

FEASIBILITY OF ALKANES AS FLOW AND DIGESTIBILITY MARKERS, AND THE EFFECTS OF SODIUM BICARBONATE ON SITE OF DIGESTION AND METABOLISM IN THE GASTROINTESTINAL TRACT OF SHEEP

A. R. Askar

Animal and Poultry Nutrition Department, Desert Research Center, P.O. Box 11753, El-Matareya, Cairo, Egypt

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SUMMARY

Two experiments were conducted to study the evaluation of long chain n-alkanes as flow and digestibility markers and the effect of sodium bicarbonate (SB) on the metabolism and digestion throughout the gastrointestinal tract in lambs fed high concentrate diets using Ytterbium-chloride (YbCl₃) as a flow marker. Eighteen and twelve Raza Aragonza lambs were used for experiment 1 and 2, respectively. Lambs were individually employed directly after finishing the fattening period (24 kg live weight), continuing on same diet, with free access of whole barley grain and protein supplement. Each experiment lasted 3-week period when offered feeds and orts were recorded daily. Lambs were slaughtered after a 7-day period of daily continued dose of chromium EDTA (Cr- EDTA) and YbCl₃ (YbCl₃ only in the second experiment). Lambs were slaughtered 3 hours post feeding and the digestive tract was sectioned and their contents were sampled. Results of experiment 1 indicated that C₃₁ was the predominant alkane in the diet, followed by C₂₉ and C₃₃. Recovery of C₂₉, C₃₁ and C₃₃ in respect to YbCl₃ was much higher in abomasum and significantly dropped in ileum and rectum reflecting upon digestibility coefficients through the gastrointestinal tract sections. Rumen digestibility values using alkane C₃₁ were similar to those obtained by YbCl₃, while C₂₉ and C₃₃ showed much higher values. Digestion in small intestine was very low with using alkanes in comparison with YbCl₃. C₃₃ has a significant higher recovery in faeces as compared to C₂₉ and C₃₁. In the second experiment, two treatments were conducted, with or without SB. Adding SB did not affect the intake of DM, OM, and N but negatively affected rumen nutrient digestibility and enhanced small intestine digestibility. Therefore, the site of digestion might be affected by SB. Efficiency of rumen N utilization and microbial protein synthesis were low and not improved by SB addition, resulting in losing 48% of N intake in rumen. Rumen N degradation was high and not affected by SB addition. Results can conclude that long chain n-alkanes, particularly C₃₁, which is a useful rumen digestibility marker. However, Cr-EDTA can not be recommended as flow marker. Additionally, Cr-EDTA and long chain n-alkanes can be used as total tract digestibility markers, taking into consideration their faecal recovery. SB addition can manipulate the site of digestion through the gastrointestinal tract but does not seem to regulate protein intake and degradation in lambs. The present study suggested that the use of low degradable N source would be a solution for not selecting diets rich in protein by growing lambs fed high concentrate diets.

Keywords: *long chain n-alkane, Cr-EDTA, YbCl₃, lambs, high concentrate diet*

INTRODUCTION

Three key elements are required for sustainable research efforts in animal nutrition. The amount of feed intake (the quantity), diet composition (the quality), and nutrients actually made available for absorption in the digestive tract after rumen digestion (Dove and Mayes, 1991) which is difficult to be directly measured. Individual and metabolic cages are used to measure the individual intake and digestibility but they are not representative for the intensive production system in which feeding behaviour can alter and affect the accuracy of obtained results. Therefore, the use of nutritional markers allows monitoring of the process of interest (Kotb and Luckey 1972).

The estimation of digestibility throughout the digestive tract, and microbial protein contribution to post-ruminal flow requires an appropriate flow and microbial markers (Askar *et al.*, 2005). The characteristics of an ideal marker and procedures for its use have been reviewed earlier (Kotb and Luckey 1972; Faichney 1975; Owens and Hanson, 1992). Several markers have been used, either as internal or external (Mayes and Dove, 2000). Internal markers have several advantages over external markers, including their performance and representation in feed and digesta. Long chain n-alkanes are present naturally in the cuticular surface wax of plants and have recently reported to estimate feed intake, composition, and digestibility in grazing animals (Mayes and Dove, 2000; Valiente *et al.*, 2003; Dove and Mayes, 2005) but their application as flow (Askar *et al.*, 2005) and transit (Giráldez *et al.*, 2006) markers has been limited with scarce information (Mayes *et al.*, 1988; Ohajuruka and Palmquist, 1991).

On the other hand, the quantity of nutrients digested in rumen is a function of two rates, the rate of passage and the rate of digestion in which sodium bicarbonate (SB) has been reported to affect both of them. Inclusion of SB is recommended to improve rumen pH (Santra *et al.*, 2003; Tripathi *et al.*, 2004) and increase dilution rate in the rumen, as well as the rate of the passage (Harrison *et al.*, 1975; Kellaway *et al.*, 1978; Okeke *et al.*, 1983; Owens and Goetsch, 1986). This leads to improve the efficiency of microbial protein synthesis (Harrison *et al.*, 1975; Chamberlain and Thomas, 1980; Mees *et al.*, 1985), enhance bacterial fixation of ammonia (Newbold *et al.*, 1988 and 1991), and increase dietary nutrients by-passing ruminal degradation (Okeke *et al.*, 1983, Kovacik *et al.*, 1986). A little is known about the effect of SB on rumen and post rumen metabolism and digestibility in lambs fed on high concentrate diets.

