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In vitro ruminal fermentation of diets containing wheat straw and date pits as forage

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Abstract. The aim of this study was to analyze the effect of partial replacement of wheat straw (WS) by date pits (DP) in diets with different forage:concentrate (F:C) ratio. Three feeds [WS, DP and a commercial concentrate (CON)] were used to formulate six diets according to a 3 × 2 factorial arrangement of treatments (3 F:C ratios and 2 forage sources). The diets had F:C ratios of 100:0, 80:20 (80) or 60:40 (60) with either WS or a 80:20 WS:DP mixture (MIX) as forage, and were designated as WS (100% WS), 80WS, 60WS, MIX, 80MIX and 60MIX. Samples (500 mg) of each diet were incubated with 50 ml of buffered rumen fluid at 39ºC. Final pH, production of volatile fatty acids (VFA), total gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) were determined after 24 h. The neutral detergent fibre (NDF) content of WS and DP was 71.1 and 86.5% (dry matter basis), but NDF lignification was greater for DP (26.7% of NDF) than for WS (15.3% of NDF). Compared with WS, the ruminal fermentation of the MIX diet resulted in greater ($P<0.05$) butyrate proportions (11.7 *vs.* 14.0%) and lower ($P<0.05$) gas production (2768 *vs.* 2588 µmol) but there were no differences ($P>0.05$) in the rest of the fermentation parameters. For both forages (WS and MIX), increasing the proportion of CON in the diet increased ($P<0.05$) the production of gas and total VFA, the molar proportion of butyrate and the OMAF. There were no differences ($P>0.05$) between 80WS and 80MIX diets in any fermentation parameter. Compared with 60WS diet, the fermentation of 60MIX diet resulted in greater production of total VFA (3061 *vs.* 3264 µmol), but no differences were detected in the rest of the parameters measured. The final pH, methane production and NH_3-N concentrations ranged between 6.91 and 7.00, 434 and 516 µmol, and 330 and 359 mg/L, respectively, with no differences ($P=0.059$ to 0.121) among diets. The results indicate that WS can be replaced by DP at 20% of the forage portion of the diet without any detrimental effect on ruminal fermentation. Furthermore, in diets with 60:40 F:C ratio, incorporating DP resulted in greater VFA production. If this result is confirmed in vivo, it would be of great interest in sheep practical feeding.

Keywords. Date pits – Wheat straw – Ruminal fermentation – Sheep.

Fermentation ruminale* in vitro *de rations contenant de la paille de blé et des noyaux de dattes

Résumé. *L'objectif de cette étude était d'analyser l'effet du remplacement partiel de la paille de blé (WS) par les noyaux de dattes (DP) dans des rations avec différents rapports fourrage:concentré (F:C). Trois aliments [WS, DP et du concentré commercial (CON)] ont été utilisés comme ingrédients pour formuler 6 rations selon un dispositif factoriel (3 rapports F:C et deux sources de fourrages). Les rations avaient des rapports de F:C de 100:0, 80:20 (80) ou 60:40 (60) avec soit WS ou 80:20 du mélange WS:DP (MIX) comme fourrage, et étaient désignées comme WS (100% PB), 80 WS, 60 WS, MIX, 80MIX et 60MIX. Des échantillons (500 mg) de chaque ration ont été incubés avec 50 ml de jus de rumen tamponné à 39ºC. Le pH final, la production d'acides gras volatils (AGV), de gaz et de méthane, la concentration de NH_3-N et la matière organique apparemment fermentée (MOAF) ont été déterminés après 24 h de fermentation. La teneur en NDF de WS et DP était de 71,1 et 86,5% (DM), mais la lignification de la NDF était plus importante pour les DP (26,7% de NDF) que pour WS (15,3% NDF). Par comparaison à WS, la fermentation ruminale du mélange a permis d'avoir des proportions plus élevées ($P<0,05$) en acide butyrique (11,7* vs. *14,0%) et plus faibles ($P<0,05$) en gaz (2768* vs. *2588 µmol) mais il n'y avait pas de différence ($P>0,05$) entre les deux rations pour les autres paramètres de fermentation. Pour les deux fourrages (WS et MIX), l'augmentation de la proportion du CON dans la ration a augmenté ($P<0,05$) la production de gaz et les acides gras volatils totaux, ainsi que la proportion molaire du butyrate et la matière organique apparemment fermentée. Aucune différence ($P>0,05$) entre les rations 80WS et 80MIX n'a été* *enregistrée au niveau des paramètres de fermentation. Par comparaison avec la ration 60WS, la fermentation de la ration 60MIX a généré une plus grande production d'AGV totaux (3061* vs. *3264 µmol), cependant, aucune différence n'a été détectée pour le reste des paramètres mesurés. Le pH final, la production de méthane et les concentrations de NH_3-N ont varié entre 6,91 et 7,00, 434 et 516 µmol, et 330 et 359 mg/L, respectivement, sans aucune différence (P=0,059 à 0,121) parmi les rations. Les résultats indiquent que WS peut être remplacé par DP à raison de 20% de la proportion du fourrage de la ration sans aucun effet négatif sur la fermentation ruminale. Par ailleurs, dans les rations 60:40 F:C, l'incorporation des DP a résulté en une plus grande production d'AGV. Si ces résultats sont confirmés* in vivo*, ils pourraient être d'un grand intérêt dans la pratique.*

