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Isoquinoline Alkaloids and the Ionophore Monensin Supplemented Alone or Combined on Ruminal Fermentation and Nutrient Digestibility in Steers Fed a High-Energy Diet

[1]Jesús D. Urías-Estrada, [1]Beatriz I. Castro-Pérez, [1]Alfredo Estrada-Angulo, [1]Soila Gaxiola-Camacho, [1]Elizama Ponce-Barraza, [2]Alberto Barreras, [3]Luis Corona, [4]Richard A. Zinn, [5]Iván G. Martínez-Álvarez, [5]Jorge Soto-Alcalá and [2,5]Alejandro Plascencia

[1]Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma de Sinaloa. Blvd. San Ángel s/n, Fraccionamiento San Benito CP 80246, Culiacán, Sinaloa, México
[2]Instituto de Investigaciones en Ciencias Veterinarias. Universidad Autónoma de Baja California. Km 4.5 carretera Mexicali-San Felipe, CP 21386, Mexicali, Baja California, México
[3]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Col. CU, Coyoacán, CP 04510, Cd. de México, México
[4]Department of Animal Science, University of California, Davis, United States
[5]Departamento de Ciencias Naturales y Exactas, Universidad Autónoma de Occidente, Unidad Regional Guasave, Avenida Universidad s/n, Flamingos, CP 81048, Guasave, Sinaloa

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Corresponding Author:
Alejandro Plascencia
Departamento de Ciencias Naturales y Exactas, Universidad Autónoma de Occidente, Unidad Regional Guasave, CP 81048, Guasave, Sinaloa, México
Email: aplas_99@yahoo.com

Abstract: The aim of this experiment was to investigate the Influence of Alkaloids (IQA) and sodium Monensin (MON) supplementation on characteristics of ruminal fermentation, microbial protein synthesis and site and extend of digestion. For this, 4 cannulated steers were used in a 4×4 Latin square design. Treatments consisted of a high-energy basal diet supplemented with: (1) No additive (Control), (2) 15 mg IQA/ kg DM, (3) 30 mg MON/kg DM and (4) combination of IQA+MON. There were no treatment effects (P> 0.10) on ruminal and total tract digestion of OM, NDF and starch. Supplemental IQA increased (P<0.01) duodenal flow of NAN (9.2%), ruminal N efficiency (10%) and postruminal N digestion (6.2%). These effects were non-significant (P > 0.20) when IQA was combined with MON. Supplemental MON decreased (P<0.02) duodenal NAN supply and ruminal N efficiency. There were no treatments effect (P>0.43) on ruminal pH. There were no MON effects, or synergism (P≥0.23), on ruminal pH and ruminal VFA proportions. Supplemental IQA increased (P≤0.05) ruminal acetate and tended (P = 0.06) to increase acetate: Propionate ratio. Compared with control, MON did not affect (P>0.10) VFA proportions. Ruminal protozoa and total bacterial counts were decreased by both, IQA and MON when offered separately. Plasma liver enzymes were not affected by treatments. The enhancement in N utilization by steers receiving IQA supplementation can be attributed to reduced ruminal N degradation and by increased microbial N flow to the small intestine. There were no additive effects of the combination of IQA plus MON on measures of digestion.

Keywords: Isoquinoline Alkaloids, Feedlot, Digestion and Fermentation, Monensin, Feed Additives

Introduction

The ionophore Monensin (MON) is commonly included in feedlot cattle growing-finishing diets to enhance feed efficiency. The enhancement in feed efficiency has been attributed, at least in part, to shifts in ruminal fermentation patterns favoring increased propionate and decreased molar proportions of acetate and butyrate (Russell *et al*., 1988). Although MON may also lead to reduced ruminal microbial efficiency, it may increase flow of non-ammonia N to the small intestine by reducing the ruminal feed protein degradation (Zinn, 1988). Nevertheless,