However, the first experiment aimed to study the validation of long chain n-alkanes as flow markers in comparison with traditional markers, such as Ytterbium chloride (YbCl₃) and chromium-EDTA (Cr-EDTA), while the second experiment dealt with the effect of SB inclusion on nutrient utilization and metabolism throughout the gastrointestinal tract, using YbCl₃ as a flow marker.

MATERIALS AND METHODS

Animals and treatment

Experiment 1

Eighteen growing Raza Aragonesa male lambs, with an average body weight of 24.7 kg (SE = 0.48), were employed in individual cages to study the feasibility of n-alkanes as flow and digestibility markers throughout the gastrointestinal tract compared with YbCl₃ and Cr-EDTA as traditional markers. All animals were fed on whole barley grain and protein supplement ad libitum in separated troughs. Protein supplement was formulated from a mixture of soybean meal and barley grain to obtain approximately 34% of crude protein (Table 1).

Table (1). Chemical composition (g/kg DM), purine bases (μmol PB/g DM), and n-alkanes content (mg/kg DM) in feed ingredients.

	Protein supplement	Barley grain
Chemical composition (g/kg DM)		
OM	858	975
CP	341	100
EE	16.5	22.5
NDF	155	197
ADF	82.7	51.8
Purine bases (μmol PB/g DM)	12.8	8.83
N-alkanes content (mg/kg DM)		
C ₂₁	-	-
C ₂₃	0.62	1.51
C ₂₄	-	-
C ₂₅	1.54	3.16
C ₂₆	-	-
C ₂₇	1.20	2.72
C ₂₈	0.04	1.09
C ₂₉	3.34	9.24
C ₃₀	0.06	-
C ₃₁	3.71	11.13
C ₃₂	-	-
C ₃₃	1.61	2.67

Experiment 2

Twelve growing Raza Aragonesa male lambs, with an average body weight of 24.1 kg (SE = 0.11), were employed in individual cages to study the effect of adding SB on metabolism, and digestion throughout the gastrointestinal tract system. Lambs were divided into two treatments, 6 in each, and fed the same diet as in experiment one except for protein supplement that was mixed, either without (treatment 0) or with 30 g SB / kg dry matter (treatment 30) as proposed by Askar *et al.* (2006). Moreover, when lambs were

fed similar diet with free access of SB in separated troughs on the side, Askar *et al.* (2002) found that female selected much SB than male lambs with an average of 17 g SB / kg dry matter intake for both sexes (38.8 g SB per kg protein supplement). This selected amount was significantly decreased in male at the last 2 weeks of the fattening period (around 30 g SB per kg protein supplement) which has been taking into consideration in the present study.

Experimental procedures

Lambs, in both experiments, were moved to individual cages directly after finishing the fattening period (24 kg live weight or 8 weeks of fattening after early weaning at 6 weeks of age, see Askar *et al.*, 2006) in which the same diet was fed before and after fattening.

Both experiments lasted 3-week period during which offered feed and orts were recorded daily. Lambs were slaughtered after a 7-day period of daily continuous dose of 80 ml of Cr-EDTA (2821 mg Cr/L) and 20 ml of YbCl₃ (4960 mg Yb/L), YbCl₃ was used in the second experiment only. The daily amount of marker solution was infused at 6 hours intervals. At that period, feeds were offered at levels very close to ad libitum to avoid much feed refusal and reduce fluctuations in feed intake. Offered feed and orts were recorded daily and sampled to determine the dry matter contents by drying at 65°C for 48 h. An appropriate pooled sub-sample for each animal was also preserved for further analysis.

After the marker infusion period, animals were slaughtered by exsanguination previous intramuscular administration of 0.3 mg Xilacine/kg live weight (Xilagesic® 2%) and 10 mg/kg live weight of Tiopental (Tiobarbital® BRAUN). Lambs were slaughtered 3 hours post feeding in sets of 3 for the first experiment and 4 (2 per treatment) for the second experiment. The digestive tract was sectioned and their contents were sampled. The flow of abomasal, ileum, and rectum were determined by YbCl₃ and Cr-EDTA (only YbCl₃ in the second experiment), and then used to validate the use of n-alkanes as flow and digestibility markers in experiment one or to study the nutrient utilization and metabolism throughout the gastrointestinal tract in experiment two. Whole rumen content was strained and liquid fraction was centrifuged at 500xg for 10 min, then bacteria was extracted from the supernatant fluid by centrifugation at 20.000xg, for 20 min at 4°C. Bacterial pellets were rinsed with saline solution (9 g NaCl/L, at 4°C), then centrifuged again. Samples of bacterial pellets, and abomasum, ileum, and rectum contents were stored at -20°C and freeze dried for later analysis.

Analytical procedures

Dry matter content of feed ingredients, was determined by drying at 105°C for 24 hours. In feed ingredients, digesta, and faeces, organic matter was detected by ashing at 550°C for 8 h, total N content by the Kjeldahl method, using Se as catalyst, and non ammonia N (NAN) also by Kjeldahl method but after NH₃ evaporation by 1M NaOH addition. In feeds, ether extract was determined following the method of the Association of Official Analytical Chemists (2005), and neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as described by Van Soest *et al.*, (1991).

