Actes Inst. Agron. Vet. (Maroc) 2003, Vol. 23(2-4): 87-93

Internal markers and water balance in sheep fed straw-based diets

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(Received on 11/02/2003 ; Accepted on 17/06/2003)

الواسمات الداخلية والتوازن المائي عند الغنم المغذى أساسا بالقش

في حالة الحميات المبنية على القش، قدرنا فعالية توقع الهضومية لثلاث واسمات داخلية هي الرماد العقيد في الحمض و الخشبين و الرماد المتبقي من استخلاص الخشبين. بحثنا أيضا عن العلاقة بين استعمال عناصر الغذاء و استعمال الماء. قدمت أربعة أنواع من قش الشعير مع مكمل لثمان رخلات محبوسات في صنادق هضمية حسب تناول اختياري أو محدد (75% من الإختياري) بعد مدة التكييف وزنا الطعام المقدم و الطعام المعاف و الروث و قسنا حجم البول و أخذنا عينات من هذه العناصر. كما سجلنا حجم الماء المشروب و الماء المتبخر، عينات الروث مزجت بشكل متناسب أو غير متناسب مع حجم الروث المطروح.كان الرماد العقيد في الحمض أحسن دليل على الهضومية. الخشبين كان دليلا مقبولا خصوصا خلال التناول المحدد. فعالية كل الواسمات كانت أشد عند توقع هضومية أنصاف السيليلوز. بينما لم تؤثر طريقة مزج العينات في توقع هضومية المادة الجافة العضوية، كان لها أثر بالغ في توقع هضومية البروتينات الخام، مما قد يدل على أن إفراز الواسمة منتظم في حين أن إفراز البروتينات الخام يتغير من وقت جمع لآخر. تحديد التناول حسن دقة التوقع. نسبة الماء المشروب المطروحة في الروث ارتبطت عكسيا مع هضومية الألياف و مع نسبة الماء المشروب المطروحة في البول. رطوبة الروث ارتبطت عكسيا مع نسبة البروتينات الخام في الوجبة. تحديد التناول قلل من استهلاك الماء ورفع نسبة الماء المشروب والمطروح في البول.

الكلمات المفتاحية: واسمة داخلية – استعمال الماء– هضومية – قش – ضأن

Marqueurs internes et bilan hydrique chez les ovins nourris à base de paille

Pour les rations à base de paille, on a évalué l'efficacité, dans la prévision de la digestibilité, de trois marqueurs. On a aussi cherché les relations entre l'utilisation des nutriments et le bilan hydrique de l'animal. Quatre types de paille ont été apportés à des agnelles à deux niveaux d'ingestion. L'insoluble chlorhydrique était le meilleur estimateur de la digestibilité. La lignine (ADL) était un marqueur satisfaisant. Tous les marqueurs étaient particulièrement efficaces pour l'estimation de la digestibilité de l'hémicellulose. Le mélange des échantillons fécaux proportionnellement aux volumes des déjections a augmenté la précision de l'estimation de la digestibilité des MAT mais pas celles de la MS et de la MO. La restriction de l'ingestion a amélioré l'estimation. La portion de l'eau bue qui est perdue via les fèces était négativement corrélée avec la digestibilité de la paroi cellulaire (NDF) et avec la portion de l'eau bue perdue dans l'urine. L'humidité des fèces était négativement corrélée avec la teneur de la ration en MAT. La restriction de l'ingestion a réduit la consommation d'eau et a augmenté la fraction de cette eau perdue dans l'urine.