Mots-clés. *Noyaux de dattes – Paille de blé – Remplacement – Fermentation ruminale.*

I – Introduction

Algeria, with more than 20 million sheep, 3.8 million goats, 1.65 million cattle and 0.30 million camels (FAOSTAT, 2010) has an insufficient animal feed production, and livestock production relies heavily on imported and subsidized feed. To cope with this problem, Algeria decided to adopt in the medium-long term a politic favoring the national production of feeds, and consequently reducing imports until reaching the self-sufficiency level (MADR, 2003). In this context, the use of crop residues and agro-industrial by-products would play an important role. These categories include, among other feeds produced in Algeria, cereals straw and date palm by-products, mainly date pits (DP). Date pits become available in high quantities when pitted dates are produced in packing plants or in industrial date processing plants based on juice extraction, as Algeria has over 12 million palm trees and ranked in the top 10 of the date producers worldwide (FAOSTAT, 2010). At the rural level one may find some accumulation of DP when immature dates are pitted before sun-drying (Barreveld, 1993). To achieve an efficient utilization of any by-product, it is necessary to know its chemical composition and nutritive value. The aim of this study was to analyze the effect of partial replacement of wheat straw (WS) by DP in diets with different forage:concentrate ratio.

II – Materials and methods

1. Feeds and diets

Three feeds (WS, DP and a commercial concentrate) were used as ingredients to formulate six diets according to a 2 × 3 factorial arrangement of treatments. The diets had forage:concentrate ratios (dry matter (DM) basis) of 100:0, 80:20 (80) or 60:40 (60) with either WS or a 80:20 WS:DP mixture (MIX) as forage, and were designated as WS (100% WS), 80WS, 60WS, MIX, 80MIX and 60MIX. Ingredient composition of concentrate was barley, maize, wheat bran, soybean meal, NaCl and mineral-vitamin premix in the proportions of 270, 270, 277.3, 180, 1.7 and 1 g/kg, respectively (DM basis). Chemical composition of feeds and experimental diets is given in Table 1.

2. *In vitro* incubations

Samples of each feed and diet were ground through a 1-mm screen, and accurately weighed (500 mg) into 120-ml serum bottles for *in vitro* incubations. Ruminal fluid was obtained from four rumen-cannulated Merino sheep fed medium-quality alfalfa hay *ad libitum*. Ruminal contents of each sheep were obtained before the morning feeding, mixed and strained through four layers of cheese-cloth into an Erlenmeyer flask with an O_2-free headspace. Particle-free fluid was mixed with the buffer solution of Goering and Van Soest (1970) in a proportion 1:4 (v:v) at 39ºC under continuous flushing with CO_2. Bottles were prewarmed (39ºC) prior to the addition of 50 ml of buffered ruminal fluid into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39ºC for 24 h. Four incubation runs were performed on different days, so that each treatment was conducted in quadruplicate.