Marker preparation and analysis

Cr-EDTA was prepared following the method described by Downes and McDonald (1964), in which 71 g $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ were weighed and dissolved in 1 L distilled water, additionally 100 g disodium salt of the ethylenediaminetetraacetic acid (EDTA, $\text{Na}_2\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) were dissolved in 1.5 L of distilled water and added to the first solution. The mixture was heated until boiling with small glass balls as anti-bump, and kept boiling gently for 1 hour using a reflux system. Once the solution was cooled down, the small excess of EDTA was neutralized with 20 ml 1M CaCl_2 (7.35 g $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ in 50 ml distilled water) and pH was adjusted between 6 and 7 with 5 M NaOH. Then, the final volume up to 5 L with distilled water was made, and allow to stand at room temperature for 12-18 hours. Solution was filtered by Whitman filter paper (82 g/ m^2) before its use.

On the other hand, ytterbium chloride solution was prepared by a suitable dilution of $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water.

The analysis of Cr and Yb in flow and faeces samples was determined according to de Vega and Poppi (1997). Briefly, 15 mL of a 5:1 mixture of nitric acid: perchloric acid was added to 0.2 g dry matter of sample, and allow to stand overnight. Then, samples were gradually heated in a sand bath until their fully digestion. Once cooled down, samples were quantity transferred to 25 ml volumetric flasks by adding distilled water. After precipitation of silica, a 10 ml aliquot was taken and transferred to a plastic vial for atomic absorption spectrophotometric analysis.

N-alkanes extraction and analysis

For alkane extraction, 1.0 or 1.5 g of digesta flow and feed samples was weighed into 200 x 20 mm thick-walled screw-topped Pyrex test-tubes, adding 100 mg for flow, and 25 mg for feed samples. The internal standard solution was heptane containing 1 mg/g of C_{22} and C_{34} alkanes). Then extraction of n-alkanes was carried out following the technique described by Mayes *et al.* (1986), with the modifications suggested by Oliván and Osoro (1999).

Alkane analysis was carried out by on-column injection of 0.2 μl of the elute onto a 30 m x 0.53mm HP-1 capillary column (1.5 μm thickness) in an Agilent 6890 gas chromatograph fitted with an automatic injector and flame ionization detector (45 and 450 ml/min for H_2 and air, respectively). The carrier gas was helium (10 ml/min) as it was the make-up gas to the detector (45 ml/min). After an initial hold time of 0.2 min at 230°C the injector temperature was increased by 6°C/min to 303°C during 18 min. The oven was kept at 230°C for 0.2 min and then the temperature was increased by 6°C/min to 300°C, which were kept for a further 18 min. The detector oven was maintained at 350°C. Peak area data was processed using a Chemstation computer program (integrator). Detector response factors for individual n-alkanes were determined by injecting onto the chromatograph a standard n-alkane mixture (C_{21} - C_{36} inclusive) after every 8 sample extracts.

Microbial protein contribution:

The microbial contribution to abomasal NAN and rumen degradation were estimated using purine bases (PB) as microbial marker. Purine bases in isolated bacteria and abomasal digesta were analysed by HPLC following the technique reported by Martín-Orue *et al.* (1995). Microbial contribution was estimated by dividing the PB/N ratio in abomasal digesta by the same ratio in rumen bacterial extract as described by Askar *et al.*

(2005).

Statistical analysis

In both experiments, data were analysed as a one way analysis of variance with six replicates for each treatment. All analyses were made with the SAS package (SAS, 2000) and least significant differences were used to compare the means.

RESULTS AND DISCUSSION

Experiment 1

Feed intake and n-alkanes content in diet and digesta flow

Lambs dry matter intake was about 4.19% (SE = 0.157) as kg body weight (BW) bases, in which 51.7 (SE = 2.21) and 41.5 (SE = 3.60) g dry matter / kg BW^{0.75} for barley grain and protein supplement, respectively, with total feed intake of 93.2 (SE = 3.44) g dry matter / kg BW^{0.75}.

The present study indicated that only odd chain n-alkanes were present in a significant amount in the diet, and alkane C₃₁ was the most abundant alkane, followed by C₂₉ and C₃₃. Alkane C₃₁ is accounted for by 35 and 31% of the total for barley grain and protein supplement, respectively. However, even chain n-alkanes were also detected in feed ingredients but in a minor amount. Results are in agreement with Van Soest (1994) who reported that over 90% of n-alkanes have odd numbers of carbon atom, with C₂₉, C₃₁, and C₃₃ alkanes being dominant in most plant species. Therefore, these three alkanes were selected to be evaluated as a flow and digestibility markers.

The quantitative importance of C₂₉ and C₃₁ has been previously reported in concentrates (Piasentier *et al.*, 1995; Valiente *et al.*, 2003) and in forages (Dove and Mayes, 1991 and 1996; Dove, 1992; Pueyo *et al.*, 2005). In general, alkane content in concentrates is always less than that detected in forage (Mayes *et al.*, 1986; Valiente *et al.*, 2003) in which adding concentrates to the diet (mixed diet) leads to reduce faecal concentration of alkanes as a simple dilution effect (Malossini *et al.*, 1994).

Odd n-alkanes content (mg/kg dry matter) in diet and digesta flow of abomasums, ileum, and rectum are presented in Table (2). Concentrations of alkanes C₂₉, C₃₁, and C₃₃ was much higher in faeces and ranked as diet < abomasum and ileum < rectum. Increasing the concentration in faeces, than that found in abomasum, ileum, or diet, is logical and complies with the natural dilution of digesta through the gastrointestinal tract.