the magnitude of the response to supplemental MON on feed efficiency quite varied, fluctuating from nil (Zinn and Borquez, 1993; Depenbusch *et al*., 2008) up to increases of 18% (Bartley *et al*., 1979). A major factor influencing the feed efficiency response to MON is diet energy density (Barreras *et al*., 2013). Duffield *et al*. (2012), observed that the magnitude of response of MON on feed efficiency decreased from 8.1 to 3.5% during the past 4 decades, coincident with increases in diet energy density brought by decreased dietary forage levels, increased use of supplemental fat and flaking grain (Samuelson *et al*., 2016). These changes in diet formulation lend to enhanced ruminal molar proportions of propionate and decreased methanogenesis (Wang *et al*., 2018). Some phytogenic compounds, such as certain isoquinoline alkaloids (IQA, specifically quaternary-benzo (c) phenanthridine alkaloids sanguinarine and chelerythrine) have effects on ruminal fermentation that could be complementary to MON in high-energy diets. When IQA was added to a substrate comprised of 50:50 forage-to-concentrate, the in vitro molar proportion of acetate increased (Smink and van der Kolk, 2004). Similarly, supplementation of a high-energy diet with a standardized source of IQA (equivalent from 15.8 to 23.8 mg IQA/kg diet DM) increased ruminal acetate molar ratio, but decreasing butyrate in cannulated steers without affect ruminal propionate ratio (Aguilar-Hernández *et al*., 2016). These same researchers reported that supplemental IQA increased flow of non-ammonia N to small intestine due to both reduced ruminal on feed protein degradation and increased net microbial protein synthesis. These findings indicate a possible synergistic action on digestion and ruminal fermentation in cattle fed with high-energy diets supplemented with both additives combined.

For this reason, the aim of this experiment was to evaluate the effects of supplementation of isoquinoline alkaloids and MON on ruminal fermentation and nutrient digestibility in steers fed a high-energy finishing diet.

Materials and Methods

The trial was conducted at the Ruminant Metabolism Experimental Unit of the Instituto de Investigaciones en Ciencias Veterinarias of the Universidad Autónoma de Baja California located 10 km south of Mexicali City in northwestern México (32° 40' 7" N and 115° 28' 6"W). The area is about 10 m above sea level. All procedures were conducted within approved locally animal care guidelines (NOM, 1999).

Animals, Diets and Sampling

Four Holstein steers [302±15 kg initial shrunk Live Weight (LW)] were fitted with a 3.8 cm i.d. ruminal Tygon "T" cannula and a 1.9 cm i.d. Tygon "T" duodenal cannula (situated approximately 6-cm from pyloric sphincter) with the aim to examine the effects of feeding a combination of Isoquinoline Alkaloids (IQA) and Monensin sodium (MON) in finishing diets on the characteristics of ruminal fermentation and digestive function. Steers were housed in an indoor facility in individual pens (3.9 m^2), with a concrete floor covered by a neoprene carpet, automatic waterers and individual feed bunks. Chromic oxide was used as an indigestible marker to estimate nutrient flow and digestibility. Chromic oxide (3.5 g/kg of diet air-dry basis) was premixed with minor ingredients (MON, urea and mineral supplement) in a 2.5 m^3 capacity concrete mixer (mod 30910-7, Coyoacán, Mexico) for 5 min and then, the final product was incorporated after that steam-flaked corn was added to the mixer. Ingredient composition, chemical analysis and calculated dietary net energy (NASEM, 2016) of the basal diet are shown in Table 1. All steers received *ad libitum* access to the basal diet (Control) for 3 wk before the initiation of the experiment. To avoid feed refusals during experimental period, daily feed intake (as feed basis) was restricted to 90% of observed ad libitum intake during last 7-d of preliminary period (6.9 kg as-fed basis, equivalent to 2.28% of average shrunk initial LW). Treatments consisted of a steam-flaked corn-based finishing diet supplemented as follows: (1) Basal diet with no additives (Control), (2) basal diet plus 15 mg IQA/kg diet DM, (3) basal diet plus 30 mg MON/kg diet DM and (4) basal diet plus 15 mg IQA and 30 mg MON (IQA + MON)/kg diet DM. Source of IQA was Sangrovit RS (Phyto biotics; Futtermittelzusatzstoffe GmbH, Eltville, Germany) containing a standardized mixture of isoquinoline alkaloids, specifically quaternary-benzo (c)- phenanthridine alkaloids, sanguinarine and chelerythrine in a 2:1 ratio with a concentration of 2.25% (w/w). The source of MON was Rumensin 90 (Elanco Animal Health, Greenfield, IN) containing 200 g MON/kg of product. The daily dosage of additives was weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ). Supplemental IQA was added (top-dressed) in equal proportions (2.3 g of product per serving) to the basal diet at time the morning and evening feeding, while supplemental Rumensin 90 was premixed with minor ingredients (Cr_2O_3, urea and mineral supplement) before incorporation into complete mixed diets. The amount of feed of each steer was weighed on a digital scale (Ohaus, NVT 16000/1, México City, México). Diets were fed in two equal proportions at 0800 and 2000 h daily. Experimental periods consisted of 21 days, with 10 days for dietary treatment adjustment, 4 days for collection and 7 days of additive withdrawal (during this period all steers received the control diet). During the collection period, duodenal and fecal samples were taken from all steers following procedure described by Aguilar-Hernández *et al*. (2016). Briefly, samples were taken twice daily as follows: d 1, 0750 and 1350 h; d 2, 0900 and 1500 h; d 3, 1050 and 1650 h; and d 4, 1200 and 1800 h.

