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Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep [1]

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Summary — The purpose of this study was to compare the bioavailability of either multiple or single oral supplementation of different formulations of vitamin C and intra-duodenal supplementation of one form of vitamin C in sheep. Formulations used in the study were (1) ascorbic acid fine powder (AA); (2) ascorbic acid coated with ethyl cellulose (EC); (3) Rovimix STAY-C (SC); (4) sodium ascorbate (SA); (5) Rovimix C (RC). The bioavailability of vitamin C formulations was assessed by the changes in plasma ascorbic acid concentrations, area under the curve (AUC) and area under the curve above its basal concentration (AUC_{above}) values. There was no effect of single oral supplementation on bioavailability of vitamin C. Multiple dosing over a period of 28 days of oral supplementation of all five formulations resulted in higher AUC_{above} values. Furthermore, multiple oral supplementation of RC increased plasma concentrations of ascorbic acids and AUC values. Single intra-duodenal supplementation of ascorbic acid resulted in significantly higher AUC when compared with oral supplementation of the same vitamin C.

vitamin C / oral supplementation / bioavailability / sheep

Résumé — Biodisponibilité des différentes formulations de vitamine C administrée chez le mouton par voie orale ou par voie duodénale. Le présent essai avait pour objectif d'étudier différentes formulations de la vitamine C, soit en dose unique, soit en doses multiples chez le mouton, et cela pendant 28 jours. Deux expériences ont été effectuées à cet effet. L'expérience 1 a consisté à tester cinq formulations différentes de vitamine C à raison de 4 g/jour par voie orale pendant 28 jours. Pour chaque formulation, il y a eu cinq moutons traités et cinq autres moutons servant de contrôle. L'expérience 2 a consisté à tester différentes formulations en dose unique de 4 g par voie orale ou par voie duodénale. Les formulations utilisées étaient l'acide ascorbique, l'acide ascorbique protégé par

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une couche d'éthyl cellulose, le Rovimix-Stay, l'ascorbate de sodium et le Rovimix C. Les biodisponibilités ont été estimées par les concentrations plasmatiques, les dimensions des aires sous la courbe des concentrations plasmatiques (AUC), et AUC au-dessus du niveau de base. Il ressort de cette expérience 1 que l'administration des différentes formulations en doses multiples par voie orale eut en général pour effet d'augmenter les valeurs plasmatiques de la vitamine C ainsi que les aires sous la courbe de concentration plasmatique de la vitamine C. Dans l'expérience 2, on n'a observé aucune différence significative sur la biodisponibilité entre les différents traitements administrés. L'examen des données indique clairement que le groupe traité par voie duodénale a eu des AUC plasmatiques de vitamine C largement supérieures à celles des autres groupes traités par voie orale.

vitamine C / supplémentation / biodisponibilité / mouton

INTRODUCTION

L-ascorbic acid is biosynthetically formed in almost all mammals studied except in man, several other primates and guinea pigs (Hornig, 1975). Calves (Palludan and Wegger, 1984) and perhaps lambs do not synthesize endogenous vitamin C until approximately 3 weeks of age, making them dependent on dietary vitamin C during this period. Vitamin C is a known antioxidant and thereby protects the structural integrity of the cells of the immune system (Bendich, 1993). The amount of vitamin C required to enhance immune responses in ruminants may be many times greater than that provided in normal intakes for nutritional needs. Therefore, some form of vitamin C supplementation may be beneficial to ruminants, especially newborns, to enhance resistance against infectious diseases.

