 350 g/kg UCH diet. Whereas dry matter and neutral detergent fibre digestibility increased linearly and quadratically ($P < 0.05$), feed:gain ratio and total N excretion decreased linearly with 350 g/kg UCH diet. Faecal N (linear and quadratic, $P < 0.01$) and crude protein (CP) digestibility, digestible CP and ruminal pH (linear, $P < 0.01$) decreased, but urinary N, ruminal total N and ammonia N and serum urea N (linear, $P < 0.05$) increased with increasing UCH level. Urea-ammoniated cowpea husk is a complete substitute for DBG in goat diet but 350 g/kg UCH optimised the productive performance and metabolic welfare.

Introduction

Due to consistent increase in population of humans, agriculture in sub-Saharan African countries is intensifying in order to feed the teeming population. This has led to the increasing expansion of agro-industrial activities with concomitant accumulation of a large quantity of poor-quality lignocellulosic crop residues, which constitute environmental nuisance when not properly disposed. These crop residues can be harnessed and converted to feed ingredients for livestock, especially ruminant animals, to ameliorate the problem of dry season feeding and reduce landfill and environmental pollution resulting from their disposal (Kholif et al. 2017)

Cowpea husk (CH), the outer coverage or pod after the removal of cowpea seeds by threshing, is copiously available in Nigeria, particularly the savannah zone, due to extensive cultivation of cowpea (*Vigna sinensis*) as a staple food. Though CH is used for feeding ruminants, its use, like other crop residues, is constrained by its low nutritive value.

Among the various strategies to improve the nutritive value of crop residues, urea treatment has been found to be technically feasible and adopted at the farm level due to its ease of handling and potential to enhance fermentation and nutritional properties (Olafadehan and Adebayo 2016; Rodrigues et al. 2019). However, at present, no information on the feeding value of urea-ammoniated CH (UCH) for goats is available. It was hypothesized that urea-ammonisation of CH would enhance its nutritive, and replacement of UCH for dried brewers' grains (DBG) in diets for goats would improve the performance of the animals without compromising their welfare. This study investigated effects of feeding UCH on voluntary intake, ruminal fermentation, digestibility, N utilisation, serum metabolites and growth performance of Red Sokoto goats.

Materials And Methods

Preparation of test ingredient

Four kg of urea dissolved in 100 litter water was carefully sprinkled on 100 kg DM of CH and thoroughly mixed together. The urea-ammoniated CH was filled into a large drum, compacted to make it air tight, sealed tightly with polyethylene sheets and overlaid with a heavy object. The ensiled CH was left unopened for three weeks after which the resulting UCH was dried on a clean concrete floor under a shade to a constant weight, milled and bagged.

Animals and treatments

Eighteen 8-month-old uncastrated Red Sokoto goats, with live weight (LW) of 13.4±0.59 kg, were randomly divided into 3 groups of similar LW. Goats, housed in individual slatted floor cages equipped with facilities for collection of faeces and urine, were treated against internal and external parasites and bacterial infection before starting the experiment.

The experimental diets comprised three levels of UCH: 0 (control), 350 and 700 g/kg, substituting 0, 500 and 1000 g/kg DBG, in low-cost rations. Treatments contained (g/kg): (700 DBG + 300 cassava peel meal (CPM), 2 (350 DBG + 350 UCH + 300 CPM) and 3 (700 UCH + 300 CPM). Goats, fed at 5% of their LW with daily allowance for 20% refusals, were randomly assigned to one of three treatments in a completely randomized design (CRD). The experiment lasted for 70 d excluding 14 d adjustment period. Feeding was twice daily (08:00 h and 16:00 h), and water was available *ad libitum*. Goats were weighed at the beginning of the experiment and weekly before morning feeding to adjust feed offered as the LW changed.

Digestibility, nitrogen balance and chemical analysis

During the last week of the experiment, samples of feeds, orts, faeces and urine voided were collected and weighed every morning before feeding for 7 days. Samples of the faeces and urine (10% of daily production) were pooled for each animal for the 7-d period and sub-sampled for analysis. Urinary samples were acidified with 20 ml of concentrated H_2SO_4 to prevent ammonia losses and frozen pending chemical analysis.