Cr-EDTA and YbCl₃ as reference and flow markers

The YbCl₃ was selected for the validation of n-alkanes as a flow marker because the recovery of Cr-EDTA in respect to Yb throughout the gastrointestinal tract was very low (P<0.01), particularly in abomasum and ileum as compared to rectum (0.30, 0.49, and 0.64 (SE = 0.028), respectively, Fig. 1). Moreover, YbCl₃ was selected because it is associated with the digesta solid fraction. This marker is assumed to adhere to the particulate phase of the digesta (Faichney, 1995). Therefore, n-alkanes are known to be associated to the solid phase (Mayes *et al.*, 1988, 1997; Marais *et al.*, 1992; Giráldez *et al.*, 2006). The contrary is true for Cr-EDTA which is associated with the liquid phase of digesta (Faichney, 1975;

Van Soest, 1994). Kotb and Luckey (1972) recommended that Cr is more suitable as a digestibility marker than as a passage marker. This is because Cr passes more rapidly from the rumen than coarse fiber and tends to be associated with the movement of the liquid fraction. On the other hand, about 4 – 5% of Cr-EDTA is absorbed by ruminants and excreted in the urine (Van Soest, 1994). This absorbed quantity increased at higher osmotic pressures obtained by feeding more rapidly fermentable matter (Dobson *et al.*, 1976) as it may be expected in the current study on lambs fed high concentrate diet.

Table (2). Odd n-alkanes content (mg/kg DM) in diet and digesta flow of abomasum, ileum, and rectum.

N-alkanes (mg/kg DM)	Diet (D)	Gastrointestinal tract sampling site		
		Abomasum (A)	Ileum (I)	Rectum (R)
C ₂₃	1.10 (SE = 0.028)	2.51 (SE = 0.092)	2.04 (SE = 1.305)	2.55 (SE = 0.161)
C ₂₅	2.26 (SE = 0.073)	4.20 (SE = 0.184)	4.23 (SE = 1.515)	4.01 (SE = 0.261)
C ₂₇	2.05 (SE = 0.044)	4.82 (SE = 0.201)	3.90 (SE = 0.189)	5.07 (SE = 0.331)
C ₂₉	6.65 (SE = 0.169)	14.44 (SE = 0.963)	10.55 (SE = 0.615)	16.46 (SE = 1.327)
C ₃₁	7.87 (SE = 0.214)	13.34 (SE = 0.497)	12.51 (SE = 0.657)	17.53 (SE = 1.165)
C ₃₃	2.19 (SE = 0.031)	5.71 (SE = 0.373)	5.88 (SE = 0.287)	8.47 (SE = 0.429)

Total tract digestibility by using Cr-EDTA was significantly ($P < 0.01$) different than that obtained by YbCl_3 (72.4 vs. 82.5% (SE = 0.93), for Cr-EDTA and YbCl_3 , respectively, Fig. 2). Total tract digestibility coefficients estimated by YbCl_3 were similar to those obtained in metabolic cages by Askar *et al.* (2006), on lambs fed similar concentrate diets and at similar age, while those of Cr were much lower. This lower digestibility reflected the lower recovery of Cr as shown in Fig. 1, and is in agreement with our adjacent study (Keli *et al.*, 2009) that reported 34% drop in Cr recovery in respect to Yb in sheep located in metabolic cages. The authors attributed this drop to the analytical procedure as reported by Vicente *et al.* (2004).

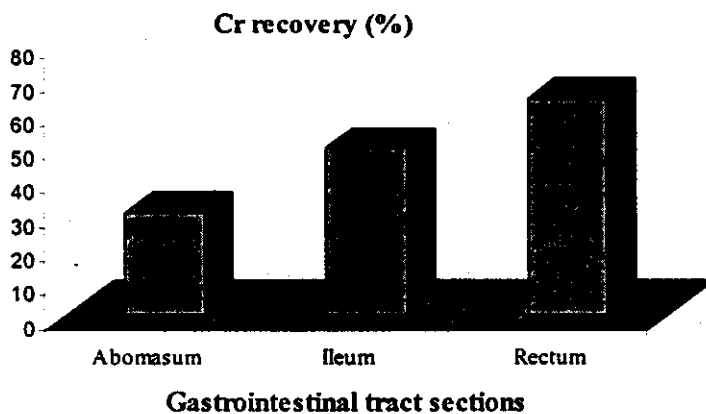


Figure (1). The recovery of Cr in respect to Yb throughout the gastrointestinal tract sections in lambs fed on high concentrate diet.