Individual samples consisted of approximately 500 mL of duodenal chyme and 200 g (wet basis) of fecal material. Samples from each steer and within each collection period were prepared for analysis. During the final day of each period, ruminal samples were obtained to measure microbial populations (protozoa, cellulolytic bacteria and total bacterial). Ruminal samples were prepared and stored by the method described by Dehority (1984) and by Mendoza *et al*. (1993). During the final day of each collection period, ruminal fluid was obtained, via the ruminal cannula, from each steer at 4 h after feeding. Ruminal sample was taken from the ruminal ventral sac by vacuum pump (Cole Parmer Instrument, Vernon Hill, IL) using a tygon tube (1.9 cm i.d.; USP Lima, Ohio). Ruminal fluid pH was determined on fresh samples. Samples were then strained through four layers of cheese cloth. For VFA analysis, 2 mL of freshly prepared 25% (w/vol) meta-phosphoric acid was added to 8 mL of strained ruminal fluid, centrifuged (17,000 × *g* for 10 min) and supernatant fluid stored at -20°C. Upon completion of the trial, ruminal fluid was obtained from all steers and composited for isolation of ruminal bacteria via differential centrifugation (Bergen *et al*., 1968) as follows: (1) ruminal fluid was diluted 50:50 with 0.16N saline (37°C) agitate gently for about 30 seconds and strained through 4 layers of cheesecloth; (2) strained fluid was promptly transferred into centrifuge bottles and spun at 2000 × g for 10 min at 10°C; (3) supernate was decanted and centrifuged at 43,000 × *g* for 20 min at 10°C and (4) supernate was decanted and the pellet isolated, oven-dried (70°C) and then ground with a mortar and pestle. The microbial isolate served as the purine: N reference for the estimation of microbial N contribution to chyme entering the small intestine (Zinn and Owens, 1986). Additionally, during the final day of each period, blood samples (5 mL) were taken via jugular vein 5 h post-feeding in order to determine the enzymes Gamma-Glutamyl Transferase (GGT) and aspartate Aminotransferase (AST), analyzed enzymatically on a Beckman Olympus AU640 auto analyzer (Myko Analytical, Lake Tapps, WA, USA). These enzymes were measured as indicators of possible liver damage due to supplemental additives.

Table 1: Composition of basal diet and the additives supplementation

	Treatments[1]			
Item	Control	IQA	MON	IQA + MON
Ingredient composition (% DM basis)				
Steam-flaked corn	72.00	72.00	72.00	72.00
Dried distillers' grains with solubles	4.80	4.80	4.80	4.80
Sudan grass hay	12.00	12.00	12.00	12.00
Tallow	2.00	2.00	2.00	2.00
Molasses	6.00	6.00	6.00	6.00
Urea	1.00	1.00	1.00	1.00
Isoquinoline alkaloids mixture[2]	--	++	--	++
Monensin[3]	--	--	++	++
Chromium oxide	0.35	0.35	0.35	0.35
Limestone	1.50	1.50	1.50	1.50
Trace mineral salt[4]	0.40	0.40	0.40	0.40
Nutrient composition (% DM basis)[5]				
Crude protein	12.01	12.01	12.01	12.01
Starch	55.00	55.00	55.00	55.00
NDF	15.57	15.57	15.57	15.57
Calculated net energy (Mcal/kg)[6]				
Maintenance	2.18	2.18	2.18	2.18
Gain	1.52	1.52	1.52	1.52