The DM of feed and faeces was determined by drying in a forced-air oven at 60 ^oC for 48 hr. The dried samples were ground using a Wiley mill to pass a 1-mm screen and analysed for the proximate constituents according to the AOAC (2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to the methods of Van Soest et al. (1991). Non-structural carbohydrate (NSC), organic matter (OM), hemicellulose, cellulose and metabolisable energy (ME) were calculated.

Rumen fluid collection and analysis

On the last day of the experiment, about 100 mL of rumen liquor was collected 3 h post-morning feeding from the ventral sac of each goat using a stomach tube. The pH was measured immediately using a digital pH meter. The liquor was filtered through four layers' cheesecloth. A subsample of 5 mL was preserved in 5 mL of 0.2 M HCl for NH_3-N analysis according to AOAC (2005), and another 0.8 mL subsample was mixed with 0.2 mL of a solution containing 250 g of metaphosphoric acid/L for the total volatile fatty acid (VFA) analysis by titration after steam distillation. Microbial protein synthesis (MPS) was calculated using the equations of Chen and Gomes (1992):

Microbial N yield (MN) = 32 g/kg x digestible organic matter fermented in the rumen (DOMR), where

DOMR = digestible organic matter intake × 0.65

Blood collection and analysis

On the last day of the experiment, blood samples (10 mL) were taken in the morning (4 h after feeding) from the jugular veins of all the goats using Vacutainer tubes without anticoagulants. The samples were allowed to cloth at room temperature for ≥2 h and centrifuged at 4000 × g at 4°C for 20 min. Serum was separated into 2-ml Eppendorf vials. Blood serum samples were analysed for concentrations of total protein, albumin, urea-N, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP), glucose and cholesterol using specific kits (Stanbio Laboratory, Boerne, TX, USA), following the manufacturer's instructions. Globulin concentration was calculated by subtracting albumin value from its corresponding total protein value.

Statistical analysis

Data were analysed as a CRD using SPSS Base 17 (SPSS software products, USA). Polynomial (linear and quadratic) contrasts were used to determine responses to increasing level of UCH.

Results

Chemical composition of untreated and urea-treated ensiled cowpea husk

Urea-ammoniated CH had higher CP and EE but lower OM, NDF, ADF, hemicellulose, cellulose and NSC relative to the non-ammoniated CH (Table 1).

Table 1
Chemical composition (g/kg DM unless otherwise stated) of non-ammoniated and urea-ammoniated cowpea husk

	CH	UCH
Dry matter (g/kg fresh matter)	931	910
Crude protein	139	220
Organic matter	903	892
Ether extract	7.1	8.0
Non-fibre carbohydrates	170	162
Neutral detergent fibre	586	502
Acid detergent fibre	454	360
Acid detergent lignin	148	128
Hemicellulose	132	142
Cellulose	306	232

CH non-ammoniated cowpea husk, *UCH* urea-ammoniated cowpea husk

Chemical composition of experimental diets

The diets were isonitrogenous but the NSC content increased while the OM, EE, NDF and ADF contents decreased with increasing dietary UCH level (Table 2).

Table 2
Ingredient and chemical composition of the experimental diets (g/kg DM)

	Level of urea-ammoniated cowpea husk		
	0	350	700
Ingredient			
Cassava peel meal	300	300	300
Dried brewer’s grains	700	350	-
Urea-ammoniated cowpea husk	-	350	700
Chemical composition			
Crude protein	175	173	172
Organic matter	954	944	934
Ether extract	34.9	12.6	10.0
Non-structural carbohydrate	171	210	226
Neutral detergent fibre	570	548	526
Acid detergent fibre	388	366	302

Voluntary Intake, Nutrient Digestibility, Nutritive Value And Growth Performance

Intake of DM was unaffected by treatments. Whereas UCH diets increased average daily gain (ADG), they decreased feed:gain ratio (quadratic, $P < 0.01$) (Table 3). Digestibility of DM and NDF was increased linearly and quadratically ($P < 0.05$) in UCH diets. Digestibility of CP decreased linearly ($P = 0.002$) with increasing UCH level. Treatments increased OM and ADF digestibility relative to the control, resulting in a quadratic ($P < 0.05$) trend. Digestible CP decreased, as the UCH level increased (linear, $P = 0.001$). 350 g/kg UCH diet increased quadratically ($P = 0.008$) digestible organic matter and ME.