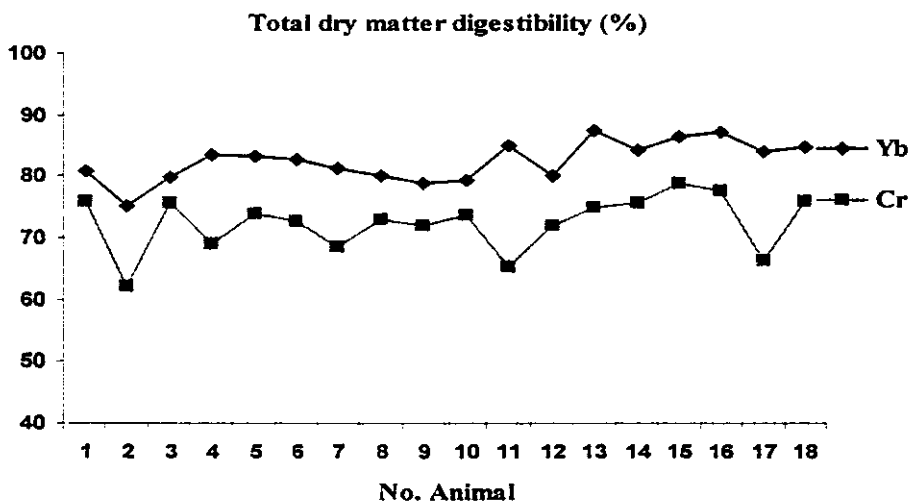


Figure (2). Total dry matter digestibility (%) estimated by Cr-EDTA and YbCl₃ in eighteen slaughtered lambs fed on high concentrate diet.

The present experiment also does not support the use of Cr-EDTA as flow marker with lambs fed high concentrate diet but it may be used as a digestibility marker after correction for its faecal recovery.

Long chain n-alkanes as flow and digestibility markers

Concentration of long chain odd n-alkanes, C₂₉, C₃₁ and C₃₃, in diet and gastrointestinal tract sections is related to that of Yb and presented in Table (3). From this relation, recovery of n-alkanes in respect to Yb is considered. Recovery was getting much higher in abomasum (1.62, 1.27, and 1.94 (SE = 0.064), P<0.01, for C₂₉, C₃₁, and C₃₃, respectively) in comparison with Yb. However, when Yb flow in rumen was adjusted for 0.05 rate of passage (AFRC, 1992; Askar *et al.*, 2006), recovery of C₃₁ was approximately similar to that of Yb, while those for C₂₉ and C₃₃ were still much higher (0.93 vs. 1.19 and 1.42 (SE = 0.050), P<0.01, respectively, Table 3).

Table (3). The relation between long chain n-alkanes (C₂₉, C₃₁ and C₃₃), and Yb in diet, and in digesta flow of abomasum, ileum, and rectum, and the recovery of n-alkanes in respect to Yb.

	Diet	Gastrointestinal tract sampling site			
	(D)	Abomasum (A)	Ileum (I)	Rectum (R)	
Relation between n-alkanes and Yb					
C ₂₉ / Yb	0.062	0.099	0.022	0.026	
C ₃₁ / Yb	0.073	0.092	0.026	0.028	
C ₃₃ / Yb	0.020	0.039	0.012	0.014	
Recovery in respect to Yb					
	A / D (0.05)*	A / D (0.00)	I / D	R / D	SE
C ₂₉ / Yb	1.19	1.62 ^a	0.36 ^b	0.43 ^b	0.067
C ₃₁ / Yb	0.93	1.27 ^a	0.36 ^b	0.39 ^b	0.034
C ₃₃ / Yb	1.42	1.94 ^a	0.60 ^b	0.67 ^b	0.081

^{a,b}Means without a common superscript letter in the row differ (P < 0.05) among digesta sites.

* (0.05) = Recovery of n-alkanes in abomasum, taking into consideration 5% rate of passage in rumen.

Concentrations of n-alkane in abomasum and ileum (Table 2), which is an indicator for alkane loss in between both sites, were found to be similar, in spite of the fact that they should be different. This was confirmed by a significant drop (P<0.01) in long chain n-alkanes recovery in ileum (1.61 vs. 0.44 and 0.50 (SE = 0.040) in abomasum vs. ileum and rectum, respectively, Table 3). This suggests that the major disappearance of n-alkanes has been occurred in between duodenum and terminal ileum. It is likely that the incomplete recovery is due to absorption from the small intestine as suggested by Mayes *et al.* (1988) who investigated the fate of natural alkanes in the gastrointestinal tract, using sheep fitted with cannulae. They concluded that little disappearance of alkanes occurred in the fore-

stomach and that absorption occurred mainly in the small intestine. Similar findings were reported by Ohajuruka and Palmquist (1991) and confirmed by Keli (2006).

Similar rumen digestibility observed using C₃₁ and Yb (Table 4) were observed, while C₂₉ and C₃₃ gave significant (P < 0.01) higher rumen digestibility values. This confirmed that C₃₁ can be used as a flow marker to estimate rumen digestibility and supported the previous results reported by Askar *et al.* (2005) on growing lambs fed similar concentrate diets. They attributed its use to that C₃₁ was the most abundant alkane in the diet and is the only alkane found in both barley grain and protein supplement, assuming its full recovery in duodenum (Mayes *et al.*, 1988; Ohajuruka and Palmquist, 1991). Moreover, higher recovery of alkanes in abomasums in respect to Yb suggests that rumen microflora does not metabolize alkanes as suggested by Dove and Mayes (1991). In vitro study by Keli *et al.* (2008) supported our results and demonstrated that there is neither synthesis nor degradation of n-alkanes by ruminal bacteria. They also reported, in coherent with the current study, that long chain n-alkanes can be confidently used as duodenal flow or rumen transit markers regardless of their incomplete faecal recovery. This supports the use of long chain n-alkanes as duodenal flow (Askar *et al.*, 2008) or as rumen transit markers (Giráldez *et al.*, 2004 and 2006; and Keli *et al.*, 2009).