Mots clés: Marqueur interne - Bilan hydrique - Digestibilité - Paille - Ovin

Internal markers and water balance in sheep fed straw-based diets

In straw based diets, the efficiency of three internal markers to predict digestibility was investigated, as well as the correlation of nutrient utilization with animal water balance. Four types of barley straw were fed, at two intake levels, with a supplement, to ewe lambs. Acid insoluble ash was the best digestibility estimator, whereas ADL was a satisfactory one. All markers were particularly adequate in determining hemicellulose digestibility. When fecal samples were composited proportionally to the fecal output, digestibility predicting accuracy was improved for CP; but not for DM and OM. This suggests a variation in the nutrients excretion patterns. Prediction accuracy was improved by intake restriction for all markers. Water intake and insensible water loss (in ml/(d.kg mbw)) were both positively correlated with digestible cellulose intake. The fraction of water intake lost through feces was negatively correlated with NDF digestibility, and with the fraction of water intake lost through urine. Fecal moisture was negatively correlated with ration CP content. Limiting ration intake reduced water intake and the fraction of water intake lost through feces, and raised the fraction of water intake lost through urine.

Key words: Internal marker - Water balance - Digestibility - Straw - Sheep

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INTRODUCTION

Low quality forages, especially cereal straws, are widely used in animal feeding around the world, particularly during the dry season (Chermiti, 1999). Because of their poor nutritive value, they are commonly treated in order to improve their nutrient availability and utilization (Kerley *et al.*, 1986; El-Yassin *et al.*, 1991; Diouri & Wiedmeier, 2002). In a previous study (Diouri & Wiedmeier, 2000), intake and digestibility of treated barley straw-based diets were measured as well as rumen fermentation characteristics. In such diets, we were interested in two more measurements which make the main objectives of the present work:

- to assess the efficiency of acid insoluble ash (AIA), ADL and ADL insoluble ash (ADLIA) as internal markers for digestibility estimation of straws.
- to correlate parameters of water and nutrients utilization. Water intake is important in the digestion of fiber, which is the main component of straws. Moreover, low quality forages intensive use is usually accompanied by water shortage.

MATERIAL & METHODS

Four different treated straws were used (Diouri & Wiedmeier, 2000): i) Control, ii) 3% NH_3, iii) 3% NH_3 after rehydration of straw to 15% moisture with water, iv) 3% NH_3 after rehydration of straw to 15% moisture with a H_2O_2 solution (to reach a level of 0.32% DM of H_2O_2).

A stack of ten 23 kg straw bales was randomly assigned to each of the four treatments. One stack was treated with enough water to bring the moisture level to 15%. Another stack was treated with a H_2O_2 solution to rehydrate to 15% moisture. All four stacks were then placed in separately sealed 6 mm thick plastic bags. NH_3 was then introduced, in the gaseous form, into three of the four bags (the two rehydrated and one of the non-rehydrated stacks), through a perforated pipe at 3% of DM. The fourth stack was set aside to serve as a control. After ammoniation, the bags were sealed for approximately 21 days (during a moderately cold late autumn) and then opened to allow excess ammonia to escape. Straw was then chopped and mixed.

Eight (4 Navajo, 2 Columbia, and 2 Black-faced) yearling ewe lambs (30 to 51 kg) were used. After proper adaptation to a straw-based diet, sheep were placed in elevated metabolism crates equipped with stainless steel feeders, watering troughs, and feces-urine collection apparatus.

Treatments were administered in a split plot in a 4*4 Latin square design with repeated measures. Two animals were allocated to each treatment. Each of the 4 trial periods was composed of two subperiods. In the first subperiod (main plot), sheep had ad libitum access to straw in order to determine the intake. A supplement, composed of ground faba bean (*Vicia faba*), and fortified with vitamins and minerals as needed to meet nutrient requirements (NRC, 1985), was top dressed. The supplement intake was gradually adjusted to represent approximately 25% of the ration in order to eliminate the possible negative associative effect between concentrate and roughage. Daily rations were given in 2 equal portions at 0800 and 1600. Diets were fed for a 14-d adaptation period followed by a 5-d collection period. In the second subperiod (split-plot), consisting of a 5-d adaptation and a 5-d collection, sheep received the same diet but their ration was limited to 75% of each animal's ad libitum intake.

