

## THE EFFECT OF USING DIFFERENT LEVELS OF MANGO SEED KERNEL ON DIGESTION COEFFICIENTS, RUMEN FERMENTATION AND METHANE EMISSION IN SHEEP DIET

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### SUMMARY

The purpose of this study was to determine the optimal ratio for improved dietary utilization by evaluating the effects of varying levels of mango seed kernel added to sheep diets on the nutritional value, digestion coefficients, and rumen fermentation. Four concentrate feed mixtures (CFM) were used to feed the Barki sheep: the first had a basal diet without mango seed kernels (MSK), the second had 5% MSK, the third had 10% MSK, and the fourth had 20% MSK. Using fully grown rams, the parameters of digestion were estimated, while rumen fermentation, the breakdown of dry and organic matters, and methane production using three ewes with rumen fistulas. The following are the outcomes:

MSK diets had higher rates of nutrients digestion efficiency than the control diet which reflected on TDN values but not effect on DCP value. While, there was no statistically significant difference among MSK rations. The diet containing 10% MSK showed the higher retained nitrogen and its percentage in the diet; there were no statistically significant differences in the amount of nitrogen consumed between the diets. The pH values did not significantly differ among diets. The control diet yielded the higher rumen ammonia concentration, whereas the MSK diets produced the lowest. The third diet (10% MSK), which producing the most microbial protein, had the higher rate of ammonia and volatile fatty acid production. Adding MSK to rations increased the decomposition of the degradable fraction (b) in the rumen and the efficiency of dry matter decomposition (EDDM), and showed higher rates in those contained 10% MSK. The control ration showed a higher rate (c) of decomposition of crude protein in the rumen and thus the higher efficiency in decomposition protein (EDCP). In contrast, the non-degradable protein part (RUP) was higher in MSK containing diets. Methane production was significantly higher in the control diet than in diets containing MSK, and its percentage fell as the percentage of MSK in the diet increased. Addition of MSK at a rate of 5, 10 and 20%, resulted in a decrease in methane gas production by about 22.83, 30.32 and 39.95% respectively. Therefore, it was recommended to incorporate MSK in complementary diets for small ruminants at the rate of 10% with the possibility of increasing the rate to 20% without any negative effect on animal health.

**Keywords:** Mango seeds, rumen fermentation, methane production, digestion coefficients, sheep.

### INTRODUCTION

It is difficult to provide enough fodder to meet the needs of producing animals because of the fierce competition between humans, animals, and poultry for feedstuffs. This is especially true in developing nations where conventional ingredients needed to make animal diets are scarce. Lack of traditional feed sources poses a serious issue for livestock in many nations throughout the world. This challenge in feeding animals may be lessened depending on the nutritional value, accessibility and animal acceptability of the unconventional feedstuffs. Mango residues, among other fruit and vegetables wastes, are highly nutritious and easily accessible in the area, which has piqued the interest of many researchers who are interested to using them as animal feed. Residues account for forty to fifty percent of the mangos' weight. They have significant concentrations of vital minerals and may provide nutritional benefits (Fowomola, 2010). However, the enormous volumes of waste generated during the industrial processing of mangoes result in serious problems with environmental contamination (El-Kholy *et al.*, 2008). It was

recently found that in addition to having an amino acid balance in the kernel, fruit seeds, like those from mangos, have good physicochemical qualities (Hassan *et al.*, 2013).

Mango seed kernels (MSK) were found to be a good source of proteins (6-7%), fat (11%), ash (2%) and carbohydrates (77%), when measured on a dry weight basis (Diarra, 2014). According to Yatnatti *et al.* (2014), mango kernels are also high in pro vitamin A (15.27 IU), vitamin E (1.30 mg/100 g db), vitamin C (0.56 mg/100 g db), and vitamin K (0.59 mg/100 g db). They also contain 368 mg/100 g of potassium, 210 mg/100 g of magnesium, and 170 mg/100 g of calcium. According to Fowomola (2010), the relative concentrations of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, and B<sub>12</sub> were 0.08, 0.03, 0.19, and 0.12 mg/100 g db, respectively. However, it contains cyanogenic glucosides, oxalates, and trypsin inhibitors in addition to relatively high tannin content (Sultana and Ashraf, 2019). There is a lack of information regarding the use of mango seeds in livestock feeding, so more investigation is needed to ascertain the effects of mango seed kernels on rumen fermentation, animal performance, health, and income. There is a comparatively high intestinal digestibility of the ruminally undegradable protein (RUDP) fraction (Punj, 1988). It is a useful tool for increasing mating and fertility (Farag *et al.*, 2022). According to Diarra *et al.* (2011), MSK has a metabolizable energy (ME) value that is similar to that of maize because it is high in lipids and starch. Furthermore, due to its high tannin content, which can range from 5 to 7%, 6% DCP, and 70% TDN, MSK can be included as an ingredient in livestock feeds (Sindhu *et al.*, 2002 and Omer *et al.*, 2019). The aim of this study was to determine the feeding value, fermentation features, gas production, and methane emission with varying levels of mango seed in order to attain the most dietary appropriate level in sheep rations.