Table (4). Rumen, post-ruminal, and total digestibility estimation in lambs fed high concentrate diet, using odd n-alkanes in comparison with YbCl₃.

Digestibility (%)	N-alkanes			Yb	SE
	C ₂₉	C ₃₁	C ₃₃		
Rumen	51.25 ^b	40.09 ^c	59.57 ^a	40.37 ^c	2.037
Rumen + intestine	35.32 ^c	36.20 ^c	61.72 ^b	78.77 ^a	2.078
Total	56.72 ^c	52.72 ^c	73.44 ^b	84.16 ^a	1.854

^{a,b,c}Means without a common superscript letter differ (P < 0.05) among markers.

No significant difference was observed between ileum and rectum in respect to long chain n-alkane recovery (Table 3). Faecal recovery of n-alkanes is much higher for C₃₃ compared to those for C₃₁ and C₂₉ (0.67 vs. 0.39 and 0.43 (SE = 0.064) in rectum, respectively). Mayes *et al.* (1986), Dove and Oliván (1998), and Keli (2006) reported that when the carbon-chain length increases, the faecal recovery of n-alkanes increases. Recovery values were reflected in faecal digestibility (Table 4) in which digestibility values obtained by C₃₃ was about only 13% reduction than those obtained by Yb. The present study suggested the use of long odd chain n-alkane as total tract digestibility marker after correction for their faecal recovery. Askar *et al.* (2003) validated the use of odd long chain n-alkanes as a digestibility marker in growing lambs fed similar diets. They indicated that no significant differences were found between estimated digestibility using odd long chain alkane and the observed ones by total faecal collection. Findings are in agreement with those reported by Ordakowski *et al.* (2001) who indicated that accurate estimates of digestibility in horse can be obtained by using individual odd chain alkane or by using all plant wax alkane after adjusting for the mean recovery of odd chain alkane. They also indicated that digestibility values were not significantly differed than those observed by total collection.

Experiment 2

Effect of sodium bicarbonate

Intake and digestibility and metabolism through the gastrointestinal tract

The first experiment confirmed that long chain n-alkanes, particularly C₃₁, can be used as abomasal flow markers but its recovery was significantly dropped in between abomasum and ileum which will not allow its use to estimate the post-ruminal digestibility. In this regard, the second experiment was dealing with using YbCl₃ as a flow marker, avoiding the use of alkane, to study the effect of SB on metabolism and digestion throughout the gastrointestinal tract.

Effect of adding SB on dry (DM) and organic (OM) matter intakes for last 7 days before slaughter together with rumen, post rumen metabolism and digestion, using YbCl₃ as a flow marker, are presented in Table (5). Similar amount of DM, OM, and crude protein intakes were observed with both treatments. This indicated that lambs were intake similar amounts of barley grains and protein supplement in both treatments. Also, results indicate that SB did not affect the protein intake that was 218 and 214 g protein per kg DM intake in treatments 0 and 30, respectively. Similar results were obtained by Askar *et al.* (2002) in lambs fed similar high concentrate diets with or without SB, either incorporated in the diet or presented as free access on the side in an independent trough. Moreover, Mandebvu and Galbraith (1999) and Santra *et al.* (2003) observed that inclusion of SB in lambs fed on high concentrate diet did not affect either DM intake or plane of nutrition.

Abomasal flow of DM tended ($P < 0.10$) to be increased, while that of OM was significantly ($P < 0.05$) increased by SB addition. This was reflected in rumen digestibility that was negatively ($P < 0.05$) affected by SB addition. The negative effect of SB on DM and OM digestibility in rumen is probably due to increasing rumen rate of passage and dilution rate (Harrison *et al.*, 1975; Newbold *et al.*, 1991; Isaacson *et al.*, 1975; Owens and Goetsch, 1986). Results were supported by increasing the flow of nutrients to abomasum that is probably enhanced post rumen digestion as shown in Table (5). This is in agreement with the findings of Okeke *et al.* (1983) and Kovacik *et al.* (1986) who reporting that SB enhanced dietary nutrients bypassing rumen and may decrease their degradability. Kawas *et al.* (2007) suggested a reduction in rumen digestibility by SB inclusion in a diet as a result of increased NDF excretion and therefore reduce its total digestibility, in addition to lower acetate to propionate ratio. However, rumen digestibility values obtained in the current study by YbCl₃ were similar to those obtained by using C₃₁ as flow marker on lambs fed similar diets (44 – 46%, Askar *et al.*, 2008) which confirmed the results of the first experiment dealing with the validation of C₃₁ as a rumen digestibility marker.

Small intestine digestibility of DM and OM was positively ($P < 0.05$) affected by SB addition. This was probably enhanced by increasing flow of nutrients going out from rumen to small intestine as explained above (Table 5). Similar digestibility values between treatments were observed up to ileum for DM, 77.1 vs. 79.0 (SE = 2.24) and OM, 80.9 vs. 82.2 (SE = 1.33), respectively for treatments 0 and 30, in which the negative effect of SB on rumen digestibility was compensated in higher digestibility values in small intestine (Table 5). At the same trend, post rumen digestibility increased ($P < 0.01$) by adding SB, while faecal digestibility was quite similar. Little information is known concerning the effects of SB on small intestine or post rumen digestibility in growing lambs fed high

concentrate diet, while its effect on total tract digestibility has been reported. In this regard and in agreement with the present results, Santra *et al.* (2003) and Tripathi *et al.* (2004) found that SB inclusion had no effect on total tract digestibility of dry and organic matter in lambs fed high concentrate diet. Similar findings were reported by Kovacik *et al.* (1986) and Leventini *et al.* (1990) with lambs fed 50% concentrate in diet.

