which were conducted 3 and 5 d after weaning. No calf was presented twice with the same ingredient. Oro-sensorial preferences were calculated as the mean difference in feed consumption every 30 min over a 6-h period. The most preferred energy ingredients were wheat, sorghum, corn, and barley; whereas oats, rice, and corn gluten feed were the least preferred (Table 1). Wheat was the most preferred ingredient in all assays but one. On the other hand, corn gluten feed was the least preferred ingredient in all assays but one.

Table 1. Significant differences (*P*< 0.05) in consumption (g/30 min) due to oro-sensorial preferences of several ingredients

Feed1[a]	Feed2[a]	Diff.[1]	Feed1	Feed2	Diff.[1]
W	CGF	10.0	C	R	48.9
W	R	77.9	C	O	44.5
W	O	23.1	C	WM	8.6
W	WM	28.8	C	B	-12.8
W	B	16.8	B	CGF	9.9
W	S	44.2	B	R	59.3
S	CGF	3.5	B	O	66.1
S	R	33.4	B	WM	-16.5
S	O	42.3	WM	CGF	19.5
S	B	21.0	WM	R	19.7
S	C	12.8	WM	O	-55.3
C	CGF	47.4	R	CGF	12.1

[1] Feed1 - Feed2; [a]C = Corn; B = Barley; CGF = Corn gluten feed O = Oats; R = Rice; S = Sorghum; W = Wheat; WM = wheat middlings

Key Words: palatability, preferences, intake

576 High dietary iron negatively impacts gene products important in iron and manganese metabolism in young calves. S. L. Hansen*, M. S. Ashwell, R. S. Fry, and J. W. Spears, *North Carolina State University*, *Raleigh*.

Fourteen weaned Holstein calves were used in a 56-d experiment examining the impacts of high dietary iron (Fe) on proteins involved in Fe and manganese (Mn) metabolism. Calves were stratified by weight and randomly assigned to one of two diets: 1) no supplemental Fe (normal Fe) or 2) 750 mg supplemental Fe/kg DM (high Fe). Blood was collected on d 0, 35 and 56. At the end of the trial 6 calves per treatment were harvested and liver and duodenal scrapings were collected for analysis. Feeding a diet high in Fe decreased ($P < 0.05$) average daily gain, dry matter intake and feed efficiency. Hemoglobin and serum Fe concentrations did not differ due to dietary treatment. Feeding a diet high in Fe increased concentrations of Fe in the liver ($P = 0.03$), but did not affect heart or duodenal Fe. Duodenal Mn concentrations were lowered ($P = 0.05$) by feeding a high Fe diet, but liver and heart Mn concentrations were not affected. As determined by real-time RT-PCR, relative hepatic expression of the gene which encodes the Fe regulatory hormone hepcidin was 5-fold greater ($P < 0.01$) in calves fed high dietary Fe. Hepcidin is released in response to increased Fe status and binds to the Fe export protein ferroportin causing it to be degraded, thereby reducing dietary Fe absorption. Confirmation of this result was achieved through Western blotting of duodenal protein which revealed that expression of ferroportin was decreased ($P = 0.03$) due to high dietary Fe. Duodenal expression of divalent metal transporter 1 (DMT1), a Fe import protein which can also transport Mn, tended (P = 0.13) to be reduced by high dietary Fe. In summary, feeding calves a diet high in Fe induced a signal cascade (hepcidin) designed to reduced absorption of Fe (via reduced expression of ferroportin and DMT1) in a manner similar to that reported in rodents. Additionally, reduced levels of DMT1 protein appeared to decrease duodenal Mn, suggesting that Mn is also a substrate for DMT1 in the bovine.

