Polyunsaturated fatty acid type and ratio in lipid supplements alter the *in vitro* dry matter digestibility of kikuyu grass (*Cenchrus clandestinus*)

JuliÃ¡n A C Vargas and Martha Olivera A

[1] **Grupo de InvestigaciÃ³n en NutriciÃ³n Animal, Universidad Nacional de Colombia, Carrera. 30 No. 45-03, BogotÃ¡ D.C, Colombia**
jcastillov@unal.edu.co
[2] **Grupo de InvestigaciÃ³n BiogÃ©nesis, Universidad de Antioquia, Carrera.75 No. 65-87, MedellÃn, Colombia.**

Abstract

Two experiments were conducted to investigate the effects of polyunsaturated fatty acid (PUFA) type and ratio in lipid supplements on the *in vitro* dry matter digestibility (IVDMD) of kikuyu grass. For this purpose, kikuyu grass (*Cenchrus clandestinus*) was incubated into rumen inoculum with a) 18 ÂµL of linoleic (LA) and alpha-linolenic (LN) acid mixtures (LA:LN = 100:0, 75:25, 50:50, 25:75 or 0:100) (Experiment 1), or b) 18 ÂµL of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acid mixtures (DHA:EPA = 100:0, 75:25, 25:75, and 0:100) containing a constant LA:LN 75:25 ratio (experiment 2). Differences between treatments were analyzed as a completely randomized design using the MIXED procedure of SAS. Also, for experiment 1, IVDMD was regressed to the ratio between LA and LN concentrations (LA/LN) in the incubation systems at 0 h of digestion, using the REG procedure of SAS.

In experiment 1, the supplementation with LA pure and mixed with LN in LA:LN= 75:25, 50:50, and 25:75 ratios, decreased by 3.4% the IVDMD of kikuyu grass, whereas the supplementation with LN pure, increased by 3.1% the IVDMD ($P < 0.01$). Also, a polynomial model was built to predict the IVDMD from the LA/LN ratio into incubation systems ($P < 0.01$): IVDMD = - 0.118 Ã— $(LA/LN)^3$ + 1.45 Ã— $(LA/LN)^2$ – 4.81 Ã— (LA/LN) + 69.7. In experiment 2, the supplementation with a 75:75 LA:LN mixture combined or not with 100:0, 75:25, and 25:75 DHA:EPA mixtures, decreased by 4.2% the IVDMD of kikuyu grass, while the supplementation with EPA pure decreased by 2.3% the IVDMD ($P < 0.01$). These results suggest that LA and DHA may be more detrimental than LN and EPA during the ruminal dry matter digestibility of kikuyu grass. Also, our study indicated that the IVDMD of kikuyu grass can be predicted, from the ratio between LA and LN concentrations in the incubation systems at 0 h of digestion. This information may be used for optimizing the design of lipid supplements in ruminant nutrition.

Keywords: *docosahexaenoic acid, eicosapentaenoic acid, forage, linoleic acid, linolenic acid, ruminal digestion*

Introduction

Forages form a major part of ruminant diets in most animal production systems (Van Soest 1994; Glasser et al 2013). However, they are commonly supplemented with lipids to enhance the energy density of rations in ruminants (Coppock and Wilks 1991).

In the last years, the use of vegetable (VOs) and fish (FOs) oils as lipids supplements under grazing conditions has increased, due to VOs are rich in linoleic (c9, c12-18:2; LA) and ?-linolenic (c9, c12, c15-18:3; LN) acids, while FOs are rich in docosahexaenoic (c4, c7, c10, c13, c16, c19-22:6; DHA) and eicosapentaenoic (c5, c8, c11, c14, c17-20:5; EPA) acids (Palmquist 1996; Harfoot and Hazlewood 1997; Jenkins et al 2008). A fraction of LA, LN, DHA, and EPA is biohydrogenated by ruminal microorganisms (Harfoot and Hazlewood 1997), and the other fraction is transferred to milk and meat, improving the nutritional quality of ruminant derived products (Bauman et al 1999; Chilliard et al 2001).

Although the effect of distinct levels of VOs and FOs on forage digestibility has been evaluated (Scholljegerdes et al 2004; Jal? et al 2009; Duckett and Gillis 2010), there is a knowledge gap about the effects of LA, LN, DHA, and EPA pure or mixed on the dry matter digestibility of forages. Therefore, we investigated the effects of polyunsaturated fatty acid (PUFA) type (i.e., LA, LN, DHA, and EPA) and ratio in lipid supplements on the *in vitro* dry matter digestibility (IVDMD) of kikuyu grass.

Material and methods

All procedures were approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Science at Universidad Nacional de Colombia (Act 01 – 2010).