Few data are available on the concentration of ascorbic acid in plasma following oral administration of vitamin C to sheep. This is due to the fact that rapid and pronounced destruction in the rumen of ingested ascorbic acid was reported as early as 1940 (Knight et al, 1941). Further, the ascorbic acid in feed is unstable and is broken down especially by heavy metal ions. Several stable ascorbic acid preparations, ethyl cellulose coated ascorbic acid (EC), Rovimix STAY-C (SC), sodium ascorbate (SA), and Rovimix C coated with silicone (RC) have recently been approved for use in feed mixtures. Although these preparations are more stable in feed compared to ascorbic acid, their resistance to ruminal degradation is unknown. The highest increase in the blood plasma concentration of ascorbic acid in 1- and 2-week old lambs was recorded after 5 h of oral administration of ascorbic acid at the rate of 50 mg/kg BW (Kolb et al, 1993). The objective of this study was to compare the bioavailabilities of different formulations of vitamin C following either multiple or single dosing in sheep. In addition, oral and duodenal supplementation of ascorbic acid were compared to determine the degradability of vitamin C in the rumen.

MATERIALS AND METHODS

Fifty Canadian Arcott breed wethers (45 to 50 kg BW; 1-year old) were used in the multiple dosing experiment, while 30 sheep of similar breed were used in the single dosing experiment. Management practices and diets were as reported earlier (Hidiroglou and Batra, 1996). All sheep were fed ad libitum a diet consisting of 40% timothy and alfalfa silage, 40% hay, and 20% corn silage (DM basis). The diet contained 15.2% CP, 1.18% crude fat, 38.8% ADF, 0.89% Ca, 0.28% P, and 0.24% Mg (DM basis). The animals were housed in individual pens (1.3 m^2) in a barn with controlled temperature maintained at 21 ± 1 °C. Feed and water were provided ad libitum. Animals were cared for according to the guidelines developed by the Canadian Council of Animal Care.

Experiment 1 tested the effect of multiple oral supplementation of different formulations of vita-

min C. Five different vitamin C formulations, at the rate of 4 g of ascorbic acid equivalent per day were fed orally during a 28-day period. Blood samples were taken at 0 , 4 and 7 h, and 3, 7, 10, 14, 17, 21, 24 and 28 d following the administration of vitamin C. The vitamin formulations were provided by Hoffmann-La Roche Inc and consisted of: (1) ascorbic acid fine powder (AA); (2) ascorbic acid coated with ethyl cellulose, Type EC (EC); (3) Rovimix STAY-C (SC); (4) sodium ascorbate (SA); (5) Rovimix C (RC). The AA is a fine white or slightly yellow odorless powder with a tart taste. This product contains a minimum of 99% ascorbic acid and is stable in dry state. The EC is an ethyl cellulose coated form of ascorbic acid. It is a free flowing powder containing a minimum of 97.5% ascorbic acid. The SC is a fine powder containing mono-, di- and tri-phosphate esters of L-ascorbic acid in a suitable carrier. It provides a minimum of 15% ascorbic acid by weight equivalent to 150 g of ascorbic acid per kg of DM. This mixture is specially formulated for use as a stabilized source of vitamin C in feeds of all animal species. The RC is a white or slightly yellow powder and contains 96% ascorbic acid. This product is coated with silicone.

Experiment 2 was conducted to determine the effect of single oral supplements of five different formulations of vitamin C and intra-duodenal supplementation of one form of vitamin C (AA). The formulations of vitamin C used in experiment 2 were the same ones tested in experiment 1. A single dose of each formulation equivalent to 4 g of ascorbic acid in capsule form was given to five sheep. Blood sample collection started at 8.00 am and were taken at 0, 10 and 30 min, once every hour up to 7 h, and 24 h after vitamin C supplementation.

Blood samples were centrifuged and plasma was separated. Plasma samples were immediately mixed with 2.55% metaphosphoric acid in the ratio of 1:2. The sample mixture was frozen at – 70 °C until analysis within 1 week. The ascorbic acid concentrations in samples were measured by high performance liquid chromatography using an electrochemical detector (Behrens and Nadere, 1987).