Key Words: cattle, iron, manganese

Ruminant Nutrition: Rumen Microbiology

577 Metagenomics analysis reveals shifts in functional profiles and population dynamics of rumen microbial communities in response to developmental and dietary changes. R. W. Li*[1], M. E. Sparks[1], Y. Huang[2], W. Li[2], E. E. Connor[1], R. L. Baldwin VI[1], C. Li[1], and T. Sonstegard[1], [1]*Unisted States Department of Agriculture, Agricultural Research Service, Bovine Functional Genomics Laboratory*, *Beltsville, MD*, [2]*University of California*, *San Diego*.

Physiologists have long attempted to manipulate rumen fermentation to enhance fibrolytic capacity and reduce proteolytic and methanogenic outputs of the rumen ecosystem. However, successful ruminal fermentation manipulation requires a solid understanding of rumen microbes and their interactions. ~8 gigabases of DNA from 6 metagenomes were generated using next-generation technologies. ~70% of short reads could be assembled into contigs using Velvet. ORFs were predicted using MetaGene, and resultant proteins were annotated using Pfam, TIGRfam and COG. Higher level metagenome features, such as phylogenetic profiles, functional compositions and population dynamics, were compared between relevant rumen microbial communities. For example, 34,948 and 14,700 proteins identified from 2 of the 6 communities studied (#911 and #103) belong to roughly the same number of Pfam protein families (1,983 and 1,835, respectively), of which ~80% were identical. Interestingly, 414 protein families were unique to #911 (from calf fed hay) while 266 families unique to #103 (from calf fed milk only). Thus, differences in biological functions and ecosystem outputs may be deduced. Both glycoside hydrolase (GH) families 70 and 42 were unique to #911, suggesting ruminal degradation of cellulose and xylans as a primary function of this community. One of the most abundant families was GH family 43, which contains arabinase activities for degradation of plant cell wall arabinan, reflecting more active fibrolytic activities in #911. Moreover, unique expression of >12 other GH families in the #911 community demonstrates its character for glycosidic bond hydrolysis. In contrast, Proteobacteria were abundant in #103, as evidenced by its unique expression of cytochrome cbb3 oxidases exclusive to this phylum. Many protein families typical of aerobic prokaryotes were also abundant in this community. In addition, #103 expressed proteins involved in N metabolism, such as a urea transporter and urease, which can be advantageous due to roles NH3 plays in microbial growth and maintaining rumen pH.

Key Words: metagenomics, rumen, microbial

578 pH dynamics and bacterial community composition in the rumen of lactating dairy cows. A. Palmonari*[1], D. M. Stevenson[2],

D. R. Mertens[2], C. W. Cruywagen[3], and P. J. Weimer[2], [1]*DIMORFIPA, University of Bologna, Bologna, Italy*, [2]*USDA-ARS-U.S. Dairy Forage Research Center, Madison, WI*, [3]*Department of Animal Science, University of Stellenbosch, Stellenbosch, Republic of South Africa*.

The effect of pH dynamics on ruminal bacterial community composition (BCC) was studied in 8 ruminally-cannulated Holstein cows fitted with indwelling electrodes that recorded pH every 10 min over a 2.4-d period. Cows were fed a silage-based TMR supplemented with monensin. Ruminal samples were collected each day just before feeding, and at 3 and 6 h after feeding. Solid and liquid phases were separated at collection, and extracted DNA was subjected to PCR amplification followed by Automated Ribosomal Intergenic Spacer Analysis (ARISA). Although cows displayed widely different pH profiles (mean pH= 6.11 to 6.51, diurnal pH range = 0.45 to 1.39), correspondence analysis of the ARISA profiles revealed that 6 of the 8 cows had similar BCC. The 2 cows having substantially different BCC from the other 6 had intermediate mean pH values (6.30 and 6.33) and intermediate diurnal pH ranges (averaging 0.89 and 0.81 pH units). These two cows displayed the lowest milk fat percentages of the 8, along with markedly higher ruminal populations of one bacterial OTU (Operational Taxonomic Unit) and reduced populations of two other OTUs. Cloning and sequencing of a DNA segment from the elevated OTU revealed phylogenetic similarity to *M. elsdenii*, a species known to produce t10-c12 conjugated linoleic acid (CLA, a known regulator of mammary lipogenesis). The higher populations of both *M. elsdenii* and OTU246 in these 2 cows were confirmed using quantitative real-time PCR with species-specific primers, and the population sizes of these two taxa were highly correlated (r^2=0.99, p<0.001). By contrast, the relative population sizes of *Streptococcus bovis* and genus *Ruminococcus*, two taxa expected to respond to ruminal pH, did not differ among cows (mean = <0.01% and 10.6%, respectively, of rRNA gene copy number, determined by real time-PCR). The results indicate that cows with widely differing pH profiles can have rather similar ruminal BCC, and that low milk fat can occur at intermediate ruminal pH. The results support recent reports that milk fat depression is associated with shifts in BCC in rumine, and are specifically related to the relative population size of *Megasphaera elsdenii*.