Nitrogen utilization

Nitrogen intake was similar between treatments and SB did not have any effect on non ammonia nitrogen (NAN) reached abomasum (Table 5). The current results indicated that

Table (5). Intake, flow, efficiency of microbial protein synthesis, apparent rumen nitrogen degradation, and digestion throughout the gastrointestinal tract in slaughtered lambs fed on high concentrate diet with or without adding sodium bicarbonate by using YbCl₃ as a flow marker and purine bases as microbial marker.

Item	Sodium bicarbonate (g/kg protein supplement)		SE	Significant
	0	30		
Intake, g / day				
DM	1071	1085	45.0	
OM	984	998	40.9	
N	37.4	37.1	2.87	
Abomasal flow, g / day				
DM	591	663	29.3	Ns
OM	513 ^b	594 ^a	26.3	*
NAN	19.3	19.3	2.33	
Microbial N	13.2	12.9	1.34	
Rumen digestion, %				
RDMD	44.7 ^a	39.0 ^b	1.72	*
AROMD	47.8 ^a	40.4 ^b	2.15	*
TROMD	62.4 ^a	55.3 ^b	2.58	Ns
Efficiency of MP				
g N / kg AROMD	28.3	32.5	3.17	
g N / kg TROMD	21.5	23.4	1.79	
Rumen N degradation, %	83.8	82.8	4.14	
Small intestine digestion, %				
DM	32.3 ^b	40.0 ^a	1.98	*
OM	33.0 ^b	41.9 ^a	1.50	**
Post-rumen digestion, %				
DM	38.2 ^b	43.6 ^a	1.86	Ns
OM	37.2 ^b	43.6 ^a	2.18	Ns
Total digestion, %				
DM	82.9	82.5	1.23	
OM	85.0	84.0	1.13	

^{a,b,c}Means without a common superscript letter differ (P < 0.05).

Ns = not significant (P<0.05), * = Significant (P<0.05), ** = Significant (P<0.01)

DM = Dry matter; OM = Organic matter; NAN = Non-ammonia nitrogen; RDMD = Rumen dry matter digestibility; AROMD and TROMD = Apparent and true rumen organic matter digestibility.

efficiency of rumen N utilization was very low in both treatments and was not affected by SB, resulting in losing almost 48% of N intake in rumen and recovered only 52% as NAN in abomasum. This inefficient N utilization justifies the lamb selection of diets rich in

protein in both treatments, although rumen N degradation was high (Table 5). Similar findings were reported by Rodríguez *et al.* (2007) and Askar *et al.* (2008) on lambs fed similar concentrate diet at free choice. They found that amount of NAN reached the abomasum (53% of N intake, Askar *et al.*, 2008) is very close to the predicted metabolizable protein (MP) required for the observed average daily gain (AFRC, 1993). This suggests that diets rich in protein were selected to cover their MP requirements as mentioned by Askar *et al.* (2008). However, protein could be selected as a source of rumen degradable N (Tolkamp *et al.*, 1998) but high rumen N degradation observed, in the present study, seems to not support this hypothesis. A high rumen N degradation has been recently reported by Askar *et al.* (2008) in lambs fed similar concentrate diets. This high degradation was associated with high rumen ammonia concentration which is supported the results of present study. Also, James *et al.* (2001) suggested that sheep does not seem to regulate their intake to cover the required rumen degradable N. On the other hand, protein can be also selected as a buffer to reduce acidosis as suggested by Phy and Provenza (1998) but similar protein intake observed between treatments, with or without SB in the current study, does not support this hypothesis.

Inclusion of SB has been reported to enhance rumen microbial protein synthesis in ruminant fed high concentrate diets (Chamberlain and Thomas, 1980; Mees *et al.*, 1985) and to enhance rumen bacterial fixation of ammonia (Newbold *et al.*, 1991). This is due to its effect on increasing rumen rate of passage (Harrison *et al.*, 1975) and dilution rate (Newbold *et al.*, 1991; Harrison and McAllan, 1980; AFRC, 1992). In both treatments, the efficiency of microbial protein synthesis was similar between treatments and only numerically increased by SB addition (Table 5). In general, values for the efficiency of microbial protein observed were lower than those reported for mixed diets (ARC, 1980). This supports other results with sheep (Askar *et al.*, 2008) and cattle (Beauchemin *et al.*, 2001; Vicente *et al.*, 2004a) fed high-concentrate diets.

Results of the second experiment together with the previous work of Askar *et al.* (2008) and Rodríguez *et al.* (2007) concluded that lambs fed on high concentrate diet preferred diets rich in protein and have high rumen N degradation with low rumen N utilization and less efficiency of microbial protein synthesis. The current study suggests the use of less N degradation source as a solution to stop selecting diets rich in protein by growing lambs fed on high concentrate diet.