The data on vitamin C concentrations in plasma were analyzed by repeated measure analysis of variance using the SAS general linear model procedure (SAS, 1985). The following linear model was used:

$$Y_{ijk} = \mu + T_i + S_k(Ti) + Dj + (TD)_{ij} + e_{ijk}$$

where Y_{ijk} is plasma vitamin C concentration; μ is the overall mean; T is the effect of treatment; S(T) is the effect of sheep within treatment; D is the time of sample collection; (TD) is the interaction of treatment with time; and e is the error term.

Area under the curve (AUC) values is considered to be the best parameter to assess the bioavailability of the product. AUC was calculated using the trapezoidal rule applied to the observed data (Gibaldi and Perrier, 1982). Area under the curve above its basal concentration (AUC_{above}) was calculated for each animal in order to use each sheep's control concentration as its own reference value. One-way analysis of variance was used for AUC data. The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y is the AUC; μ is the overall mean; T is the effect of treatment; and e is the error term.

RESULTS

The average plasma vitamin C concentration and standard deviation during the 28 days of multiple dosing of different formulations are shown in table I. Only the RC group significantly increased plasma concentration of vitamin C compared with the control group. Multiple comparisons performed by the LSD method showed that group RC was significantly ($P < 0.05$) different from other groups. No differences existed among other groups (table I).

As shown in table I, the control group had an AUC_{above} close to 0, suggesting that the average concentration over 28 days was equal to the basal concentration. In contrast, all five treatments resulted in higher AUC_{above} values, indicating that all five formulations increased plasma vitamin C concentrations.

AUC and AUC_{above} values between 0 and 7 h after single oral dosing of different formulations are reported in table II. There were no differences between the five formulations supplemented orally. However,

Table I. Means and standard deviations of plasma concentration (μg/mL) and AUC (ug/d/mL) of vitamin C in supplemented and control groups.

Group	*Plasma*		*AUC*		AUC_{above}	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
AA	8.19[a]	1.03	233[a]	28	34.1[a]	13.7
EC	6.83[a]	1.14	195[a]	31	20.7[a]	7.9
SC	7.48[a]	0.72	213[a]	16	27.8[a]	10.0
SA	7.70[a]	0.95	222[a]	23	23.7[a]	13.9
RC	10.60[b]	2.08	304[b]	61	47.2[a]	25.2
Control	7.38[a]	1.53	207[a]	43	0.0[b]	8.1

[a, b] Means in the same column with different superscripts are different ($P < 0.05$).
AUC = area under the plasma vitamin C concentration curve, AUC_{above} = area under the plasma vitamin C concentration curve above its basal concentration, AA = ascorbic acid fine powder, EC = ethyl cellulose coated ascorbic acid, SC = Rovimix STAY-C, SA = sodium ascorbate, RC = Rovimix C coated with silicone.

Table II. Means and standard deviations for AUC and AUC_{above} during 0 to 7h (μg/h/mL) for a single oral and intra-duodenal administration of vitamin C.

Pathway	*Product*	*AUC*		AUC_{above}	
		Mean	*SD*	*Mean*	*SD*
Oral	AA	2.46[a]	0.38	0.35[a]	0.28
	EC	2.42[a]	0.44	0.15[a]	0.07
	SC	2.03[a]	0.55	0.20[a]	0.12
	SA	2.08[a]	0.23	0.09[a]	0.18
	RC	2.28[a]	0.46	0.38[a]	0.17
Intra-duodenal	AA	4.60[b]	1.37	2.58[b]	1.31

[a, b] Means in the same column with different superscripts are different ($P < 0.05$).
AUC = area under the plasma vitamin C concentration curve, AUC_{above} = area under the plasma vitamin C concentration curve above its basal concentration, AA = ascorbic acid fine powder, EC = ethyl cellulose coated ascorbic acid, SC = Rovimix STAY-C, SA = sodium ascorbate, RC = Rovimix C coated with silicone.

intra-duodenal supplementation of ascorbic acid fine powder resulted in significantly higher AUC and AUC_{above} values compared with oral supplementation of the same form of vitamin C.