## MATERIALS AND METHODS

El-Noubaria Research Station, Animal Production Research Institute, Egypt was the site of this study. Mango seeds were gathered from juice factories, soaked in water for three days, then allowed to air-dry for forty-eight hours. The broken seeds were then manually extracted to extract the kernel. Following sun drying, the kernel was ground in mills to pass through a 1 mm filter and then sealed in containers until their chemical analyses and addition to the sheep's diet. Specifically, four isonitrogenous concentrate feed mixtures (CFMs) were created: 1) CFM without MSK (control), 2) CFM (5) with 5% MSK, 3) CFM (10) with 10% MSK, and 4) CFM (20) with 20% MSK. Table 1 displays the compositions of the experimental CFMs as well as the chemical analyses of the MSK and CFMs. While chemical analyses of MSK and CFM's are presented in Table (2).

**Table (1): Feed ingredients (%) of experimental CFM's (on dry matter basis).**

Feed ingredients, %	MSK levels (%)			
	CFM	CFM <sub>(5)</sub>	CFM <sub>(10)</sub>	CFM <sub>(20)</sub>
Yellow corn	39	35	33	24
Soybean meal	10	10	10	10
Wheat bran	25	23	19	18
Uncorticated cotton seed meal	17	18	19	19
Mango seed kernel	-	5	10	20
Molasses	5	5	5	5
Limestone	2	2	2	2
Salt	1.5	1.5	1.5	1.5
Mineral premix*	0.5	0.5	0.5	0.5

\*Premix composition per kilogram: vitamin A, 500,000 IU; vitamin D<sub>3</sub>, 10,000 IU; vitamin E, 100 mg; Ca, 190,000; P, 90,000; Na, 50,000; Cu, 300 mg; Fe, 3,000 mg; Mn, 2,000 mg; I, 100 mg; Co, 100 mg; Se, 1 mg; Mg, 19,000 mg; BHT antioxidant, 3,000 mg

For each diet, three Barki rams with an average live body weight of  $53 \pm 1.30$  kg were used in four metabolic trials related to digestibility and nitrogen balance. Every trial had four-week duration: three weeks were dedicated to preparation, and one week was used to collect urine and feces. The sheep were given free access to water and were fed twice a day at 8 and 16 hours. According to NRC (1994), the CFM was given to each animal. They were fed ad libitum corn silage and rice straw (4:1 on DM basis). The chemical composition of urine, feces, and feeds was assessed using techniques outlined in the A.O.A.C. (2002). Subsamples (20%) of urine and feces were collected once a day and kept at  $-18^{\circ}\text{C}$

until analysis. Samples of feces were dried for 72 hours at 60 °C. On a Wiley mill grinder, feed and fecal samples were ground through a 1 mm screen, and a 50 gm. (ration / group) sample was taken for analysis. According to A.O.A.C. (2002), the nitrogen (N) content of the urine samples was examined, whereas the crude protein (CP), crude fiber (CF), ether extract (EE), and ash were measured in the feed and feces samples. The components of the cell wall (NDF, ADF, and ADL) were identified in accordance with Van Soest *et al.* (1991). Hemicellulose and cellulose were calculated by differences. About 200 mg (DM) of ground samples were extracted in 10 ml of aqueous acetone (7:3 v/v) in a water bath kept at 39–40 °C for 90 minutes in order to determine the secondary metabolites (Makkar, 2000). According to Makkar and Becker (1993), total phenolic components (TPC) were measured using Folin-Ciocalteu-reagent 2N (Sigma®–Aldrich, El-Safua Co., Alexandria, Egypt) based on known tannic acid concentrations as the calibration curve (Sigma®–Aldrich). Total tannins (TT) were calculated using Makkar's methodology (2003). The amino acid content of MSK, corn silage, and CFMs was determined using an amino acid analyzer (Model 121) in accordance with Moore *et al.*'s (1958) report.

For every diet, rumen liquor samples were obtained from the three fistulated Barki ewes (weighing  $42.00 \pm 1.5$  Kg BW) at 0, 1, 3, and 6 hours following their morning meal. A pH meter (Orian 680) was used to test the acidity of the collected rumen liquor. Four layers of chesses cloth were used to strain the samples, and AL-Rabbat *et al.* (1971) 's methodology for determining ammonia nitrogen (NH<sub>3</sub>-N) was followed Warner (1964) provided an estimate of the concentration of total volatile fatty acids (VFAs). The total count of bacteria was done in accordance with Difco (1984). The roll-tube method was used to study cellulolytic bacteria (Hungate, 1969). Using the model equation developed by Borhami *et al.* (1992), the amount of microbial protein synthesized (gMP/day) in the rumen of sheep fed the experimental ratios was determined.