Table 1. Chemical composition (g/100 g dry matter) of feeds and experimental diets

	Organic matter	Crude protein	Neutral-detergent fibre	Acid-detergent fibre	Hemicellulose	Cellulose	Lignin
Feeds							
Wheat straw	91.8	4.44	71.1	41.2	29.9	30.3	10.9
Date pits	99.0	5.11	86.5	62.1	24.4	38.9	23.1
Concentrate	93.5	12.85	58.9	7.18	51.7	5.05	2.13
Diets†							
WS	91.8	4.44	71.1	41.2	29.9	30.3	10.9
80WS	92.2	6.13	68.7	34.4	34.3	25.3	9.12
60WS	92.5	7.81	66.2	27.6	38.6	20.2	7.37
MIX	93.3	4.58	74.2	45.3	28.8	32.0	13.3
80MIX	93.3	6.23	71.1	37.7	33.4	26.6	11.1
60MIX	93.3	7.89	68.0	30.1	38.0	21.2	8.84

†WS: wheat straw; MIX: 80:20 wheat straw:date pits; 80WS: 80:20 wheat straw:concentrate; 60WS: 60:40 wheat straw:concentrate; 80MIX, 80:20 MIX:concentrate; 60MIX, 60:40 MIX:concentrate.

Bottles were withdrawn from the incubator 24 h after inoculation, total gas production was measured in each bottle using a pressure transducer and a calibrated syringe, and a gas sample (about 15 ml) was stored in a vacuum tube before analysis for CH_4 concentration. Bottles were then uncapped, the pH was immediately measured and the fermentation was stopped by swirling the bottles in ice. One ml of the bottle content was added to 1 ml of deproteinizing solution (10% of metaphosphoric acid and 0.06% crotonic acid; w/v) for volatile fatty acids (VFA) analysis and another 1 ml was added to 1 ml of HCl for NH_3-N analysis.

3. Analytical procedures, calculations and statistical analyses

Dry matter, ash and N were determined according to the AOAC (1999). Neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin analyses were carried out according to Van Soest *et al.* (1991) and Goering and Van Soest (1970). NH_3-N concentration was determined by a colorimetric method and VFA concentrations by gas chromatography as described by Carro *et al.* (1999). Methane was measured as described by Martínez *et al.* (2010).

The amounts of VFA produced were obtained by subtracting the amounts present initially in the incubation medium from those determined at the end of the incubation period. The amount of organic matter apparently fermented (OMAF) in each bottle was estimated from net productions of acetate, propionate and butyrate as described by Demeyer (1991).

Data were analysed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The effects of type of forage (WS and WSDP), concentrate level (0, 20 and 40%) and the interaction type of forage x concentrate level were considered fixed, and incubation day effect was considered random. Significance was declared at $P<0.05$, whereas $P<0.10$ values were considered as trends and discussed. When a significant effect of treatment ($P < 0.05$) was detected, differences among means were tested using the Tukey's multiple comparison test.

III – Results and discussion

Organic matter, CP, NDF and ADF contents of WS and DP were in the range of those previously reported by others (Sipos *et al.*, 2010; Boudechiche *et al.*, 2009; Habib and Ibrahim, 2009). These results confirm DP as a good source of cellulose (38.9% of DM), but with a higher lignification compared with WS (26.8 and 15.3% of NDF, respectively).