During the collection periods, total urine and fecal outputs were gravitationally separated, by a tilted screen, at the time of their excretion. Urine volumes and fecal weights were recorded twice daily at the normal feeding times. Urine was stabilized in the collection container with mercuric chloride ($HgCl_2$). A fecal sample of each collection output was dried at 60°C for 72 h and then ground to pass through a 2 mm screen. Dry matter content was calculated. Composites of fecal samples, within ewe and period, were then made. The amount of each sample that went into the composite was proportional (p) to that sample's portion of the total output. Other fecal composites were made to simulate experiments where internal markers are measured. These composites were not proportional (np) to the fecal outputs, and contained equal amounts of each fecal sample. Urine samples were placed in whirl-pack bags, composited, stored at -20°C, then freeze dried.

Feed (straw and supplement) was weighed at each feeding. Refusal was weighed back to determine intake. Starting 1 d before the beginning of the collection periods, samples of feed and refusals were taken at each feeding. These samples were ground to pass through a 1 mm screen and proportional composites were made as was described with feces samples.

All feed, ort, and fecal composite samples were analyzed for laboratory dry matter (DM), OM (AOAC, 1990), CP (Hach *et al.*, 1985), ADF, NDF, ADL (Goering & Van Soest, 1970), AIA (Van Keulen & Young, 1977), and ADLIA (which was the ash remaining after ADL analysis).

Water intake was determined by measuring water given and water remaining at each feeding. To account for evaporation, a container like those used by the animals, was charged with water and left in the open. This water's volume was measured after a fixed period of time. Representative fresh fecal and urine samples were also left in the open and remeasured to determine water evaporation from feces and urine per unit of time.

Data were analyzed using the regression and the general linear model procedures of SAS (1988).

Since some animals could not stand the limited intake and were removed from experiment early, only animals who went through the two intake levels were considered for statistical analysis in order to have a balanced design.

RESULTS & DISCUSSION

1. Internal markers

The ration and feces composition is given in Table 1. ADL exceeds 6% in the ration, and can therefore be used as a marker (Van Soest, 1987). The level of ADLIA is higher than that of AIA. This is probably because in ADLIA analysis, acid is applied in the presence of organic matter (ADF) which may prevent some minerals from solubilization. This suggestion is suppported by the lower recovery rate for ADLIA than for AIA (Table 2). A similar trend was reported by Undersander *et al.* (1987) for AIA and ADF insoluble ash.

A part from ADLIA, the recovery of all markers (Table 2) exceeded 100%. These recovery rates were closely related to digestibility coefficients (Table 3). No significant difference was found between AIAp and AIAnp recovery rates. This resulted in a similar ability to predict digestibility that will be discussed later.

Total-fecal-collection digestibility was better estimated by AIA than ADLIA (Tables 3 & 4). ADLIA is determined from a smaller sample size (1 g vs. 5 g) and requires more analytical steps than AIA. This could lead to more analysis errors.

ADL was also an efficient marker because of its high concentration in the ration. This was especially true at the limited intake level (Table 4), where no ort was left (higher ADL content) and where the recovery rate neared 100%. However, there was more variability among straws in concentration and recovery of ADL than those of AIA (Table 5) favoring the latter over the former. This variability among straws suggests that no marker was consistently accurate and agrees with the findings of Undersander *et al.* (1987).

Table 1. Average ration and feces composition

Nutrient[1]	Forage	Suppl.	Ort[2]	Ration	Feces
DM	92.65	90.33	92.08	92.16	30.88
			% DM basis		
OM	92.30	96.04	92.99	93.11	88.69
OMnp	-	-	-	-	88.41
CP	6.46	21.14	6.00	10.11	9.47
CPnp	-	-	-	-	10.81
Cellulose	45.89	11.21	47.77	37.19	32.86
Hemicellulose	24.91	19.01	27.68	23.14	20.25
ADL	7.65	2.24	8.47	6.23	16.05
ADLIA	3.42	0.66	2.56	2.85	6.42
AIA	2.82	1.16	1.62	2.60	7.09
AIAnp	-	-	-	-	7.31

ADLIA: ADL insoluble ash;
AIA: acid insoluble ash;
OMnp, CPnp and AIAnp:
OM, CP & AIA determined in non-proportional feces composites ;
[2] When available (mainly at *ad libitum* intake)