DM and CP degradation kinetics of the experimental rations were ascertained using the nylon bags technique (Mehrez and Ørskov, 1977). For every incubation period, two 7 × 15 cm polyester bags with 45 µm pore size were utilized. Each bag held about 6 g of air-dried CFMs that had been ground to a 2 mm size. The bags were incubated for 3, 6, 12, 24, 48, and 72 hours before being taken out of each animal's rumen. After that, they were gently squeezed and rinsed in tap water until the water turned clear. By freezing at -20°C, microorganisms adhered to the residual sample were eradicated (Kamel *et al.*, 1995). Two bags were washed for fifteen minutes under running water in order to determine zero-time washing losses (a). By fitting the disappearance values, Ørskov and McDonald's (1979) equation  $P = a + b(1 - e^{-ct})$  could be used to estimate the DM and CP degradation kinetics (in each bag). By fitting the disappearance values, Ørskov and McDonald (1979) proposed the equation  $P = a + b(1 - e^{-ct})$ , which represents the disappearance after time t, and this allowed for the estimation of the DM and CP degradation kinetics (in each bag). The rapidly degraded fraction (a), the slowly degraded fraction (b), and the rate of degradation (c) are the definitions of the least-squares estimates of soluble fractions. With k representing the out flow rate, the effective degradability (ED) of the tested rations was calculated using McDonald's (1981) equation, which reads  $ED = a + bc/(c + k)$ . The method described by Menke and Steingass (1988) was used to calculate in vitro gas production. The buffer and mineral solutions are prepared and then placed in a water bath that is continuously flushed with CO<sub>2</sub> at 39 °C. Egyptian clover hay and a commercial concentrate mixture diet were given to three fistulated sheep, and the sheep's rumen fluid was collected into a thermos flask that had been slightly heated beforehand. The rumen fluid underwent four layers of gauze and was flushed with CO<sub>2</sub>. It was then combined and added to the buffered mineral solution (1:2 v/v) that was maintained in a water bath at 39 °C. A syringe equipped with plungers was filled with precisely weighed 200±10 mg of the air-dried feed ingredients. After pipetting 30 ml of buffered rumen fluid into each syringe containing the feed samples, the syringes are immediately submerged in a water bath that has been heated to 39 °C. Three syringes filled solely with buffered rumen fluid were used as the blank and incubated. The syringes were gently shaken every two hours, and the incubation was stopped after the gas volume was recorded for the entire ninety-six hours. The amount of gas produced was measured following 3, 6, 9, 12, 24, 48, 72, and 96 hours of incubation period. Cumulative gas was expressed as milliliters of gas produced per 200 mg of DM after blanks were taken into account. The exponential model of Ørskov and McDonald (1979) was fitted to the cumulative gas production GAS (Y) at time (t) in the following way: Gas (Y) is equal to  $a + b(1 - \exp^{-ct})$ , where t is the incubation period, a, b, and c are the gas production rates from the immediately soluble, insoluble, and insoluble fractions, respectively. Using a syringe with a capacity of 5 ml, 4 milliliters of 10 ml of NaOH were added immediately after the syringes containing the entire gas from the water bath were removed. Methane in gas was measured in accordance with the method outlined by Jouany (1994). The material was put into the silicon tube, which was then fastened to the 100 ml syringe. Then, as the clip was opened, the NaOH was progressively released. After shaking the contents, the calibrated body of the syringe was used to measure the amount of methane.

**Statistical analysis:**

The study's data on methane production, rumen fermentations, and digestion coefficients were statistically analyzed using SAS (2011) for PC. The model that follows was put through:

$$Y_{ijkl} = \mu + \text{WK} + E_{ijkl} + \mu + T_i + a(T)$$

Where: WK is the fixed effect of week when K = 1,2,...,8; E<sub>ijkl</sub> is random error; Y<sub>ijkl</sub> is the parameter under analysis;  $\mu$  is the overall mean; T<sub>i</sub> is the fixed effect of treatment; T<sub>ij</sub> is the random effect of animal (j) within treatment (i). Additionally, one-way analysis of variance was applied to the digestibility trial data, rumen study data, and lamb performance data, as stated by Steel and Torrie (1980). General Linear Models were used for statistical procedures, which were adapted by SAS (2011) for PC. According to Duncan (1955), significant differences in means were separated using the LSD test.

**RESULTS AND DISCUSSION****Chemical analysis and composition:**

Table 2 shows the cell wall components and chemical analyses of CFMs, MSK, rice straw (RS) and corn silage (CS). The outcomes demonstrated that MSK is a distinct feed, mostly made up of fiber, fat, and protein. According to Diarra (2014), MSK has moderate protein contents (6–10%), which are thought to be adequate to satisfy the needs of the majority of ruminants (Fowomola, 2010). More MSK was added to the CFMs in order to increase the ether extract (EE) content. This might have been caused by their moderate contents in MSK relative to corn, which had a lower EE content (4.30 vs. 9.04%) than MSK. On the other hand, Omer *et al.* (2019) found that MSK and yellow corn had ash values of 1.40 and 2.69, respectively. Okoruwa *et al.* (2015) found that chemical composition of MSK was 5.90% CP, 0.89% CF, 5.46% EE, 2.25% ash, and 76.06% NFE. Mango seed kernel was contained 70 g/kg of CP and 128 g/kg of EE as mentioned by Farag (2002). The total phenolic content of MSK was found to be 10.45% (Table 2). Other mango cultivars had reported total phenolic contents ranging from 112 to 44,760 mg/100 g seed (Abdalla *et al.*, 2007; Ribeiro *et al.*, 2008 and Sogi *et al.*, 2013). These variations in phenolic contents could be caused by the different standard equivalents that were used, the location, the extraction conditions, and the cultivars of mangos. Mango skin and kernel contain 4.89 and 6.84% of total phenols, respectively, according to Huber *et al.* (2012). The study determined that the total tannin content of MSK was 6.82%.