Table 3
Feed intake, growth performance, nutrient digestibility and nutritive value in goats fed urea-ammoniated cowpea husk (UCH) diets

Variable	**Level of UCH (g/kg)**			**SEM**	***P*-value***	
	0	350	700		Linear	Quadratic
Dry matter intake (g/d)	575	569	562	23.6	0.597	0.951
Average daily gain (g/d)	68.1	78.6	69.2	2.97	0.127	0.008
Feed:gain ratio	8.44	7.24	8.15	0.56	0.112	0.007
Apparent digestibility (g/kg)						
Dry matter	622	683	668	1.3	0.014	0.018
Crude protein	698	665	626	1.4	0.002	0.797
Organic matter	652	724	685	1.7	0.131	0.008
Neutral detergent fibre	528	590	571	1.6	0.040	0.029
Acid detergent fibre	411	475	467	1.9	0.065	0.022
Nutritive value (g/kg)						
Digestible crude protein	12.2	11.5	10.8	0.24	0.001	0.939
Digestible organic matter	62.9	68.3	63.7	1.84	0.600	0.008
ME (MJ/kg DM)	10.1	10.9	10.0	0.26	0.595	0.008

ME metabolisable energy

*Significant at the 5% probability level

Nitrogen Use Efficiency

Diets had no effect on N intake. Nitrogen balance and retention (N balance as g/kg N intake) had quadratic responses (P < 0.01) (Table 4). While faecal N decreased linearly and quadratically (P < 0.01), urinary N increased linearly (P < 0.001) with increasing UCH proportion. Total N excretion decreased linearly (P = 0.038) in UCH diets.

Table 4
Nitrogen utilisation in goats fed urea-ammoniated cowpea husk (UCH) diets

Variable (g/d)	**Level of UCH (g/kg)**			**SEM**	***P*-value***	
	0	350	700		Linear	Quadratic
N intake	16.10	15.91	15.72	0.38	0.087	0.976
Faecal N	4.73	3.76	3.68	0.26	< 0.001	0.001
Urinary N	1.85	1.90	2.77	0.28	< 0.001	0.067
Total N excretion	6.57	5.66	6.45	0.15	0.038	0.134
N balance	9.52	10.25	9.27	0.26	0.548	< 0.001
N retention (g/kg of N intake)	592	644	590	0.53	0.096	< 0.001
*Significant at the 5% probability level						

Rumen Fermentation And Microbial Protein Synthesis

Ruminal pH decreased linearly (P = 0.001), whereas NH_3-N and total N increased linearly (P < 0.01), as dietary UCH level increased (Table 5). Total VFA, DOMR and MPS were increased quadratically (P < 0.05) in 350 g/kg UCH diet.

Table 5
Rumen fermentation characteristics and microbial protein synthesis in goats fed urea-ammoniated cowpea husk (UCH) diets

Variable	**Level of UCH (g/kg)**			**SEM**	***P*-value***	
	0	350	700		Linear	Quadratic
pH	6.80	6.55	6.50	0.39	0.001	0.650
Ammonia nitrogen (mg/L)	94.9	95.4	109	3.2	0.009	0.296
Volatile fatty acid (mmol/L)	95.0	105	99.0	2.97	0.223	0.023
Total nitrogen (mg/L)	1283	1453	1577	42.9	< 0.001	0.556
DOMR (g/d)	234	253	233	10.1	0.840	0.004
Microbial protein synthesis (g/d)	47.0	50.6	46.5	2.04	0.836	0.004
DOMR digestible organic matter fermented in the rumen						
*Significant at the 5% probability level						

Serum Metabolites

Except for serum urea N, glucose and AST which were affected, other serum metabolites did not respond to the diets (Table 6). Serum urea N increased linearly (P = 0.02) with increasing dietary UCH level but AST and glucose increased quadratically (P < 0.05) in 350 g/kg UCH diet.