Key Words: ARISA, BCC, ruminal pH

579 Effect of supplemental carbohydrate source and level on in vitro gas production estimates. A. Britos*[1], N. Pomiés[1], J. L. Repetto[2], and C. Cajarville[1], [1]*Department of Animal Nutrition, Faculty of Veterinary, UdelaR, Montevideo, Uruguay*, [2]*Department of Bovines, Faculty of Veterinary, UdelaR, Montevideo, Uruguay*.

The aim of this work was to study the effect of source and level of supplemental carbohydrate mixed with forage on in vitro gas production. Substrates were composed of mixtures of 30, 70 and 100% of supplement (citrus pulp (CP), corn grain (C) or barley grain (B)) and high quality temperate pasture, also incubated with no supplemental carbohydrate source (0%). 0.5g of each substrate was weighed in triplicate (n=21) into 125mL fermentation flasks and 38.5mL of N-free media was added the evening before inoculation of 10mL of inoculum. Rumen fluid was collected from a lactating cow after 15 d of adaptation to a forage diet. Gas measurements were made at 2, 4, 6, 8, 10, 12, 18, 24, 48, 72 and 96h post-inoculation. Data were fitted to V=Vf/{1+exp[2+4*Rf*(T-L)]}+Vs/{1+exp[2+4*Rs*(T-L)]} where V is total gas volume (mL/g DM incubated), Vf is fast pool volume (mL/g DM incubated), Vs slow pool volume (mL/g DM incubated), Rf and Rs are specific rates (h^{-1}) of fast and slow pools respectively, T is time, and L is lag (h). Parameter means were analyzed by PROC GLM considering carbohydrate source, level and its interaction, and separated by LSmeans procedure of SAS®. All parameters were affected by carbohydrate source, level and its interaction. In 0% level fast pool volume provided 77% of the total gas volume. The total gas volume increased between 15% (corn) to 37% (barley), when the supplement reached 70%. *Acknowledgements: funded by PEDECIBA Biología; PDT 78/12; FCE2007_119 ANII.*

Table 1.

S	I	V	Vf	Rf	Vs	Rs	L
	0	189^{a}	146^{a}	0.09^{a}	43.5^{a}	0.02^{a}	-0.24^{a}
CP	30	194^{a}	156^{a}	0.09^{a}	38.4^{a}	0.02^{a}	0.14^{ac}
	70	230^{b}	149^{a}	0.12^{b}	80.9^{b}	0.04^{b}	0.37^{bc}
	100	227^{b}	131^{b}	0.12^{b}	96.2^{c}	0.03^{b}	0.29^{bc}
	0	189^{a}	146^{a}	0.09^{a}	43.5^{a}	0.02^{a}	-0.24^{a}
C	30	204^{ab}	137^{abc}	0.11^{bc}	66.8^{b}	0.03^{b}	0.57^{b}
	70	218^{b}	123^{bc}	0.10^{ac}	94.7^{c}	0.03^{b}	1.22^{c}
	100	219^{b}	123^{c}	0.11^{bc}	96.2^{c}	0.03^{b}	2.62^{d}
	0	189^{a}	146^{a}	0.09^{a}	43.5^{a}	0.02^{a}	-0.24^{a}
B	30	191^{a}	147^{a}	0.10^{ac}	43.8^{a}	0.02^{b}	1.21^{b}
	70	259^{b}	206^{b}	0.09^{a}	52.8^{b}	0.02^{a}	1.38^{b}
	100	260^{b}	211^{b}	0.10^{bc}	48.5^{ab}	0.02^{a}	2.72^{c}
P(**S**)		**	*	*	*	*	*
P(**S**×**I**)		**	*	*	*	*	*