[1]C = control (no additive), IQA = isoquinoline alkaloids mixture, MON = monensin, IQA + MON = combination IQA plus monensin
[2]Dose at 15 mg/ kg of feed (dry matter basis)
[3]Dose at 30 mg/kg of feed (dry matter basis)
[4]Trace mineral salt contained: $CoSO_4$, .068%; $CuSO_4$, 1.04%; $FeSO_4$, 3.57%; ZnO, 1.24%; $MnSO_4$, 1.07%, KI 0.052%; and NaCl, 92.96%
[5]Dietary chemical composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other
[6] Based on tabular Net Energy (NE) values for individual feed ingredients (NASEM, 2016)

Sample Analysis and Calculations

Feed, duodenal and fecal samples were subject to the following analysis: Dry matter (method 930.15); ash (method 942.05) and Kjeldahl N (method 984.13) following the procedures published by AOAC (2000). Neutral detergent fiber [aNDFom, corrected for NDF-ash, incorporating heat stable α-amylase (Ankom Technology, Macedon, NY) at 1 mL per 100 mL of NDF solution (Midland Scientific, Omaha, NE)] was determined following the procedures described by Van Soest *et al*. (1991) and chromic oxide (Hill and Anderson, 1958) and starch (Zinn, 1990). In addition, ammonia-N (method 941.04; (AOAC, 2000) and purines (Zinn and Owens, 1986) were determined in duodenal samples. Concentrations of VFA in ruminal fluid were determined by gas chromatography (Zinn, 1988). The counting procedures for total protozoa and total bacterial were performed according to Dehority *et al*. (1989). Cellulolytic bacteria was cultured and counted by the method described by Van Gylswyk and Hoffman (1970). Bacterial and protozoal counts are expressed as log10/mL.

Total DM flow to the duodenum and fecal excretion were estimated using Cr2O3 as an external marker. Feed, duodenal and fecal OM was determined by difference between DM and ash content. Microbial Organic Matter (MOM) and Microbial Nitrogen (MN) leaving the abomasum (as obtained from a duodenal cannula placed approximately 6 cm from the pyloric sphincter) were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter truly fermented in the rumen was considered equal to the OM intake minus the difference between the amount of total OM reaching the duodenum and the MOM reaching the duodenum. Feed N escape to the small intestine is considered equal to the total N leaving the abomasum minus the sum of ammonia-N plus MN reaching duodenum and, thus, includes any endogenous N contributions. Ruminal microbial efficiency was estimated as duodenal MN, g /kg OM fermented in the rumen and protein efficiency represent the duodenal non-ammonia-N, g/g N intake.

Statistical Design and Analysis

Treatment effects on characteristics of digestion were analyzed as a balanced 4 × 4 Latin square design in a 2 × 2 factorial arrangements using the MIXED procedure according to SAS (2004). The fixed effect consisted of treatment and random effects consisted of steer and period. The statistical model for the trial was as follows:

$$Y_{ijk} = \mu + S_i + P_j + T_k + E_{ijk},$$

where: Y_{ijk} is the response variable, μ is the common experimental effect, S_i is the steer effect, P_j is the period effect, T_k is the treatment effect and E_{ijk} is the residual error. Treatment effects were separated into the following orthogonal contrasts: (1) Non-additive vs. IQA; (2) non additive vs MON; and (3) IQA× MON interaction. In addition, means separations were performed using Fisher's LSD. Contrasts are considered significant when the P value was ≤0.05 and as tendencies when the P-value was >0.05 and ≤0.10.

Results and Discussion

Treatment effects on characteristics of ruminal and total tract digestion are summarized in Table 2. Flow of ammonia-N to the small intestine was greater (interaction, P<0.01) for non-supplemented control than for the other three treatments. Flow non-ammonia N (NAN; interaction, P = 0.04) was greater for supplemental IQA alone than for the other three treatments. When IQA was added to the control diet, duodenal flow of NAN increased (9.2%) and NH_3-N flow decreased (23.7%). In contrast, when IQA and MON were added to the Control diet no effect on NAN flow to the small intestine was observed and NH_3-N flow decreased only 18.2%. This interaction was also observed (P<0.05) in protein efficiency (NAN flow to the small intestine/N intake) and postruminal N digestion. Addition of IQA to control diet resulted in a 9.1% increase in protein efficiency and 6.1% increase in postruminal N digestion. Whereas, IQA in combination with MON did not increase (interaction, P<0.05) protein efficiency or postruminal N digestion. The basis for this response is unclear. Earlier reports indicate that MON decrease ruminal concentration of microorganisms with high proteolytic activity and it may have direct effect on protease and deaminase enzymes as well (Bergen and Bates, 1984; Russell and Strobel, 1989). On the other hand, IQA could modulate metabolism of rumen microbes with minor changes on rumen microbial population and species diversity (Petri *et al*., 2019). The effects of both additives alone, decrease the ruminal degradation of feed N; however based on the duodenal flows of NAN, ruminal scape feed N and microbial N with the combination, apparently the effects of each additive are negatively affected. More research is is needed to further assess these interactions, as well as possible interactions with other additives (including alternative ionophores) that may be supplemented in feedlot diets.