Laboratory procedures

Samples of kikuyu grass (*Cenchrus clandestinus*) with 60 days of regrowth were harvested by Hand Plucking (Cook, 1964). Forage samples were dried at 60Â°C, ground to pass a 1-mm sieve, and analyzed for crude protein (182 g/kg DM; AOAC 2006, method: 984.13), ash (117 g/kg DM; AOAC 2006, method: 942.05), and ether extract (30.0 g/kg DM; AOAC 2006, method: 930.09). Neutral detergent (609 g/kg DM) and acid detergent fibers (322 g/kg DM) were determined, according to Van Soest et al (1991) and Goering and Van Soest (1970) methods, respectively.

One rumen fistulated Holstein steer maintained on a diet of kikuyu was used as the donor of rumen fluid. The rumen fluid was taken before morning feeding, strained through two layers of cheesecloth into a flask and kept under CO_2 gas at 39Âº C, until used. The incubations were performed using 100 mL tubes containing 500 mg of kikuyu grass (W_1) supplemented with: a) 18 ÂµL of LA:LN mixtures (100:0, 75:25, 50:50, 25:75 or 0:100) (Experiment 1; n = 18) or, b) 18 ÂµL of DHA:EPA mixtures (100:0, 75:25, 25:75, and 0:100) containing a constant LA:LN 75:25 ratio (experiment 2; n = 18). Each tube was filled with 50 mL of rumen inoculum (1:4 rumen fluid: buffer McDougall proportion; McDougall 1948), gassed with CO_2, and sealed with one hole rubber stoppers (Fisherbrand™). Then, the tubes were incubated in a water bath (Blue Sland Illinois, USA) at 39ÂºC during 48 h, with occasional shaking (Tilley and Terry 1963). In both experiments, extra kikuyu tubes without the addition of PUFA mixtures were assigned as the control treatment (CT). For each treatment, three replications were prepared.

At the end of 48 h, 6 mL HCl (20%) and then 5 mL of pepsin solution (0.5 g pepsin dissolved in 100 mL HCl 0.1 N) were added gradually to each tube. Then, the tubes were incubated at 39ÂºC for 24 h. Finally, the contents were filtered, and the residues were dried at 55ÂºC until constant weight. The dry weight of residues was measured (W_2) to calculate the IVDMD of kikuyu grass, using the following equation:

Also, for experiment 1, the concentrations of LA and LN in the incubation systems at 0 h of digestion (Table 1) were determined, according to Vargas et al (2012a).

Table 1. Linoleic (LA) and alpha-linolenic (LN) acid concentrations (mean Â± SE) in the incubation systems of experiment 1, at 0 h of digestion.

Treatment[a,b]	LA (g/100 g fatty acids)	LN (g/100 g fatty acids)
CT	5.01 Â± 0.09	18.9 Â± 0.30
100:0	54.8 Â± 1.86	9.68 Â± 0.65
75:25	39.2 Â± 0.55	18.4 Â± 0.05
50:50	27.6 Â± 1.61	29.4 Â± 2.93
25:75	16.2 Â± 0.34	42.0 Â± 1.80
0:100	2.87 Â± 0.05	47.5 Â± 0.58

[a] *CT = kikuyu without lipid supplementation.*
[b] *Kikuyu supplemented with 100:0, 75:25, 50:50, 25:75, and 0:100 LA:LN mixtures*

Statistical analysis

The IVDMD differences between treatments were analyzed as a completely randomized design, using the MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). The statistical model included the treatment as fixed effect and the error as random effect. Also, a prediction model for IVDMD was built, regressing the IVDMD against the ratios between LA and LN concentrations (LA/LN) in the incubation systems at 0 h of digestion, using the REG procedure of SAS. Differences between treatments were significant at $P < 0.05$.

Results and discussion

In experiment 1, we found that supplementation with LA pure or mixed with LN in 75:25, 50:50, and 25:75 LA:LN ratios, decreased by 3.4%, the IVDMD of kikuyu grass, whereas the supplementation with LN pure increased by 3.1% the IVDMD (Figure 1; $P < 0.01$). These results suggest that LA pure or mixed with LN may be more detrimental for kikuyu digestibility than LN pure, which may be due to a differential effect between LA and LN on fibrolytic microorganisms (i.e., which mainly degrades dry matter in forages). According to Jouany et al (2007), Ribeiro et al (2007), and Vargas et al (2012a), the LA:LN ratio in lipid supplements influences FA concentration, as well as the diversity of FA isomers from LA and LN biohydrogenation. Also, Maia et al., (2010) reported that FA intermediaries have differential toxicity effects on microorganisms that degrade dry matter. Therefore, the effect of LA:LN ratio on the IVDMD of kikuyu, may be a consequence of LA:LN ratio influence on the LA and LN ruminal biohydrogenation. Thus, our results suggest that not only the inclusion level of lipid supplement, but also its LA:LN ratio may influence the dry matter digestibility of forages.