abcd within S differ P<0.05; *: P<.001; **: P<.01 S: source, I: level

Key Words: carbohydrate, temperate pasture, gas production

580 Differential chemotaxis by entodiniomorphids and isotrichids toward glucose after incubation with emulsified polyunsaturated fatty acids. H. L. Diaz*, A. M. Stalford, K. N. Barr, and J. L. Firkins, *The Ohio State University, Department of Animal Sciences, Columbus*.

We previously documented extensive chemotaxis by isotrichid (IS) protozoa toward glucose; however, entodiniomorphids (EN) had less pronounced, but dose-responsive, chemotaxis to the same concentrations. We hypothesized that prolonged exposure to fat would blunt chemotaxis toward glucose and be increasingly inhibited when mixed ruminal protozoa were previously incubated for 0, 3, or 6 h in the presence of 0, 0.5, 1.0 or 2.0% (v/v) emulsified polyunsaturated fatty acids (PUFA; Liposyn II™). After preincubation, 20 mL of ruminal fluid were inoculated to beakers that contained capillary tubes previously filled with either saline or 1000 m*M* glucose (G1000) or else unfilled and uncapped (positive control; POS). Counts in capillary tubes at 20 min were corrected for 0-min blanks and $\log_{10}$-transformed to normalize data. For only POS, there was an interaction (*P*=0.01) for PUFA × time. At 3h, PUFA decreased counts with both linear (L) and quadratic (Q) responses (*P*<0.01); however, at 6h, counts decreased linearly (*P*<0.01; Q was *P*>0.20) with increasing PUFA. Counts for saline or G1000 were covariate-adjusted for the respective POS counts to assess chemotaxis irrespective of changing protozoal abundance. For EN, there was a PUFA × glucose presence × time interaction (*P*<0.05). At 3 h, the main effect mean was greater (*P*<0.01) for G1000 than saline. At 6 h, the interaction (*P*<0.01) between PUFA and presence of glucose was explained by a cubic (*P*<0.01) response to PUFA for saline without effect (*P*>0.10 for all contrasts) for G1000. There were no interactions (*P*>0.20) for IS populations. For POS, increasing PUFA linearly decreased (*P*=0.01) counts. After covariate adjustment for POS, the

IS populations remained constantly chemotactic toward G1000 (main effect, $P<0.05$). Although IS populations were inhibited by fat, chemotaxis to G1000 was unchanged; in contrast, chemotaxis by EN is more complex. Fat might be inhibitory by disrupting membrane structure or more selectively by disrupting the ability to sense a glucose gradient, blinding EN to newly ingested feed.

Key Words: rumen protozoa, chemotaxis, fat inhibition

581 From Redox potential field measurement to its bioenergetic meaning in the rumen. J. P. Marden*[1,2], E. Ungerfeld[3], R. A. Kohn[4], C. Julien[1], E. Auclair[2], R. Moncoulon[1], and C. Bayourthe[1], [1]*Université de Toulouse, INRA, Castanet-Tolosan, France*, [2]*Lesaffre Feed Additives, Marquette-Lez-Lille, France*, [3]*Agriculture and Agri-Food Canada, Lethbridge, Canada*, [4]*University of Maryland, College Park.*