CONCLUSIONS

Results of the first experiment showed that long chain n-alkanes, particularly C₃₁, can be used as a rumen digestibility marker. Long chain n-alkanes and Cr-EDTA can be used as total tract digestibility markers after correction for their faecal recovery but the use of Cr-EDTA as a flow marker is not preferred. Experiment two concluded that SB can manipulate the site of nutrients digestion throughout the gastrointestinal tract but does not seem to regulate protein feed intake in lambs according to their recommended requirements. Inclusion of SB has a negative effect on rumen digestibility and enhances small intestine digestibility. Lambs fed high concentrate diet have low efficiency of rumen N utilization and microbial protein synthesis which lead to increase protein selected in diet

to cover the required metabolizable protein in small intestine. The second experiment suggests the use of less protein degradation source as a solution to stop selecting diets rich in protein by growing lambs fed high concentrate diet.

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جدوى استخدام الألكانات كمرقم غذائي لتقدير معدل المرور وتدفق البلعة الغذائية والهضم وتأثير بيكربونات الصوديوم على مكان الهضم والتمثيل الغذائي بطول القناة الهضمية في الأغنام

أحمد رجب عسكر

قسم تغذية الحيوان والدواجن بمركز بحوث الصحراء

الدراسة الحالية مكونه من تجربتين الأولى تهدف لدراسة جدوى استخدام الألكانات طويلة السلسلة الكربونية كمرقم غذائي لتقدير معدل المرور وتدفق البلعة الغذائية والهضم الجزئي بطول القناة الهضمية والثانية تهدف لدراسة تأثير بيكربونات الصوديوم على الهضم والتمثيل الغذائي بالكرش وبعد الكرش بطول القناة الهضمية في الحملان المغذاة على علائق مركزة. تم استخدام ثمانية عشر حمل وأثني عشر آخرين من سلالة Raza Aragoneza للتجربة الأولى والثانية على التوالي. الحملان في التجريبتين تم نقلهم الى حظائر فردية مباشرة بعد الانتهاء من فترة تسمين (٢٤ كيلوجرام وزن حي) مع الاستمرار في التغذية الفردية الحرة على نفس العليقة من حبوب الشعير الكاملة والمكمل البروتيني. كل تجربة استمرت ٣ اسابيع من التغذية الفردية مع تسجيل المقدم والمرفوض وحساب المأكول اليومي. تم ذبح الخراف بعد ٧ ايام من اعطائها جرعات مستمرة من المرمقات التقليدية من Cr-EDTA و $YbCl_3$ (فقط) $YbCl_3$ في التجربة الثانية).، وذبحت الحيوانات بعد ٣ ساعات من تقديم العليقة في الصباح وتم تحديد وفصل مقاطع أو غرف القناة الهضمية وأخذ عينات من محتواها. نتائج التجربة الأولى تشير الى أن الألكان C31 هو السائد بالعليقة من حيث الكمية والتركيز ويليها C29 و C33. أن معدل التغطية (Recovery) للألكانات طويلة السلسلة (C29, C31, and C33) بالنسبة لل Yb كانت عالية جدا في المعدة الحقيقية وانخفضت بشكل ملحوظ في نهاية الامعاء الدقيقة (اللفانفي) و المستقيم مما أثر بشكل معنوي على معاملات الهضم الجزئي بطول القناة الهضمية الناتجة من استخدام الألكانات. استخدام الألكان C31 أعطى معاملات هضم بالكرش مماثلة لتلك المتحصل عليها من ال Yb ، بينما استخدام C29 و C33 سجلوا قيما أعلى. الهضم بالأمعاء النقيية كان منخفض جدا مع استخدام الألكانات بالمقارنة بال Yb . الألكان C33 سجل أعلى معدل تغطية في الروث (Fecal recovery) مقارنة بال C29 و C31. في التجربة الثانية تم استخدام معاملتين، مع أو بدون بيكربونات الصوديوم. اضافة البيكربونات لم تؤثر على المأكول من المادة الجافة والمضوية والنتروجين ولكن أثرت بالسلب على معدل هضم المواد الغذائية بالكرش وبالإيجاب على معاملات الهضم بالأمعاء الصغيرة. كفاءة استخدام النيتروجين وكفاءة بناء البروتين الميكروبي بالكرش كانت متدنية ولم تتحسن باضافة البيكربونات للعليقة، مما أدى إلى فقد حوالي ٤٨٪ من النيتروجين المأكول بالكرش. معدلات هدم النيتروجين بالكرش كانت عالية ولم تتأثر باضافة البيكربونات.

النتائج المتحصل عليها تشير إلى أن الألكانات طويلة السلسلة، وخاصة C31، يمكن أن تستخدم كمرقم غذائي لتقدير الهضم بالكرش ولا توصى باستخدام Cr-EDTA كمرقم غذائي لتقدير معدل المرور وتدفق البلعة الغذائية. Cr-EDTA والألكانات طويلة السلسلة الكربونية يمكن استخدامها كمرقم لتقدير الهضم الكلي بعد الأخذ في الاعتبار معدل التغطية بالروث (Fecal recovery). اضافة بيكربونات الصوديوم يمكن أن تعدل أو تغير من مكان الهضم بطول القناة الهضمية ولكن لا يبدو أن لها القدرة في تنظيم المأكول من البروتين. الدراسة الحالية تقترح استخدام مصدر بروتيني ذات معدل هدم قليل بالكرش كحل لوقف تقصير أو اختيار الحيوان للعلائق الغنية بالبروتين في الحملان المغذاه على علائق مركزة.