DISCUSSION

The dosage and route of vitamin C administration need to be determined before vitamin C can be supplemented to overcome nutritional deficiencies and enhance immune function. Although it has been known since 1940 (Knight et al, 1941) that ruminal fluid has a strong destructive effect on L-ascorbic acid, the exact species of microorganisms involved is yet to be identified. Despite extensive loss of L-ascorbic acid in the rumen, oral supplementation is still preferred to other routes. Intravenous administration is effective but labour-consuming. Subcutaneous and intramuscular administration of ascorbic acid are also efficient,

but could cause marked local irritation at the injection sites, as observed in horses (Löscher et al, 1984).

Bioavailability of vitamin C from different formulations has been compared before on animals other than sheep. Ascorbyl palmitate gave both highest plasma concentration and greatest AUC for ascorbic acid, when compared to a similar dosage of ascorbyl palmitate, ascorbyl stearate and formulated ascorbic acid in horses (Snow and Frigg, 1987). On the other hand, Johnston and Luo (1994) found no differences in relative bioavailability of three commercially available vitamin C tablets (ascorbic acid, ester-C, and ascorbic acid with bioflavonoids) in humans. There is a limited absorption of ascorbic acid at high dose levels and levels of ascorbic acid in the tissues cannot be increased appreciably by ingesting large doses of vitamin C (Rivers, 1987). Supplementation of crystalline L-ascorbic acid and L-ascorbyl-2-polyphosphate in broiler chicken diet and drinking water found no consistent differences in plasma ascorbic acid concentrations, relative to vitamin source (Pardue et al, 1993).

In this study, no increase in plasma ascorbic acid concentrations or AUC was observed after single oral supplementation of five different formulations. Only multiple oral supplementation of RC significantly increased plasma concentrations of ascorbic acids and AUC values. Furthermore, all five formulations resulted in higher AUC_{above} values, relative to the control group. These differences between single and multiple supplementation might reflect differences in cellular uptake of vitamin C by tissues. In humans, plasma ascorbic acid concentrations were directly correlated to tissue ascorbic acid values prior to tissue saturation (Johston and Luo, 1994). In this study, it is very likely that sufficient storage of ascorbic acids in tissues prior to supplementation did not occur and this would compromise the effect of single oral vitamin C supplementation, when judged only by changes in plasma ascorbic acid concentrations. On the other hand, multiple oral supplementation may saturate tissues at least partially with ascorbic acids and subsequently elevate plasma ascorbic acid levels. The increase in AUC_{above} values after multiple oral supplementation of five formulations supports this assumption. Concentration of ascorbic acid in plasma in the control group was lower than reported for sheep (Richetti, 1987), but was similar to the one reported for calves just after birth (Hidiroglou et al, 1995). Thus, ascorbic acid supplementation that fails to significantly elevate plasma ascorbic acid should not be easily dismissed as biologically insignificant. Based on our results, it seems that RC was more effective in increasing plasma ascorbic acid concentrations and AUC values compared with other vitamin C formulations. Supplementation of 80 g of ascorbyl-2-polyphosphate or 20 g ascorbic acid equivalent for 31 days to dairy cattle significantly increased plasma levels of ascorbic acid over the control animals (MacLeod et al, 1996). They suggested that ascorbyl-2-polyphosphate was a rumen stable source of ascorbic acid supplementation.

In summary, single oral supplementation of five different formulations of vitamin C did not result in a significant increase in plasma vitamin C concentration. Comparison between single oral and duodenal supplementation supports the notion that vitamin C is quickly destroyed in the rumen. Multiple oral supplementation of five formulations of vitamin C increased AUC_{above} values suggesting that gastrointestinal absorption of ascorbic acid in sheep is efficient, if ascorbic acid can reach the duodenum. Therefore, supplementation of rumen-protected ascorbic acid is feasible for use in ruminants.

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