Table 6
Serum metabolites of goats fed urea-ammoniated cowpea husk (UCH) diets

Variable	**Level of UCH (g/kg)**			**SEM**	***P*-value**	
	0	350	700		Linear	Quadratic
Urea-N (mmol/L)	4.71	6.55	7.46	0.88	0.02	0.566
Creatinine (mmol/L)	101	102	111	4.7	0.081	0.476
Aspartate transaminase (U/L)	117	130	118	3.0	0.672	0.003
Alanine transaminase (U/L)	16.8	17.0	17.0	2.16	0.946	0.584
Alanine phosphate (U/L)	24.8	19.7	20.2	2.49	0.109	0.237
Total protein (g/L)	73.5	72.0	70.5	4.00	0.479	0.998
Albumin (g/L)	34.0	30.5	33.3	3.05	0.818	0.282
Globulin (g/L)	36.5	41.5	40.2	4.08	0.396	0.410
Albumin/globulin ratio	0.94	0.74	0.86	0.99	0.604	0.272
Glucose (mmol/L)	2.54	2.80	2.58	0.08	0.676	0.017
Cholesterol (mmol/L)	2.25	2.40	2.18	0.120	0.521	0.083
*Significant at the 5% probability level						

Discussion

The improved CP but decreased NDF and ADF contents of UCH relative to the non-ammoniated CH concur with earlier investigations on urea-ammonisation of crop residues (Olafadehan and Adebayo, 2016; Abera et al. 2018; Okunade et al. 2018). Urea, a non-nitrogen protein source, improved the CP of UCH obviously due to incorporation of N from urea. The reduction in NDF and ADF in UCH is attributable to absorption of the ammonia generated from urea into the cell wall and chemical break down of the ester bonds between lignin and hemicellulose and cellulose (Aruwayo 2018).

Similar CP content and reduced fibre contents of the UCH diets relative to the control diet indicate that urea-ammonisation of the low-quality CH improved its nutritive value by upgrading its CP content to a parallel level and reducing its fibre contents to those of the DBG, a moderately-high protein ingredient.

Similar feed intake of goats was unexpected because of the lower fibre level and improved nutrient digestibility, two major critical determinants of feed intake in ruminants, of UCH diets relative to control diet. It appears that ammonia concentration in the UCH was high and compromised the aroma of the UCH diets. However, the effect was not too pronounced as to compromise feed intake. Generally, urea is converted to ammonia during ensiling; therefore, to mitigate its effect on the silage palatability and toxicity, it was dried before use. Rodriguez et al. (2019) also reported unaffected intake with feeding of urea-ammoniated crop residues.

The increased ADG of goats fed 350 g/kg UCH diet is the consequence of its improved ruminal fermentation, MPS, nutrient digestibility, nitrogen utilisation and ME, in concurrence with earlier reports (Olafadehan et al. 2020). Improved nutrient digestibility, N utilisation, MPS and energy concentration imply more nutrient, particularly CP, and energy availability for growth. Reduced feed/gain ratio of 350 g/kg UCH diet indicates superior feed utilisation efficiency and nutritive value, suggesting positive associative effect between both DBG and UCH, the two protein sources in the diet.

The increase in apparent digestibility of the UCH diets can be attributed to their low fibre and high NSC which perhaps improved ruminal conditions for effective degradation. Both fibre and NSC play important role in determining ruminal condition and fermentation (Olafadehan and Adebayo 2016; Olafadehan et al. 2016). Similarly, high NSC has been has reported to support ruminal microflora activity and promote nutrient digestibility (Olafadehan and Adebayo 2016). Reduced CP digestibility of the UCH diets suggests enhanced ruminal proteolysis of the dietary CP, diminishing the proportion of protein available for digestion in the lower gut (Olafadehan and Adebayo 2016; Olafadehan et al. 2014a).