**Table (2): Chemical composition (%) of MSK, CFM's, corn silage and rice straw (on dry matter basis).**

Item	MSK	CFM	CFM <sub>(5)</sub>	CFM <sub>(10)</sub>	CFM <sub>(20)</sub>	CS	RS
<b>Chemical analysis:</b>							
OM	97.54	91.88	91.85	91.93	91.76	88.31	85.12
CP	9.18	15.74	15.77	15.75	15.74	7.98	3.86
CF	2.68	7.86	7.31	7.91	7.67	26.79	38.71
EE	9.04	3.85	4.35	4.51	5.14	2.10	1.56
Ash	2.96	8.12	8.15	8.07	8.24	11.69	14.88
NFE	76.14	64.43	64.42	63.76	63.21	51.44	40.99
<b>Fiber fractions:</b>							
NDF	30.60	34.08	34.29	34.13	34.23	48.94	67.04
ADF	14.12	19.30	19.48	19.72	19.63	30.55	48.51
ADL	4.50	3.07	3.25	3.35	3.40	7.09	11.19
Hemicellulose	16.48	14.78	14.81	14.41	14.60	18.39	18.78
Cellulose	9.62	16.23	16.23	16.37	16.23	23.46	37.32
<b>Phytonutrients</b>							
Total phenols	10.45	-	0.56	1.22	2.49	-	-
Total tannins	6.82	-	0.36	0.73	1.52	-	-

MSK: mango seed kernel; CFM: concentrate feed mixtures; CFM<sub>(5)</sub>: concentrate feed mixtures containing 5% MSK. CFM<sub>(10)</sub>: concentrate feed mixtures containing 10% MSK ; CFM<sub>(20)</sub>: concentrate feed mixtures containing 20% MSK.

CS: corn silage; RS: rice straw.

**Digestibility and nitrogen balance trials:**

Table (3) displays the experimental diets' dry matter intake, nutrient digestibility, nutritive value, and nitrogen utilization. The feed intake of the experimental groups was the same as that of Omer *et al.* (2019), who found that replacing maize with ground MSK slightly decreased total DMI (g/d/day). On the other hand, Patel *et al.* (2004) showed that when the sheep ration was expressed as g/kg W 0.75, adding 25% of MSK increased the DMI significantly but only slightly in the DMI when expressed as kg/100 kg. Meanwhile, Aragão *et al.* (2012) found no changes in nutrient intake when they investigated the effects of replacing corn in sheep diets with mango meal. The nutrient digestibility coefficients of DM, OM, CP, CF, and NFE were significantly ( $P < 0.05$ ) increased when MSK was added to the control ration, whereas the digestibility of EE was significantly ( $P < 0.05$ ) decreased. Nonetheless, the feeding values of the experimental rations mirrored these findings. The nutritional values and digestibility results match those reported by Omer *et al.* (2019), who observed that feeding sheep rations that replaced maize with MSK had a significant ( $P < 0.05$ ) impact on the nutrient digestibility coefficients of nutrients, with the exception of EE digestibility, which was significantly ( $P < 0.05$ ) decreased when compared to the control. According to the same pattern, Saiyed *et al.* (2003) found that adding 25% MSK to the ration of weaned children did not significantly alter any of the nutrient digestibility coefficients or nutritive values. According to Bueno *et al.* (2020), this adaptation manifests itself in some ruminants, like goats, as salivary glands that secrete copious amounts of mucus-containing enzymes that can bind to condensed tannins (CT), increasing palatability and freeing up more diet proteins for digestion. Refer to the study of nitrogen intake it was either slightly above or close to the NRC's (1994) recommended levels. Sheep in the R2 (10% MSK) group were able to retain 7.05 g/d of nitrogen, which was less than the control group. Differences in the amount of nitrogen retained could be explained by differences in the digestibility and composition of amino acids in various protein sources. The higher percentage of dietary nitrogen retained for R2 may be due to the more digestible protein's higher ( $P < 0.05$ ) nitrogen utilization. This supported the conclusion made by Saiyed *et al.* (2003). However, Anigbogu *et al.* (2006) discovered that substituting maize offal with MSK at 25, 50, 75, and 100% reduced nitrogen retention considerably ( $P < 0.05$ ) when compared to the control one. Different levels of MSK inclusion in the rations, however, had indicated insignificant ( $P > 0.05$ ) effects on the nutrients digestibility.

**Table (3): Dry matter intake (g/h/d), digestibility coefficients and nutritive values (TDN and DCP) of the experimental diets fed to sheep (means ± SE).**