The results of the *in vitro* fermentation of WS, DP and concentrate with ruminal fluid from sheep are shown in Table 2. The VFA production for DP was 1.3 times lower ($P<0.001$) than that for WS, which is consistent with the higher NDF and lignin content of DP (Table 1). Compared with WS, *in vitro* fermentation of DP resulted in lower ($P<0.05$) proportions of acetate and propionate, but higher ($P<0.05$) proportions of butyrate and other minor VFA (isobutyrate, valerate and isovalerate) and acetate/propionate ratio. In contrast, there were no differences ($P>0.05$) in methane production between WS and DP. Final concentrations of NH_3-N were lower ($P<0.05$) for DP than for WS, despite of their similar CP content (5.11 and 4.44 g/100 g of DM for DP and WS, respectively). This difference may be due to a lower CP degradability of DP compared with that of WS. As expected, fermentation of concentrate resulted in higher ($P<0.001$) VFA production and lower ($P<0.001$) acetate/propionate ratio than fermentation of WS and DP, which agrees well with the lower NDF and ADF content of concentrate compared with that of WS and DP. The higher CP content of the concentrate resulted in higher ($P<0.05$) NH_3-N concentrations in the cultures with concentrate as substrate than those in the cultures with DP (361 and 316 mg/L, respectively), but in all cultures NH_3-N concentrations were above the minimum level necessary for maximal rate of fermentation (Mehrez *et al.*, 1978) due to the use of a nitrogen-enriched buffer for the in vitro incubations. The higher ($P<0.05$) NH_3-N concentrations observed in the cultures with WS compared with those with DP could not be explained by differences in CP content, as both feeds had similar CP content (4.44 and 5.11 g/100 g of DM for WS and DP, respectively). Digestibility of CP of DP has been reported to be very low (Al-Yousef *et al.*, 1993) and even negative values have been found in some studies (El-Shazly *et al.*, 1963). A low CP degradability of DP would explain the lower NH_3-N concentrations observed in the cultures with DP.

Table 2. Final pH, production of of volatile fatty acids, gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) after *in vitro* fermentation of wheat straw, date pits and concentrate samples (500 mg) in batch cultures of mixed ruminal microorganisms for 24 h (n=4)

Item	Wheat straw	Date pits	Concentrate	SEM	P value
pH	6.97^{b}	7.10^{c}	6.76^{a}	0.014	<0.001
VFA, volatile fatty acids (µmol)					
Acetate	1613^{b}	1207^{a}	2118^{c}	28.5	<0.001
Propionate	565^{b}	385^{a}	888^{c}	31.9	<0.001
Butyrate	310^{a}	308^{a}	743^{b}	14.7	<0.001
Other†	147^{a}	139^{a}	188^{b}	6.1	0.002
Total	2635^{b}	2039^{a}	3937^{c}	54.7	<0.001
Molar proportions of VFA (mol/100 mol):					
Acetate	61.3^{a}	59.5^{b}	53.9^{b}	0.75	0.001
Propionate	21.4^{b}	18.8^{a}	22.4^{b}	0.51	0.006
Butyrate	11.7^{a}	15.0^{b}	18.9^{c}	0.43	<0.001
Others†	5.58^{a}	6.73^{b}	4.78^{a}	0.238	0.003
Acetate/Propionate (mol/mol)	2.89^{b}	3.18^{c}	2.45^{a}	0.081	0.002
Gas (µmol)	2768^{b}	2109^{a}	4676^{c}	45.1	<0.001
Methane (µmol)	437^{a}	419^{a}	581^{b}	22.4	0.004
NH_3-N (mg/L)	359^{b}	316^{a}	361^{b}	6.0	0,031
OMAF (mg)	226^{b}	179^{a}	364^{c}	4.9	<0.001

SME: Standard error of the mean. †Calculated as the sum of isobutyrate, isovalerate and valerate.
[a, b, c]Mean values within a row with unlike superscript letters differ ($P<0.05$).