Increased N absorbed, balance and retention of 350 g/kg UCH diet suggests balanced ruminal N degradation, improved rumen escape protein, less total N excretion and enhanced N metabolism. Improved N use efficiency of 350 g/kg UCH diet is nutritionally and environmentally beneficial, as it can be used for mitigation of N excretion emissions, acid formation, environmental pollution and climate change. Reduced faecal N and increased urinary N excretions of the 700 g/kg UCH diet indicate enhanced ruminal CP deamination. This is because increased ruminal CP degradation reduces the amount of dietary CP available for digestibility in the lower gut and thus excretion in the faeces (Olafadehan et al. 2014a, 2016), whereas increased urinary N excretion is due to greater ruminal ammonia production, absorption, detoxification and excretion as urea in the urine (Cherdthong and Wanapat 2013; Olafadehan and Adebayo 2016; Olafadehan et al. 2016).

Reduced ruminal pH of UCH diets could be attributed to the low fibre and high NSC levels which are associated with rapid ruminal organic acids production due to lower saliva secretion occasioned by decreased rumination time (Hristov et al. 2005; Olafadehan et al. 2016; Okunade and Olafadehan 2019; Okunade et al. 2020). Increased ruminal NH_3-N and total N concentrations of UCH diets accentuate the enhanced ruminal dietary CP proteolysis. Since ruminal MPS depends on ruminal NH_3-N concentration and an adequate supply of carbohydrates as an energy source for peptide bonds synthesis (Bach et al. 2005), it is, therefore, plausible to attribute the increased MPS of 350 g/kg UCH diet to its higher energy and ruminal NH_3-N concentrations, in agreement with previous reports (Firkins et al. 2007; Olafadehan and Adebayo 2016). The diet appears to promote synchronization of the available fermentable energy and degradable N in the rumen to optimise MPS. Improved VFA of 350 g/kg UCH diet suggests enhanced DM degradation and ruminal fermentation of both structural and non-structural carbohydrates (Olafadehan et al. 2016; Olafadehan and Okunade 2018). It can also be related to the improved OM and fibre digestibility of the diet (Kholif et al 2018a).

Though few of the serum metabolites were altered by the treatments, all the measured metabolites were within optimal physiological ranges for healthy goats. Both serum urea N and ruminal NH_3-N of the UCH diets followed the same trend, in agreement with previous findings (Olafadehan et al. 2014b; Olafadehan and Okunade, 2018) where serum urea N positively correlated with ruminal NH_3-N. Serum urea N is generally reduced in ruminants fed diets with low protein content or with severe liver disease (Mahgoub et al. 2008). However, since serum urea N proteins were within the optimum physiological ranges, results suggest unaffected liver and kidney functions (Kholif et al. 2021)., Increased serum glucose concentration with 350 g/kg UCH treatment indicates enhanced energy status of goats (Olafadehan 2011) in response to the improved ruminal VFA, OM digestibility and ME energy level (Olafadehan and Okunade 2018; Kholif et al. 2018b). Diet with 350 g/kg UCH increased AST by 10% relative to the other diets; however, its normal concentration and those of other liver enzymes (ALT and ALP) indicate normal liver function or absence of hepatotoxicity. The mild effect of UCH diets on serum metabolites indicates that UCH can be used goat diets without compromising metabolic welfare.

Conclusion

The study indicates that UCH can adequately and completely replace DBG in the diet of goats without compromising their performance and welfare. However, replacement of DBG with 350 g/kg UCH improved ruminal fermentation, microbial protein synthesis, nutrient digestibility, nitrogen utilisation, energy concentration and growth performance without compromising of goats' metabolic welfare.

Declarations

Author contributions

Conceptualisation, supervision, and data analysis and curation: OAO; Design and investigation: OAO, TRO, GCO and AJS; Writing – original draft: OAO, SAO, TRO and GCO; Writing – review and editing: AEK, EEO, GA and EUA. All authors read and approved the final manuscript.

Data availability

Data are available on request from the corresponding author.

Ethics approval

Guidelines for the care and use of animals were followed as approved by Nigerian Institute of Animal Science.

Conflict of interest

Authors declare no competing interest.

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