IQA supplementation increased (5.6%, P = 0.02) flow of NAN and decreased (11.8%, *P* = 0.01) flow of NH3-N to the small intestine. The increase in NAN flow to the small intestine was due in part to increased (6.5%, P = 0.01) flow of microbial N to the small intestine. This increase is a reflection of increased (7.6%, P = 0.03) ruminal microbial efficiency (expressed as duodenal MN, g/kg OM fermented in the rumen) and increased (9.1%, *P* = 0.02) ruminal protein efficiency (expressed as duodenal non-ammonia N, g·g-1 N intake). Aguilar-Hernández *et al*. (2016) observed that in steers fed a diet similar that of the present study,

supplementation with 16.8 mg IQA/kg diet DM decreased ruminal ammonia N concentration; presumably due to decreased proteolysis and deamination of amino acids (Drsata *et al*., 1996). More recently, Petri *et al*. (2019) likewise observed decreased amino acids metabolism in IQA supplemented treatments. They noted that predicted amino acid metabolism pathways were down-regulated in all IQA supplemented groups in comparison to the control group, a key mode of action for these IQA supplementation in regard to improving ruminal amino acid bypass. The increase of net microbial N flow to duodenum in steers fed IQA may be partially explained (as indicated below) by reduced recycling of microbial protein as consequence of decreased ruminal protozoa.

There were no interactions on ruminal, posruminal and total tract digestion of OM, starch and NDF. Although IQA increased ruminal microbial efficiency, N efficiency and postruminal N digestion, it did not affect (P>0.10) ruminal, postruminal and total tract digestion of OM, starch and NDF. The absence of IQA effects on digestion of OM, starch and NDF are consistent with those obtained in previous studies performed in vivo (Aguilar-Hernández *et al*., 2016) and in vitro (Rusitec experiment; Petri *et al*., 2019).

Supplemental MON decreased (9.4%, P = 0.03) flow of ammonia-N and NAN (4.8%, P = 0.02) to the small intestine and in turn, ruminal N efficiency (5.7%, P = 0.02). The latter is attributable to decreased (7.4%, P = 0.02) ruminal microbial efficiency and associated decrease (8.6%, P = 0.02) in microbial N flow to the small intestine. Consistent with previous studies (Morris *et al*., 1990; Salinas-Chavira *et al*., 2009), supplemental MON did not affect (P>0.10) site and extent of OM, NDF and starch digestion. Comparable effects of supplemental MON on feed N degradation and microbial synthesis in feedlot steers has been reported previously (Zinn, 1987; Zinn *et al*., 1994). Due to antimicrobial properties of MON some decrease in ruminal NDF degradation can be expected. However, the effects of MON of fiber digestion has not be consistent (Salinas-Chavira *et al*., 2009). Both increases and decreases in fiber digestion have been associated with ionophore feeding (Spears, 1990). Varying effects are apparently dependent on fiber level and source. As with the present study, NDF levels are low in conventional finishing diets for feedlot. Due to high dietary starch and consequent low ruminal pH, the extent of ruminal fiber digestion is low (≤40%), independently of MON supplementation. Although, in the present study MON numerically decreased (10%, P = 0.18) ruminal digestion of NDF.

Treatment effects on ruminal pH, VFA molar proportions are shown in Table 3. There were no treatment main effects, or synergism (P>0.10), on ruminal pH. Contrary to our hypothesis, there was no treatment synergism (P>0.10), on ruminal VFA proportions. However, IQA increased (10.9%, P = 0.02) acetate molar ratio, this effect reflected a tendency of increase (P = 0.06) the acetate: Propionate molar ratio. The absence of effects of IQA on ruminal pH and total VFA production are consistent with lack of treatment effect on ruminal OM digestion. Similar findings have been reported for steers fed both medium energy diets (Petri *et al*., 2019) and high energy diets (Aguilar-Hernández *et al*., 2016; Zhang *et al*., 2019). In vitro studies of Smink and van der Kolk (2004) also showed increased acetate: Propionate molar ratio without effect on total VFA production. However, Khiaosa-ard *et al*. (2020) using a rumen simulation technique (Rusitec) noted that IQA supplementation increased ruminal propionate at dose of 8.75 mg IQA/kg DM, but at dose of 17.50 mg IQA/kg DM did not registered differences between ruminal molar proportion of VFA in a medium-energy substrate (35:55 forage concentrate ratio).

