

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

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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 μ L 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 μ L 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 ($P < 0.01$): $IVDMD = - 0.118 \times (LA/LN)^3 + 1.45 \times (LA/LN)^2 - 4.81 \times (LA/LN) + 69.7$. 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 α -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; Jalč 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 60°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 CO₂ gas at 39°C, until used. The incubations were performed using 100 mL tubes containing 500 mg of kikuyu grass (W₁) supplemented with: a) 18 µL of LA:LN mixtures (100:0, 75:25, 50:50, 25:75 or 0:100) (Experiment 1; n = 18) or, b) 18 µL 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 CO₂, and sealed with one hole rubber stoppers (Fisherbrand™). Then, the tubes were incubated in a water bath (Blue Sland Illinois, USA) at 39°C 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 39°C for 24 h. Finally, the contents were filtered, and the residues were dried at 55°C until constant weight. The dry weight of residues was measured (W₂) 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 ± 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 ± 0.09	18.9 ± 0.30

100:0	54.8 ± 1.86	9.68 ± 0.65
75:25	39.2 ± 0.55	18.4 ± 0.05
50:50	27.6 ± 1.61	29.4 ± 2.93
25:75	16.2 ± 0.34	42.0 ± 1.80
0:100	2.87 ± 0.05	47.5 ± 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 ± 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 Rennó 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 \pm 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.

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