Item	Experimental diets			
	C	R1	R2	R3
DM intake (g/h/d):				
Roughage intake	542.29 ± 7.15	537.30 ± 6.41	539.77 ± 7.11	531.99 ± 5.51
Concentrate intake	682.20 ± 9.54	680.55 ± 5.93	680.70 ± 11.89	681.45 ± 5.03
Total DMI	1224.49 ± 9.24	1217.85 ± 5.38	1220.47 ± 12.85	1213.44 ± 14.84
Digestibility coefficients (%):				
DM	64.50 ± 0.04 <sup>b</sup>	65.34 ± 0.12 <sup>a</sup>	66.09 ± 0.05 <sup>a</sup>	65.52 ± 0.11 <sup>a</sup>
OM	69.77 ± 0.05 <sup>b</sup>	70.57 ± 0.11 <sup>a</sup>	71.22 ± 0.05 <sup>a</sup>	70.91 ± 0.22 <sup>a</sup>
CP	63.81 ± 0.17 <sup>b</sup>	63.89 ± 0.15 <sup>b</sup>	64.78 ± 0.18 <sup>a</sup>	63.66 ± 0.05 <sup>b</sup>
CF	60.24 ± 0.28 <sup>b</sup>	62.03 ± 0.09 <sup>a</sup>	62.54 ± 0.06 <sup>a</sup>	62.35 ± 0.14 <sup>a</sup>
EE	62.88 ± 0.47 <sup>a</sup>	60.04 ± 0.42 <sup>b</sup>	60.06 ± 0.63 <sup>b</sup>	59.97 ± 1.72 <sup>b</sup>
NFE	74.55 ± 0.16 <sup>b</sup>	74.47 ± 0.15 <sup>b</sup>	75.93 ± 0.03 <sup>a</sup>	75.67 ± 0.45 <sup>a</sup>
Nutritive values (%):				
TDN	65.35 ± 0.05 <sup>b</sup>	65.52 ± 0.09 <sup>b</sup>	66.70 ± 0.07 <sup>a</sup>	66.42 ± 0.12 <sup>a</sup>
DCP	7.61 ± 0.05 <sup>a</sup>	7.66 ± 0.04 <sup>a</sup>	7.75 ± 0.03 <sup>a</sup>	7.41 ± 0.06 <sup>ab</sup>
Nitrogen utilization (g/h/d):				
N-intake	23.38 ± 0.28	23.38 ± 0.10	23.35 ± 0.29	23.14 ± 0.11
N-absorbed	14.92 ± 0.09 <sup>b</sup>	14.93 ± 0.01 <sup>b</sup>	15.33 ± 0.04 <sup>a</sup>	15.19 ± 0.03 <sup>a</sup>
N-retained	6.13 ± 0.15 <sup>b</sup>	6.25 ± 0.02 <sup>b</sup>	7.05 ± 0.11 <sup>a</sup>	6.61 ± 0.13 <sup>b</sup>
N- retained as % of N-intake	26.22 ± 1.21 <sup>c</sup>	26.76 ± 0.003 <sup>c</sup>	30.20 ± 0.59 <sup>a</sup>	28.57 ± 0.61 <sup>b</sup>
N- retained as % of N-absorbed	41.08 ± 2.02 <sup>b</sup>	41.87 ± 0.08 <sup>b</sup>	46.62 ± 0.79 <sup>a</sup>	45.95 ± 1.03 <sup>a</sup>

*a and b: means in the same row with different superscripts are significantly differ (P < 0.05).*

*C: Control.; R1: diet containing CFM (5); R2: diet containing CFM (10); R3: diet containing CFM (20).*

**Ruminal fermentation:**

Results in Table (4) showed that there was no significant difference ( $P > 0.05$ ) in the pH values of rumen liquor among the groups. This was in line with the findings of El-Sanafawy *et al.* (2023), who reported that adding mango seed to diets had no effect on ruminal pH. On the other hand, Omer *et al.* (2019) found that when 25 or 50% of the yellow maize in the control ration was replaced with mango seed kernel, the ruminal pH increased significantly ( $P < 0.05$ ). The results fell between the normal ranges (6.22–6.37) reported by Hungate (1966), who stated that sufficient available ammonia and a rumen pH of between 6.2 and 7.0 are necessary for cellulolytic bacteria to multiply quickly and colonize the epidermal surfaces of plant fragments in less than five minutes. The results fell between the normal ranges (6.22–6.37) reported by Hungate (1966), who stated that sufficient ammonia and a rumen pH of between 6.2 and 7.0 are necessary for cellulolytic bacteria to multiply quickly and colonize the epidermal surfaces of plant fragments in less than five minutes. According to Lu *et al.* (2015), ruminal  $\text{NH}_3\text{-N}$  concentration values showed that it was adequate for microbial growth. However, there were significant ( $P < 0.05$ ) differences in the concentration of ruminal metabolites ( $\text{NH}_3\text{-N}$ , mg/100 mL.R.L. and VFA, meq/100 mL.R.L.) between the experimental rations. The ewes fed the control ration had the higher ( $P < 0.05$ ) overall mean  $\text{NH}_3\text{-N}$  concentration in their rumen, whereas the other rations had lower ( $P < 0.01$ ) concentrations according to R2. Rumen microbes may be able to best utilize ammonia-N when there is a lower concentration of  $\text{NH}_3\text{-N}$  (Abdl-Rahman, 2010). Nonetheless, according to Mehrez *et al.* (1977), this concentration was within the range and ideal for the development of ruminal microorganisms. R2 produced ammonia and nitrogen at a higher rate than R3, which came in at 4.88 and 4.02, respectively. The R2 and R3 rations had the higher VFA values, but the R2 ration also had the highest rate ( $P < 0.05$ ). This was to be expected given that CF has a higher digestibility than C.