Lack of an influence of MON on ruminal pH, VFA molar proportions is consistent with numerous studies in which MON was supplemented in high-energy diets (Zinn *et al*., 1994; Salinas-Chavira *et al*., 2009; Felix and Loerch, 2011).

Treatments effects on ruminal microbial counts are shown in Table 4. Combining IQA with MON increased (interaction, P < 0.01) total protozoa count and nullified the effects (interaction P = 0.04) of MON on the ruminal total bacterial count. It has been previously observed that combinations of antimicrobials may act differently on microorganisms than when they are administered separately (Mitosch and Ballenbach, 2014).

There were no treatment effects (P > 0.16) on cellulolytic bacteria counts. The total bacteria count was lower (interaction, P = 0.04) for MON alone than for the other three treatments. Supplemental IQA alone decreased (interaction, P<0.01) ruminal protozoa counts compared to that of controls (28%) and MON, alone (21%). Whereas protozoal counts for control and MON plus IQA were not different. Likewise, Petri *et al*. (2019) using a rumen simulation technique (Rusitec) noted that IQA supplementation decreased ruminal protozoa population. Sanguinarine and chelerythrine, principal compounds on IQA source used here, have a significant dose-dependent antibacterial activity (since 16 µg/mL) against Gram-positive and Gram-negative bacteria when tested *in vitro* (Opletal *et al*., 2014). Protozoal recycling of microbial protein depresses ruminal microbial efficiency (net flow of microbial N to the small intestine). The decrease in ruminal protozoal counts and concomitant increase in observed microbial efficiency with IQA supplementation is consistent with this observation.

Development of MON resistance has been shown to occur in both gram-positive and negative species (Russell and Strobel, 1988). Apparently, prolonged use of MON alters the ruminal microbial ecosystem, selecting for ionophore-resistant members of the microbial population (Callaway *et al*., 2003). Although MON has consistently reduced in vitro protozoal counts, that effect is less consistently observed in vivo (Russell and Houlihan, 2003).

Table 2: Treatment effects on characteristics of ruminal and total tract digestion in cannulated Holstein steers (302 kg BW)