Figure 1. *In vitro* dry matter digestibility (IVDMD; mean Â± SE) of kikuyo grass not supplemented (CT) or supplemented
with 100:0, 75:25, 50:50, 25:75, and 0:100 ratios of linoleic (LA) and alpha-linolenic (LN) acids (LA:LN).

We found that the IVDMD can be predicted from the ratio between LA and LN concentrations (LA/LN) in the incubation systems at 0 h of digestion, using a polynomial model ($r = 0.80$; $P < 0.01$; Figure. 2). The correlation between IVDMD and LA/LN may be due to a link between the LA and LN metabolism in the rumen, and the dynamics of dry matter digestion in this compartment, which is in accordance with Maia et al (2010) findings, which reported that ATP production decreases by 66% when LA was added to growing *B. fibrisolvens* bacteria (i.e., important fibrolytic bacteria in the rumen), as well as with RennÃ³ et al (2014) and Freitas et al (2015) findings, which demonstrated that soybean oil supplementation into forage modulates nutrient digestion in dairy cows. Therefore, the LA/LN ratio into incubation systems at 0 h of digestion may be suitable for predicting the IVDMD of forages supplemented with lipids. However, additional studies are needed to confirm this assumption for different forages and scenarios.

Figure 2. Prediction of the *in vitro* dry matter digestibility (IVDMD) of kikuyu grass, from the ratios between linoleic (LA)
and alpha-linolenic (LN) acid concentrations (LA/LN) in the incubation systems, at 0 h of digestion.

In experiment 2, we found that supplementation with a 75:75 LA:LN mixture combined or not with 100:0, 75:25, and 25:75 DHA:EPA mixtures, decreased by 4.2% the IVDMD of kikuyu grass, while the supplementation with EPA pure, decreased by 2.3% the IVDMD (Figure 3; $P < 0.01$). Similarly, Vafa et al (2009) reported that FO (i.e., DHA:EPA: 75:25) decreased the IVDMD of alfalfa. Therefore, DHA pure or mixed with EPA, may be more detrimental to kikuyu digestibility than EPA pure, which may be due to EPA is more easily metabolized than DHA by ruminal microorganisms (Maia et al 2010; Vargas et al 2012b). Thus, our data suggest that is necessary to consider the DHA:EPA ratio when formulating lipid supplements rich in these PUFAs.

Figure 3. *In vitro* dry matter digestibility (IVDMD; mean Â± SE) of kikuyu grass not supplemented (CT) or supplemented with:
a) 75:75 ratio of linoleic (LA) and alpha-linolenic (LN) acids (LA:LN); b) 75:75 LA:LN mixture combined with 100:0,

75:25, 25:75, and 0:100 ratios of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids (DHA:EPA).

Conclusions

- Our results indicate that LA and DHA may be more detrimental than LN and EPA during the ruminal dry matter digestibility of kikuyu grass.

- Furthermore, our study suggest that the *in vitro* dry matter digestibility of kikuyu grass may be predicted, from the ratio between LA and LN concentrations in the incubation systems, at 0 h of digestion.

- This information may be used for optimizing the design of lipid supplements in ruminant nutrition.

Acknowledgements

The authors thank Universidad de Antioquia, the DivisiÃ³n de InvestigaciÃ³n de BogotÃ¡ (DIB) of Universidad Nacional de Colombia, and Colciencias for funding the research: “Efecto de la suplementaciÃ³n de vacas lactantes con fuentes ricas en Ã¡cidos grasos insaturados sobre el perfil de la leche, transcripciÃ³n de enzimas lipogÃ©nicas, actividad SRBP1 y su relaciÃ³n con diferentes componentes del perfil de Ã¡cidos grasos en leche” (code: 1115-452- 21319).

References

AOAC 2006 Official methods of analysis, Association of Official Analytical Chemistry (18th ed) Gaithersburg, MD, USA.

Bauman D E, Baumgard,L H, Corl, B A and Griinari J M 2000 Biosynthesis of conjugated linoleic acid in ruminants. Proceedings of American Society of Animal Science **77**: 1-15.

Chilliard Y, Ferlay A and Doreau M 2001 Review: Effect of different types of forages, animal fat or marine oils in cow’s diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livestock Production Science 70: 31-48.

Cook C W 1964 Symposium on nutrition of forages and pastures: collecting forage samples representative of ingested material of grazing animals for nutritional studies. Journal of Animal Science 23(1): 265-270.