Table 2. Recovery of internal markers (% of intake)

Marker[1]	VI[2]	LI[2]	Mean[2]
ADL	116.5[a]	103.2[a]	109.9[a]
ADLIA	107.4[b]	85.5[b]	96.5[b]
AIAp	113.5[ab]	118.7[c]	116.1[c]
AIAnp	117.0[a]	122.3[c]	119.6[c]

Internal marker: ADLIA:
ADL insoluble ash; AIA: acid insoluble ash;
fecal samples composited proportionally (AIAp) or non-proportionally (AIAnp) to the fecal outputs;
[2] Data from voluntary intake (VI), limited intake (LI) or both intakes (Mean) ;
[a, b, c] Column means, lacking a common superscript, differ (P<0.05)

Table 3. Apparent digestibility coefficients (%) as measured by total fecal collection or estimated by internal markers

Nutrient	Method[1]	VI[2]	LI[2]	Mean[2]
DM	TFC	56.9 a	58.2 a	57.5 a
	ADLIA	58.7 ab	49.1 b	53.9 b
	AIAp	61.9 bc	64.5 c	63.2 c
	AIAnp	63.0 c	65.6 c	64.3 c
	ADL	62.6 c	58.8 a	60.7 d
CP	TFC	59.9 a	61.2 a	60.6 a
	ADLIA	61.7 ab	53.1 b	57.4 d
	AIAp	64.6 bc	67.3 c	65.9 c
	AIAnp	60.2 a	63.1 a	61.6 ab
	ADL	65.3 c	62.0 a	63.6 bc
Cellulose	TFC	61.4 a	63.1 a	62.3 a
	ADLIA	62.9 ab	54.8 b	58.9 c
	AIAp	65.9 b	68.6 c	67.3 b
	ADL	66.5 b	63.5 a	65.0 ab
Hemicellulose	TFC	64.1 a	61.5 a	62.8 ab
	ADLIA	65.4 a	52.3 b	58.8 a
	AIAp	68.0 a	67.1 a	67.5 b
	ADL	68.4 a	61.5 a	65.0 b

TFC: total fecal collection, ADLIA: ADL insoluble ash,
AIA: acid insoluble ash; fecal samples composited proportionally (AIAp) or non-proportionally (AIAnp) to the fecal outputs ;
[2] Data from voluntary intake (VI), limited intake (LI) or both intakes (Mean) ; [a, b, c, d] Column means, within each nutrient, lacking a common superscript, differ ($P<0.05$).

Table 4. Correlation coefficients between digestibility coefficients determined by total fecal collection and internal markers

Nutrient	Marker[1]	VI[2]	LI[2]	Mean[2]
DM	ADLIA	0.52**	0.55**	0.38**
	AIAp	0.62***	0.79***	0.73***
	AIAnp	0.57**	0.79***	0.71***
	ADL	0.55**	0.81***	0.59***
OM	ADLIA	0.55**	0.59**	0.43**
	AIAp	0.66***	0.82***	0.75***
	AIAnp	0.61**	0.82***	0.73***
	ADL	0.58**	0.83***	0.62***
CP	ADLIA	0.48*	0.37	0.30*
	AIAp	0.80***	0.67***	0.74***
	AIAnp	0.10	-0.03	0.07
	ADL	0.67***	0.67***	0.59***
Cellulose	ADLIA	0.70***	0.77***	0.62***
	AIAp	0.86***	0.93***	0.89***
	ADL	0.77***	0.92***	0.78***
Hemicellulose	ADLIA	0.89***	0.84***	0.80***
	AIAp	0.97***	0.96***	0.96***
	ADL	0.95***	0.96***	0.94***

ADLIA: ADL insoluble ash, AIA: acid insoluble ash; fecal samples composited proportionally (AIAp) or non-proportionally (AIAnp) to the fecal outputs. [2] Data from voluntary intake (VI), limited intake (LI) or both intakes (Mean). *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 5. Effect of straw treatment on marker concentration and recovery