	-MON		+MON		IQA, mg/kg DM			MON, mg/kg DM			IQA×MON	
Item	-IQA	+IQA	- IQA	+IQA	0	15	P Value	0	30	P Value	P Value	SEM
Intake (g/d)												
DM	6,190	6,190	6,190	6,190	6,190	6,190	--	6,190	6,190	--	--	--
OM	5,893	5,893	5,893	5,893	5,893	5,893	--	5,893	5,893	--	--	--
NDF	964	964	964	964	964	964	--	964	964	--	--	--
Starch	3,405	3,405	3,405	3,405	3,405	3,405	--	3,405	3,405	--	--	--
N	119	119	119	119	119	119	--	119	119	--	--	--
Duodenal flow (g/d)												
OM	2,907	2,955	2,917	3,010	2912	2983	0.38	2931	2964	0.67	0.77	75
NDF	519	536	570	551	545	543	0.95	527	561	0.18	0.43	23
Starch	687	682	736	689	685	712	0.73	712	685	0.75	0.79	79
N	122a	134b	121a	122a	121	128	0.02	128	121	0.02	0.05	1.95
NH_3-N	3.07a	2.34b	2.28b	2.51b	2.71	2.39	0.01	2.67	2.42	0.03	<0.01	0.088
NAN	119a	131b	118a	120a	118	125	0.02	125	119	0.02	0.04	1.92
MN	83.12a	90.35b	75.92c	79.73bc	79.52	85.04	0.01	83.12	75.95	<0.01	0.20	1.18
Feed N	36.12a	40.98ab	42.37b	39.82b	39.25	40.44	0.51	38.55	41.10	0.17	0.07	1.67
Ruminal digestion (%)												
OM	64.78	65.19	63.38	62.44	64.08	63.82	0.84	64.98	62.91	0.15	0.61	1.77
NDF	39.97	38.00	33.89	36.20	36.93	37.10	0.95	38.98	35.04	0.18	0.44	3.62
Starch	79.82	78.38	79.97	79.77	79.90	79.07	0.73	79.10	79.87	0.75	0.80	3.27
Feed N	69.66a	65.58a	64.40b	66.54a	67.03	66.06	0.52	67.62	65.47	0.18	0.07	1.99
Microbial N [1]	21.82ab	23.59b	20.33a	21.68a	21.07	22.63	0.03	22.70	21.01	0.02	0.70	0.526
N efficiency [2]	1.00a	1.10b	0.99a	1.00a	1.00	1.05	0.02	1.05	0.99	0.02	0.04	0.016
Fecal excretion (g/d)												
DM	1,304	1,295	1,306	1,341	1,305	1,317	0.77	1,300	1,323	0.61	0.63	62.1
OM	1,139	1,138	1,144	1,172	1,141	1,155	0.73	1,141	1,155	0.61	0.71	52.0
NDF	449	443	464	437	456	439	0.33	446	450	0.77	0.53	16
Starch	70.91	67.28	57.71	59.78	64.32	63.53	0.89	69.10	58.75	0.11	0.62	7.70
N	32.24	28.89	30.81	31.06	31.53	29.98	0.17	31.52	29.98	0.72	0.12	1.41
Postruminal digestion (% duodenal)												
OM	60.76	61.39	60.75	61.09	60.76	61.24	0.78	60.76	61.24	0.92	0.94	2.37
NDF	13.32	16.18	18.31	20.01	15.81	18.09	0.70	14.75	19.16	0.47	0.92	7.91
Starch	89.40	90.41	91.37	91.31	90.39	90.86	0.67	89.90	91.34	0.23	0.64	1.52
N	73.62a	78.37b	74.44a	74.55a	74.03	76.46	0.05	75.99	74.49	0.19	0.05	1.32
Total tract digestion (%)												
DM	78.93	79.08	78.91	78.34	78.92	78.71	0.78	79.01	78.63	0.61	0.63	1.00
OM	80.67	80.69	80.59	80.11	80.63	80.40	0.72	80.68	80.35	0.61	0.70	0.88
NDF	48.02	48.73	46.30	49.38	47.16	49.05	0.33	48.37	47.84	0.77	0.53	2.50
Starch	97.92	98.02	98.31	98.24	98.11	98.13	0.89	97.97	98.27	0.11	0.62	0.22
N	72.92	75.73	74.12	73.91	73.52	74.82	0.17	74.32	74.01	0.72	0.12	1.18

[a,b]Means in a row with different literal differ (P<0.05)
[1]Microbial efficiency is estimated as duodenal MN, g/ kg^{-1} OM fermented in the rumen
[2]N efficiency is estimated as duodenal non-ammonia N, g/ g^{-1} N intake

Table 3: Treatment effects on characteristics of ruminal pH and volatile fatty acid proportions[1]

	-MON		+MON		IQA (mg/kg DM) Ety			MON (mg/kg DM)			IQA × MON	
Item	-IQA	+IQA	-IQA	+IQA	0	14	P-value	0	30	P-value	P-value	SEM
pH	6.03	5.91	5.92	5.86	5.97	5.89	0.19	5.97	5.89	0.24	0.63	0.61
Total VFA	75.23	74.24	75.29	73.77	75.23	74.34	0.94	75.29	73.20	0.81	0.92	4.36
VFA (mmol/100 mol)												
Acetate	48.94	55.58	48.35	53.55	48.64	54.56	0.02	52.26	50.95	0.50	0.71	1.86
Propionate	38.89	34.87	39.70	35.34	39.30	35.10	0.20	36.88	37.52	0.12	0.11	1.52
Butyrate	12.15	9.54	11.94	11.10	12.04	10.32	0.16	10.85	1.38	0.55	0.44	1.06
Acetate:Propionate	1.27	1.59	1.22	1.52	1.24	1.55	0.06	1.42	1.38	0.66	0.85	0.19

[a,b]Means in a row with different superscripts differ (P<0.05)
[1]Samples taken at 4 post-feeding

Table 4: Treatment effects on microbial population of ruminal fluid[1]