Coppock, C E and Wilks D L 1991 Supplemental fat in high-energy rations for lactating cows: effects on intake, digestion, milk yield, and composition. Journal of Animal Science 69:3826–3837.

Duckett S K and Gillis M H 2010 Effects of oil source and fish oil addition on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. Journal of Animal Science 88: 2684-2691.

Freitas J E, RennÃ³ F P, Gandra J R, RennÃ³ L N, Rego A C, Santos M V, Oliveira M D S and Takiya C S 2014 Addition of unsaturated fatty acids improves digestion of mid lactating dairy cows.

Archivos de Zootecnia. 63:563–573.

Glasser F, Doreau M, Maxin G and Baumont R 2013 Fat and fatty acid content and composition of forages: A meta-analysis. Animal Feed Science and Technology. 185:19–34.

Goering H K and Van Soest P J 1970 Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agricultural Handbook No. 379. US Government Printing Office, Washington, DC.

Harfoot C G and Hazlewood G P 1997 Lipid metabolism in the rumen. In: The Rumen Microbial Ecosystem. P. N. Hobson and C. S. Stewart, ed. Chapman and Hall, London, UK. pp. 382–426.

Jal? D, ?ertÃk M, KundrÃkovÃ¡ K and KubelkovÃ¡ P 2009 Effect of microbial oil and fish oil on rumen fermentation and metabolism of fatty acids in artificial rumen. Czech Journal of Animal Science 54(5): 229-237.

Jenkins T C, Wallace R J, Moate P J and Mosley E E 2008 Board-Invited Review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. Journal of Animal Science 86: 397-412.

Jouany J P, Lassalas B, Doreau M and Glasser F 2007 Dynamic features of the rumen metabolism of linoleic acid, linolenic acid and linseed WJ oil measured in vitro. Lipids 42: 351-360.

Maia M R G, Chaudhary L C, Bestwick C S, Richardson A J, McKain N, Larson T R, Graham I A and Wallace R J 2010 Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, Butirivibrio fibrisolvens. Microbiology 10(52): 1-10.

McDougall E I 1948 Studies on Ruminant Saliva. 1. The composition and output of sheep's saliva. Biochemical Journal 43: 99-109.

Palmquist D L 1996 UtilizaciÃ³n de lÃpidos en dietas de rumiantes. Memorias XII curso de especializaciÃ³n FEDNA, Madrid, EspaÃ±a. Available in: http://www.etsia.upm.es/fedna/publi.htm.

RennÃ³ F P, Freitas J E, Gandra J R, Maturana M, Verdurico L C, RennÃ³ L N, Barletta R V and Vilela F G 2014 Effect of unsaturated fatty acid supplementation on digestion, metabolism and nutrient balance in dairy cows during the transition period and early lactation. Revista Brasileira de Zootecnia 43:212–223.

Ribeiro C V D M, Eastridge M L, Firkins J L, St-Pierre N R and Palmquist D L 2007 Kinetics of fatty acid biohydrogenation in vitro. Journal of Dairy Science 90:1405-1416.

Scholljegerdes E J, Hess B W, Moss G E, Hixon D L and Rule D C 2004 Influence of supplemental cracked high-linoleate or high-oleate safflower seeds on site and extent of digestion in beef cattle. Journal of Animal Science 82:3577-3588.

Tilley J M A and Terry R A 1963 A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18: 104-111.

Vafa T S, Naserian A A, Heravi A R M, Valizadeh R and Danesh M M 2009 Effects of different levels of fish oil and canola oil on in vitro and in vivo nutrient digestibility. Research Journal of

Biological Sciences 4(12): 1221-1226.

Van Soest P J 1994 Nutritional Ecology of the Ruminant. 2nd edition. Cornell University Press. Itaca. United States of America.

Van Soest P J, Robertson J B and Lewis B A 1991 Methods for dietary fiber, detergent fiber and non starch polisaccharides in relation to animal nutrition. Journal of Dairy Science 74: 3583-3597.

Vargas J A C 2012a CinÃ©tica de biohidrogenaciÃ³n *in vitro* de Ã¡cidos grasos poliinsaturados en fluido ruminal. Tesis (M.Sc). Universidad Nacional de Colombia. BogotÃ¡ D.C. pp. 47-67.

Vargas J A C, Olivera M, PabÃ³n M L and Carulla J E 2012b ReducciÃ³n de la biohidrogenaciÃ³n del Ã¡cido linolÃ©ico y alfa linolÃ©nico por la adiciÃ³n de diferentes proporciones de Ã¡cido eicosapentaenÃ³ico y docosahexaenÃ³ico. Revista Colombiana de QuÃmica 41(3): 395-408.

Received 5 April 2017; Accepted 13 May 2017; Published 2 July 2017

Go to top