The results of the in vitro fermentations of the experimental diets are shown in Table 3. For both forages (WS and MIX), supplementation with concentrate resulted in increased ($P<0.05$) total VFA, acetate, propionate and butyrate productions without changes ($P>0.05$) in the production of other minor VFA. These results are in agreement with those from other studies (García-Martínez *et al.*, 2005; Martínez *et al.*, 2010) in which diets with different forage/concentrate ratios were fermented in vitro with ruminal fluid from sheep. Moreover, the differences observed between the fermentation pattern of WS and MIX as the only feed and that of the diets including concentrate closely resembled the in vivo fermentation patterns reported when sheep fed low-quality forages were supplemented with concentrates (Fondevila *et al.*, 1994; Castro *et al.*, 2002).

Table 3. Final pH, production of volatile fatty acids (VFA), gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) after *in vitro* fermentation of diets (500 mg) containing wheat straw, date pits and concentrate samples in batch cultures of mixed ruminal microorganisms for 24 h (n=4)

Item	Diet†						SEM	P value
	WS	80WS	60WS	MIX	80MIX	60MIX		
pH	6.97	6.92	6.91	7.00	6.92	6.93	0.020	0.059
VFA (µmol)								
Acetate	1613ab	1781cd	1711bc	1486a	1774cd	1892d	49.3	0.001
Propionate	565a	611ab	697bc	538a	580ab	660bc	30.1	0.017
Butyrate	310a	397b	492c	355ab	406b	523c	22.2	<0.001
Others††	147	150	161	160	152	179	10.2	0.315
Total	2635a	2940bc	3061cd	2539a	2911b	3254e	48.8	<0.001
Molar proportions of VFA (mol/100 mol):								
Acetate	61.3	60.8	56.4	58.6	61.1	58.2	1.52	0.191
Propionate	21.4	20.7	22.4	21.1	19.8	20.2	0.84	0.346
Butyrate	11.7a	13.5ab	15.9c	14.0b	13.9b	16.1c	0.60	0.001
Others††	5.58ab	5.09a	5.23a	6.27b	5.20a	5.51a	0.249	0.045
Acetate/Propionate (mol/mol)	2.89	3.00	2.68	2.78	3.11	2.90	0.146	0.394
Gas (µmol)	2768b	3203c	3510d	2588a	3136c	3513d	41.8	<0.001
Methane (µmol)	437	516	474	462	508	434	23.4	0.108
NH_3-N (mg/L)	359	341	348	330	331	341	7.3	0.121
OMAF (mg)	226ab	258bc	275cd	221a	245bc	292d	6.5	<0.001

†WS: wheat straw; 80WS: 80:20 wheat straw:concentrate; 60WS: 60:40 wheat straw:concentrate MIX: 80:20 wheat straw:date pits; 80MIX: 80:20 MIX:concentrate; 60MIX: 60:40 MIX:concentrate. ††Calculated as the sum of isobutyrate, isovalerate and valerate.
a, b, c, d, e: mean values within a row with unlike superscript letters differ ($P<0.05$).

Final pH ranged between 6.91 and 7.00 for all diets, being in the range of values for optimal cellulolytic activity (Stewart, 1977). There were no differences ($P>0.05$) between 80WS and 80MIX in any of the measured parameters, indicating that replacing 20% of WS by DP in the forage did not affect the ruminal fermentation of the diet. However, the production of acetate and total VFA was 11 and 6% higher ($P<0.05$), respectively, compared with that with the 60WS diet. There were no differences ($P>0.05$) between 60WS and 60MIX diets in the rest of the determined fermentation parameters. These results indicate that replacing WS by DP at 20% of the forage portion of the diet would be an easy means of increasing VFA production.

IV – Conclusions

The results of this study indicate that wheat straw can be replaced by date pits at 20% of the forage portion of the diet without any detrimental effect on ruminal fermentation. Furthermore, in diets with 60:40 forage:concentrate ratio, incorporating date pits in the forage portion resulted in greater volatile fatty acids and acetate production. If these results are confirmed *in vivo*, it would be of great interest in sheep practical feeding in countries having high palm by-products production. Including date pits in the diet would be especially interesting for dairy ruminants, as acetate is the main precursor for de novo milk-fat synthesis in the mammary gland.

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