Marker[1]	Control	NH_3	NH_3+H_2O	$NH_3+H_2O_2$
Content (% DM)				
AIA	2.94 a	2.85 ab	2.65 c	2.72 bc
ADL	8.35 a	7.72 b	7.55 b	7.17 c
ADLIA	3.55 a	3.46 a	3.38 a	3.41 a
Recovery rate (% intake)				
AIAp	109.5 a	117.6 b	118.9 b	118.5 b
AIAnp	113.8 a	121.5 b	121.7 b	122.1 b
ADL	100.9 a	106.8 a	114.3 b	117.9 b
ADLIA	88.5 a	95.1 a	100.9 a	95.5 a

ADLIA: ADL insoluble ash;
AIA: acid insoluble ash;
fecal samples composited proportionally (AIAp) or non-proportionally (AIAnp) to the fecal outputs.
[a, b, c] Row means, lacking a common superscript, differ ($P<0.05$).

When digestibility was predicted separately in each of the four straws (data not shown), AIA was consistently prevailing. The prediction accuracy was, however, different from one straw to another. If we add to this difference the variability in recovery rates, we can confirm that treatment changes forage behavior toward markers. This change was also shown to be caused by forage growth condition (Undersander *et al.*, 1987) and supplementation (Judkins *et al.*, 1990).

Limiting intake improved digestibility prediction by all markers. At this intake level, there is no refusal and, in turn, less analysis error accumulation and a higher marker concentration.

All markers gave better estimation of the digestibility of cellulose and hemicellulose than that of DM, OM or CP. There was more variability among straws in the digestibility of these two fiber components than that of other nutrients (Diouri & Wiedmeier, 2000). This was probably the reason of this better estimation. The same trend was reported, for cellulose digestibility, by Undersander *et al.* (1987).

In hemicellulose digestibility, not only the correlation was high, but also the digestibility estimates were similar to total collection values. Hemicellulose digestibility determined by these markers can not be used only for comparison purposes, but also to accurately estimate total collection digestibility.

Van Keulen & Young (1977) did not detect any diurnal AIA excretion pattern. Comparison of

AIAp and AIAnp (Tables 3 & 4) suggests that AIA excretion is not different from one fecal collection to another and from one day to another after an adequate adaptation period.

Therefore, as long as the diet has a high marker content (Sherrod *et al.*, 1978; Sunvold & Cochran, 1991), which was our case, and the grab samples are representative (Thonney *et al.*, 1985), AIAnp can serve as a satisfactory internal marker. CP digestibility was an exception to this rule. When determined by AIAp, it was highly significantly correlated to in vivo CP digestibility. But, this correlation was no longer significant when AIAnp was used to predict CP digestibility (Table 4). This phenomenon was not due to the ration marker concentration or recovery rate. Otherwise, it would have affected other nutrients' digestibility. It seems to be caused by feces CP content.

In fact, correlation coefficients of nutrient concentration in proportionally composited feces versus the same nutrient concentration in non-proportionally composited feces were 0.99***, 0.93***, and 0.79*** for AIA, OM and CP respectively. It seems, therefore, that CP has a different excretion pattern than other nutrients over time.

2. Water balance

Daily water intake, from feed and drinking water, varied, at voluntary intake, from 122 ml/kg of metabolic body weight in Navajo to 169 ml/(d.kg mbw) in Columbia ewes. Although this difference was significant ($P<0.05$) and did not arise from a correlation between water intake and body weight ($R=0.23$), the small number of sheep in each breed (4, and 2 respectively) does not allow a confident conclusion. This possible difference may be due to a different level of metabolism resulting either in an adaptation to water shortage, in the extensively raised Navajo, or in a high performance, in the dual-purpose breed of Columbia.

Limiting feed intake reduced water intake and the part of this water that is lost through feces; and raised the part of water intake that is lost through urine (Tableau 6). This suggests that the body tries to keep a certain water flow level for an optimal metabolism to balance the lower physiologic call for water caused by a lower feed intake. The insensible water loss fraction did not differ, since it depends mainly on environmental conditions.