**Table (4): Overall mean of rumen parameters of sheep fed the experimental diets (means  $\pm$  SE).**

Item	Experimental diets			
	C	R1	R2	R3
pH value	6.37 $\pm$ 0.08	6.31 $\pm$ 0.05	6.28 $\pm$ 0.07	6.22 $\pm$ 0.07
$\text{NH}_3\text{-N}$ concentration (mg/100mL.R.L)	14.87 $\pm$ 0.12 <sup>a</sup>	13.23 $\pm$ 0.24 <sup>b</sup>	12.42 $\pm$ 0.28 <sup>b</sup>	12.81 $\pm$ 0.14 <sup>b</sup>
Rate of $\text{NH}_3\text{-N}$ production(mg/100 mL.R.L/hr)	3.11 $\pm$ 0.15 <sup>b</sup>	3.17 $\pm$ 0.18 <sup>b</sup>	4.88 $\pm$ 0.29 <sup>a</sup>	4.02 $\pm$ 0.18 <sup>b</sup>
VFA's concentration (meq/100mL.R.L)	12.46 $\pm$ 0.17 <sup>b</sup>	12.61 $\pm$ 0.14 <sup>b</sup>	14.73 $\pm$ 0.11 <sup>a</sup>	14.35 $\pm$ 0.40 <sup>a</sup>
Rate of VFA production (meq/100 mL.R.L/hr)	3.49 $\pm$ 0.18 <sup>c</sup>	4.07 $\pm$ 0.11 <sup>b</sup>	5.44 $\pm$ 0.25 <sup>a</sup>	4.92 $\pm$ 20 <sup>b</sup>
Total bacteria, $\times 10^8$ /ml	1.11 $\pm$ 0.02 <sup>b</sup>	1.13 $\pm$ 0.02 <sup>b</sup>	1.21 $\pm$ 0.02 <sup>a</sup>	1.10 $\pm$ 0.02 <sup>b</sup>
Cellulolytic bacteria, $\times 10^6$ /ml	4.29 $\pm$ 0.13 <sup>b</sup>	4.28 $\pm$ 0.12 <sup>b</sup>	5.16 $\pm$ 0.06 <sup>a</sup>	4.25 $\pm$ 0.02 <sup>b</sup>
Microbial Protein Synthesis (g/d)	53.25 $\pm$ 1.15 <sup>d</sup>	64.52 $\pm$ 2.08 <sup>c</sup>	92.84 $\pm$ 3.92 <sup>a</sup>	83.64 $\pm$ 3.18 <sup>b</sup>

a,b,c and d: means in the same row with different superscripts are significantly differ ( $P < 0.05$ ).

According to Allam *et al.* (2006), the concentration of volatile fatty acids (VFAs) in the rumen is determined by multiple factors, including the rate of absorption, DM digestibility, rumen pH, the movement of rumen digesta from the rumen to other parts of the digestive tract, and the microbial population and activities within the rumen. Because microorganisms capture a lot of  $\text{NH}_3\text{-N}$ , the high fermentation of mango waste probably promoted microbial growth (Satter and Slyter, 1974). Since  $\text{NH}_3\text{-N}$  and minor VFA are the end products of protein degradation in the rumen, the higher  $\text{NH}_3\text{-N}$  concentrations are consistent with the greater proportion of minor VFA (Pereira *et al.*, 2008). The results obtained were consistent with those reported by Omer *et al.* (2019), who found that when Rahmani sheep were fed rations containing varying amounts of MSK, the concentration of total volatile fatty acid increased significantly ( $P < 0.05$ ), while the concentration of ammonia nitrogen decreased significantly ( $P < 0.05$ ) three hours after feeding. It appears that more bacteria are absorbing ammonia nitrogen to increase their protein, which is the cause of the drop in ammonia nitrogen in rumen liquor. This was previously believed to be caused by increased microbial activity, but it could also be caused by the rumen's fermentation and increased use of dietary energy. The amount of ammonia in the rumen lowers when ruminants' diets contain more fermentable carbohydrates (Tagari *et al.*, 1964). This might be the result of the rumen bacteria consuming more ammonia to support their growth. In this instance, the rate of TVFA production might exceed the rate of absorption via the gastrointestinal tract, leading to an increase in TVFA concentration in rumen juice (Van'tKlooster, 1986). Therefore, MP synthesis was lower ( $P < 0.01$ ) for the control ration than the other rations, with average values ranging from 53.25 to 92.84 (g/d) for R2 and MP synthesis, respectively. Adding 10% MSK to the concentrate feed mixture,

however, also increased the flow rate of microbial protein and may change the patterns of VFA formation.

**Degradation kinetics:**

Table (5) displays the ruminal degradation constants (a, b, and c) fitted with the rates of DM and CP disappearance for concentrate feed mixtures (CFM's). When compared to the control, the addition of MSK resulted in a significant linear ( $P < 0.05$ ) increase in the degradable fraction "b", rate of degradation "c", and effective degradability "ED" of DM for CFMs. These may be associated with the higher digestibility of nutrients in the rumen; however, there were no notable alterations noted in the washing loss fraction "a". As MSK levels increased, rumen un-degradable protein (RUP) increased ( $P < 0.05$ ), but the control ration showed higher CP degradable fraction ("b"), rate of degradation ("c"), and effective degradability ("ED"). Condensed tannins have been shown by Min *et al.* (2007) to reduce the amount of CP that ruminally degrades, increasing the amount of CP that reaches the small intestine and abomasum. This could be supported by the current study, since rations containing MSK had lower RDPs ( $P < 0.05$ ). On the other hand, ammonia is created by amino acid deamination during rumen fermentation (Van Soest, 1994). Nonetheless, the activity of the CTs that were found in MSK to form CT-protein complexes may have contributed to the reduction in ammonia-producing proteolysis (Pal *et al.* 2015). High-producing dairy cows and ruminants require bypass digestible proteins that going through the lower tract instead of being broken down in the rumen, according to Kleinschmit *et al.* (2007).