	-MON		+MON		IQA (mg/kg DM)			MON (mg/kg DM)			IQA × MON	
Item	-IQA	+IQA	-IQA	+IQA	0	14	P-value	0	30	P-value	P-value	SEM
Protozoa	6.16a	4.12b	5.19c	5.88a	5.68	5.00	0.02	5.14	5.54	0.10	<0.01	0.19
Total bacteria	10.53a	10.66a	10.03b	10.64a	10.28	10.65	<0.01	10.59	10.33	0.03	0.04	0.12
Cellulolytic bacteria	8.13	8.88	7.80	8.32	7.97	8.60	0.23	8.51	8.06	0.38	0.82	0.48

[a,b]Means in a row with different superscripts differ (P<0.05)
[1]Quantities of bacteria and protozoa were expressed as log10/mL

Table 5: Treatment effects on liver enzymes[1]

	-MON		+MON		IQA (mg/kg DM)			MON (mg/kg DM)			IQA×MON	
Item	-IQA	+IQA	-IQA	+IQA	0	14	P-value	0	30	P-value	P-value	SEM
Enzymes (U/L)[2]												
GGT	19.75	23.25	29.00	19.50	24.37	21.37	0.51	21.50	24.25	0.55	0.19	4.35
AST	45.25	46.75	55.50	50.00	50.35	48.37	0.67	46.00	52.75	0.19	0.47	6.39

[1]Blood sample taken via jugular vein 5 h post-feeding.

The effects of treatments on plasmatic liver enzymes concentration are shown in Table 5. There were no treatments effects on plasma liver enzymes concentration. Across treatments, the plasma concentration of GGT and AST were within normal ranges for healthy ruminants averaging 22.9 and 49.4 U/L (Radostits *et al*., 2000). Michels *et al*. (2018) reported a similar levels of plasma haptoglobin (a liver protein associated with liver inflammation) between Controls and IQA supplemented bulls that were fed a high energy diet at the rate of 12.9 mg IQA/kg DM (equivalent to 0.26 mg IQA/kg LW) over a 105-d period. Previous studies with rats and pigs have likewise have not shown toxic effects of supplemental IQA when were administered up to 7 mg IQA/kg LW (Kosina *et al*., 2004; Psotova *et al*., 2006). Stivorova *et al*. (2008) did not observe toxicological effects in rats fed a dose equivalent to 2.90 mg IQA/kg LW. In the present experiment the dose was nearly 10-fold lower (a daily intake of 100 mg IQA an equivalent to 0.30 mg IQA/kg LW) than the challenge dose used by Stivorova *et al*. (2008). It is well known that cattle are more tolerant than other species (i.e., horses and pigs) to MON ingestion. The monensin LD_1 for cattle was estimated from 5 to 6 mg/kg BW (Gonzalez *et al*., 2005), while LD_{50} was observed at doses from 21.9 to 80 mg/kg LW (Basaraba *et al*., 1999). In the present study the supplemental MON consumption was 10-fold lower than LD_1 doses determined by Gonzalez *et al*. (2005), equivalent to 0.615 mg MON/kg LW.

Conclusion

Isoquinoline Alkaloids mixture (IQA) supplementation improve of N utilization by promotes a lesser ruminal degradation of N and by greater microbial N flow to the small intestine. The inclusion of IQA to the diet increase molar proportion of ruminal acetate. IQA plus MON combination failed to be synergic on digestion nor ruminal fermentation, in opposite, had a negative associative effect on N utilization. More research is needed to further assess these interactions, as well as possible interactions with other additives (including alternative ionophores) that may be supplemented in feedlot diets. Isoquinoline alkaloids mixture (IQA) represent a strategy to improve N utilization in ruminants fed high-energy diets.

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Author's contribution

Jesús D. Urías-Estrada: Performed the experiment

Beatriz I. Castro-Pérez: Performed the experiment

Alfredo Estrada-Angulo: Analyzed and interpreted the data.

Soila Gaxiola-Camacho: Sampling and clinical laboratory analysis

Elizama Ponce-Barraza: Sampling and clinical laboratory analysis

Alberto Barreras: Collaborated with statistical analysis.

Luis Corona-Gochi: Performed the chemical and nutritive composition of feed and digesta samples

Richard A. Zinn: Provided very helpful feedback on an early draft of the paper.

Iván G. Martínez-Álvarez: Contribute in wrote early draft of the paper.

Jorge Soto-Alcalá: Contribute in wrote early draft of the paper.

Alejandro Plascencia: Designed and supervised the experiment and laboratory works, obtained and administered funding, wrote the final version of paper.

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