Table 6. Water intake and output

Water parameter[1]	VI[2]	LI[2]	Mean[2]	SE[3]	P
WI	135.6	111.8	123.7	7.08	0.02
%WF	38.4	33.3	35.8	1.90	0.06
%WU	25.3	31.9	28.6	1.96	0.02
%IWL	36.4	34.9	35.6	1.56	0.48

WI = Water intake (ml/(d.kg mbw));
%WF = percentage of water intake lost in feces;
%WU = percentage of water intake lost in urine;
%IWL = percentage of water intake in insensible water loss.
[2] Data from voluntary intake (VI), limited intake (LI) or both intakes (Mean). [3] Standard error of the LS means.

Water utilization parameters were better intercorrelated at the limited intake level; whereas, their correlations with nutrient utilization were higher at the voluntary intake level (Table 7).

Water intake was best correlated with digestible cellulose intake. The correlation coefficient is not very high, probably because of the low variability in animals, breeds and rations. Nevertheless, this correlation shows that cellulose metabolism requires relatively high amounts of water. Jacques *et al.* (1989) reported also that water intake is increased by an increased DM intake.

Table 7. Highest correlation coefficients among parameters of water and nutrients utilization

Parameter[1]	Indicator[1]	VI[2]	LI[2]	Mean[2]
WI	WU	0.66***	0.89***	0.72***
WI	IWL	0.73***	0.82***	0.77***
%WF	%WU	-0.56**	-0.76***	-0.70***
WI	DCI	0.48*	0.41*	0.52***
%WF	NDFD	-0.75***	-0.63***	-0.70***
IWL	DCI	0.59**	0.59**	0.65***
FMST	CPfed	-0.70***	-0.56**	-0.61***

WI = Water intake (ml/(d.kg mbw));
%WF = percentage of water intake lost in feces;
%WU = percentage of water intake lost in urine;
IWL = insensible water loss (ml/(d.kg mbw));
WU = amount of water intake lost in urine (ml/(d.kg mbw));
FMST = feces moisture (%);
Cpfed = ration CP content (%);
DCI = digestible cellulose intake (g/(d.kg mbw));
NDFD = NDF digestibility (%).
[2] Data from voluntary intake (VI), limited intake (LI) or both intakes (Mean).

Water intake goes out of the body through feces, urine, or insensible loss (calculated here by difference). The portion of water lost through feces is negatively correlated with NDF digestibility and with water lost through urine. The more NDF is digestible, the more water is needed to transport its products to the circulatory system instead of being lost in the feces. This water will, in turn, be excreted in the urine. The portion of water intake in insensible loss depends primarily on environmental conditions, and was not highly correlated with nutrients digestibility.

The absolute amount of insensible water loss, however, was positively correlated with digestible cellulose intake, probably because of the difference in metabolism level with different intake levels (Silanikove, 1989; Shalit *et al.*, 1991).

It is important to note that the different water utilization parameters are not independent, probably because of the interference of water secretion at different parts of the gastro-intestinal tract (Sklan & Hurwitz, 1985; Jacques, 1989).

Fecal moisture was negatively correlated with ration CP content. In the case of ammonia-treated straws, an increase in CP content reflects an improvement of treatment efficiency and, in turn, an increase in DM digestibility and intake (Diouri, 1993), leading to a greater water absorption. A higher ration CP content was also showed to cause a higher bacterial nitrogen flow (Chermiti *et al.*, 1994) requiring a greater water absorption.

CONCLUSION

In our conditions, AIA was an efficient internal marker; while ADL was a satisfactory one especially at the limited intake level. Fecal sample compositing did not seem to be essential except when predicting CP digestibility. All the markers were more efficient at the limited intake level, and for fiber digestibility prediction.

The correlations found between water utilization and nutrient utilization parameters may be used to predict those parameters. These correlations may have been higher if there were more animal and ration diversity. These correlations allow to predict digestibility or other digestion parameters without supplementary measurements or analyses.

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