**Table (5): Degradation kinetics of DM and CP in the rumen of sheep fed the experimental diets (mean ± SE).**

Item	Experimental diets			
	C	R1	R2	R3
<b>DM</b>				
A	25.53 ± 0.31	25.61 ± 0.47	25.41 ± 0.36	25.26 ± 0.51
B	47.11 ± 0.62 <sup>b</sup>	50.82 ± 0.59 <sup>ab</sup>	53.33 ± 0.68 <sup>a</sup>	52.56 ± 1.31 <sup>a</sup>
C	0.055 ± 0.001 <sup>c</sup>	0.056 ± 0.002 <sup>c</sup>	0.064 ± 0.002 <sup>a</sup>	0.059 ± 0.002 <sup>b</sup>
EDDM	50.21 ± 0.45 <sup>c</sup>	52.46 ± 0.37 <sup>c</sup>	55.35 ± 0.24 <sup>a</sup>	53.71 ± 0.37 <sup>b</sup>
<b>CP</b>				
A	21.72 ± 0.17	21.59 ± 0.26	21.46 ± 0.10	21.40 ± 0.28
B	62.83 ± 0.11 <sup>a</sup>	60.62 ± 0.59 <sup>ab</sup>	59.65 ± 0.54 <sup>b</sup>	57.61 ± 0.25 <sup>bc</sup>
C	0.0730 ± 0.002 <sup>a</sup>	0.0710 ± 0.003 <sup>a</sup>	0.0615 ± 0.002 <sup>b</sup>	0.0612 ± 0.001 <sup>b</sup>
EDCP	59.01 ± 0.64 <sup>a</sup>	57.16 ± 0.87 <sup>ab</sup>	54.36 ± 0.77 <sup>b</sup>	53.11 ± 0.52 <sup>b</sup>
RUP	40.99 ± 0.37 <sup>c</sup>	42.84 ± 0.56 <sup>b</sup>	45.64 ± 0.45 <sup>a</sup>	46.89 ± 0.34 <sup>a</sup>

a, b and c: means in the same row with different superscripts are significantly differ ( $P < 0.05$ ).

a: soluble fraction (%). b: potentially degradable fraction (%). c: rate of degradation ( $\% h^{-1}$ ).

ED: effective degradability =  $a + [bc/c + k]$ , where k is passage rate.; EDDM: effective degradability of dry matter.

EDCP: effective degradability of crude protein.; RUP = 100 - ED (Orskov and McDonald, 1979).

**In vitro gas production from concentrate feed mixtures (CFMs):**

Table (6) displays the data related to gas production. Variations in gas production were observed between the rations ( $P < 0.05$ ). Consequently, compared to R2 and R3, gas production with R1 and control was higher ( $P < 0.05$ ). On the other hand, gas production decreased as the rations' percentage MSK rose. Protein fermentation yields less gas than the fermentation of carbohydrates, but it mostly affects gas production in the first few hours of incubation when most of the protein is in the soluble fraction (Cone and van Gelder, 1999). The results of this study showed a correlation between an increase in the amount of ether extract in the sample and a decrease in overall gas production (Tables 2 and 6). Adeyemi *et al.* (2015) found that adding different oils to the gas production either had no effect at all or decreased it. These results supported their findings. When oil or cellulose was added to NDF residue, the amount of methane produced and the overall amount of gas produced either decreased or stayed the same (Drehmel, 2017). Tiemann *et al.* (2008) state that the degree to which tannin-containing plant species influence rumen fermentation depends on their chemical composition. Furthermore, Hassen *et al.* (2016) report that using *Acacia luederitzii* (high tannin), *A. haematoxylin* (moderate tannin), and *A. mellifera* (low tannin) as the substrate reduced the amount of gas produced in vitro by as much as 20, 15.5, and 7.3%.

**Methane production and emission:**

The control ration's methane production percentage was significantly higher ( $P < 0.05$ ) than that of all the other experimental rations in Table (6). On the other hand, the rations containing R1 and R2 indicated intermediate values without significant difference ( $p > 0.05$ ) between them, and the ration containing 20% MSK (R3) had the lowest recorded values. Nevertheless, when the ration's MSK levels rose, all values dropped. It was able to gracefully illustrate the connection between feed composition, rumen acidity, and methanogen activity, according to Kessel and Russell (1996). It was also reported in the current study that CFMs with higher levels of MSK introduced showed comparable variations in the proximate analysis and tannin content of experimental diets. When MSK was introduced into CFMs at 5, 10, and 20% levels, the amount of  $\text{CH}_4$  produced on a DM basis decreased significantly ( $P < 0.05$ ) by 22.83, 30.32, and 39.95%, respectively, in comparison to the control group. Many studies have looked at various tannin-containing plants to see if they can stop the synthesis of  $\text{CH}_4$ . (Bhatta *et al.* 2012; Soltan *et al.* 2012). Conversely, little is known about how MSK can have an anti-methanogenic effect when taken in conjunction with a whole diet. The ideal MSK dosages must be established in order to minimize detrimental effects on rumen fermentation while preventing enteric  $\text{CH}_4$  production as much as feasible. This could be because of the high concentrations of TT, CT, and flavonoids, which may limit *in vitro* fermentation (Huangat *et al.* 2010; Hassen *et al.* 2016). Furthermore, it has been noted that methanogenesis is inhibited by high TT and CT concentrations (Pal *et al.*, 2015). Tannins were believed to reduce rumen methanogenesis through an indirect effect through fiber digestion and a direct impact on methanogenic Archaea activity (Tavendale *et al.*, 2005).

**Table (6): *In vitro* gas production, methane production and methane depression from concentrate feed mixtures.**

Item	Experimental diets			
	C	R1	R2	R3
Gas production (ml/200mg DM)	40.10 ± 0.43 <sup>a</sup>	36.80 ± 0.25 <sup>b</sup>	34.80 ± 0.15 <sup>c</sup>	31.60 ± 0.36 <sup>d</sup>
methane production (ml/200mg DM)	11.74 ± 0.08 <sup>a</sup>	9.06 ± 0.01 <sup>b</sup>	8.18 ± 0.06 <sup>b</sup>	7.05 ± 0.02 <sup>c</sup>
Methane concentration, %	29.28	24.61	23.51	22.31
Methane depression, %	-	22.83	30.32	39.95

*a, b, c and d: means in the same row with different superscripts significantly differ ( $P < 0.05$ )*

**CONCLUSION**

According to the obtained results, it can be pointed out that MSK is thought to be good UDP sources and can enhance animal performance, mango seed kernels may be a good source of fat and energy for sheep. Mango seed kernels, on the other hand, can be added to CFMs at a maximum of 20%, meaning that they could replace some corn grains, wheat bran, and even uncorticated cotton seed meal which is an expensive ingredient and could be expected to improve animal performance feed cost. As a result, it could boost feed intake and rumen fermentation in addition to lowering methane production by up to 20% when mango seed kernels are added to the CFM without compromising animal health.

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## تأثير استخدام مستويات مختلفة من نواة بذور المانجو على معاملات الهضم وتخمرات الكرش وانبعاث الميثان في علائق الأغنام

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<sup>2</sup>قسم الانتاج الحيوانى - كلية الزراعة - جامعة طنطا - مصر.

كان الهدف من التجربة هو تقييم تأثير اضافة مستويات مختلفة من نواة بذور المانجو لعلائق الاغنام على القيمه الغذائية ومعاملات الهضم وتخمرات الكرش و انتاج غاز الميثان للوصول الى النسب المثلئ فى تغذيه الاغنام، حيث تم تغذية اغانم البرقى على اربع علائق، الاولى خاليه من نواة بذور المانجو و الثانى احتوت على 5% و الثالثه 10% و الاخيره 20% بذرة، وتم تقدير معاملات الهضم باستخدام كباش تامه النمو فى حين استخدم ثلاث نعاج مزودة بفستيوالا الكرش لتجارب تخمرات الكرش وقياساته و قد اظهرت النتائج ما يلي:

احتوت بذور المانجو على نسبه معتدله من البروتين والدهن والالياف ومع زيادة نسبتها فى العليقه يزداد معه نسبه الدهن ولكن نسبه رماد اقل عنه فى الذرة أو السيلاج كما ان بها نسبه معقوله من الفينولات والتانينات. و اظهرت معدلات كفاءة الهضم الى التحسن خاصه مع علائق البذرة عما فى عليقه المقارنه ولم يكن هناك اختلاف معنوي فيما بين علائق البذرة فيما يتعلق بمعاملات الهضم والذي انعكس بدوره على القيمه الغذائيه (TDN) وان لم يحدث تأثير معنوى على معاملات هضم البروتين الخام. لم تكن هناك فروق معنويه بالنسبه للبروتين المأكول بين العلائق ولكن العليقه المحتويه على 10% بذره اظهرت اعلى نيتروجين محتجز ونسبته للمأكول. لم يكن هناك فروق معنويه فيما بين العلائق بالنسبه لقيم الاس الهيدروجينى (pH) وكان اعلى تركيز لامونيا الكرش فى العليقه الكنترول و اقلها فى علائق البذره وكان اعلى معدل لانتاج الامونيا والاحماض الدهنيه الطياره فى العليقه الثالثه كما كانت أكثرهم إنتاجا للبروتين الميكروبي. من الملاحظ ان اضافة البذرة فى العلائق قد ازدادت معه نسبه الجزء المتحلل من المادة الجافه (b) فى الكرش وكذا كفاءة التحلل EDDM وكان اعلاه فى العليقه المحتويه على 10% بذرة فى حين اظهرت العليقه الكنترول اعلى معدل لتحلل البروتين (C)، اعلى كفاءة لتحلل البروتين (EDCP)، وعلى العكس من ذلك فقد سجلت عليقة الكنترول أقل تركيز لجزء البروتين غير المتحلل بالمقارنة بالعلائق المحتوية على البذرة وبناء على ذلك يمكن التوصيه باضافه بذور المانجو الى علائق الاغنام بنسبه 10% ومن الممكن زيادتها الى 20% بدون اى تأثيرات سلبيه على صحة الحيوان.