Anaerobic microbial metabolism is driven by free energy change (ΔG) of fermentation reactions, but ΔG can be difficult to estimate in biological systems. Ruminal metabolism is highly controlled by dynamics of dihydrogen (H_2) production and utilization, e.g. methanogenesis ($CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$; $\Delta G = \Delta G° + RT \ln [CH_4][H_2O]^2/[CO_2][H_2]^4$) is highly dependent on H_2 availability. Redox potential (E_h), in conjunction with pH, can be used to calculate equilibrium H_2 availability to study ΔG in fermentation systems. E_h is defined as the tendency of a biochemical/chemical system to oxidize (lose electrons to) or reduce (gain electrons from) another system. E_h measurements have been applied in fields as diverse as understanding fermentation in soils to ripening of cheese and maturation of wines. In the rumen, this variable was initially measured during the late 1950's in sheep and later in goats and cattle. Some studies reported a clear relationship between E_h and metabolic rate of rumen microbes when animals were fed grain and hay diets. E_h can be used in combination with pH and temperature to calculate equilibrium H_2 partial pressure (P_{H2}), based on the Nernst Equation: $rH = -\log P_{H2} = E_h/30 + 2pH$ at 312 K. Since H_2 is readily transferred among organisms in the rumen, it is generally near equilibrium in reality, or moving rapidly in that direction, and is central to the feasibility of major fermentation pathways in the rumen. Three trials: a dry cow model with 8 kg DMI/d (T8) and 2 lactating cow models with 21 (T21) and 28 kg DMI/d (T28) were used to continuously measure pH and E_h over 9 h around the morning meal to finally calculate mean ruminal P_{H2}. Lower DMI was associated with higher estimated P_{H2}, with a 10-fold significant decrease ($P<0.001$) at each feeding level (T8 vs. T21 & T21 vs. T28). Calculated P_{H2} values from simultaneous and continuous E_h and pH measurements can be valuable to study ΔG of processes highly dependent on H_2 availability, like methanogenesis, propionate formation, or even microbial growth. Processes dependent on releasing H_2 (e.g acetate and butyrate formation), are also dependent on H_2 concentration.

Key Words: ruminal redox potential, free energy, partial pressure of H_2

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582 Pharmacological amounts of nicotinic acid can reduce isoproterenol-stimulated lipolysis in cattle, but also reduce feed intake. K. S. Spivey, E. C. Titgemeyer*, and B. J. Bradford, *Kansas State University*, *Manhattan*.

Six Holstein steers (225 kg) were used in a series of experiments to determine if pharmacological supplies of nicotinic acid could reduce lipolysis stimulated by beta-agonists. The ruminally cannulated steers were fed a grain-based diet (72% corn, 12% soybean meal, 10% alfalfa) near ad libitum intake. In the first trial, nicotinic acid was continuously infused into the abomasum at 0, 2, 4, 8, 16, or 32 g/d in a 6x6 Latin square with 1-d periods. Steers were challenged with a pulse dose of isoproterenol (ISOP; 0.225 mg) with blood samples collected 8 min after ISOP to measure plasma NEFA. Results were inconclusive, and 4 of 6 steers demonstrated reductions in feed intake during the trial. In a second trial, the same steers were used in a replicated 3x3 Latin square with continual infusion of nicotinic acid in amounts of 0, 8, or 16 g/d. ISOP was dosed at 0.1125 mg, and blood samples were collected just prior to and 8 min after ISOP. Nicotinic acid at 16 g/d inhibited ($P<0.05$) the ISOP-stimulated increases in plasma NEFA concentrations, whereas 8 g/d did not. All 6 steers were then fed 60 mg/d zilpaterol-HCl, and 3 were provided a continual abomasal infusion of 16 g/d nicotinic acid, whereas 3 were infused with water. Steers given 16 g/d nicotinic acid had large reductions in feed intake, and nicotinic acid infusions were terminated after 3 d. Plasma glucose and insulin were somewhat elevated in response to nicotinic acid, even in the face of reductions in feed intake. Following discontinuation of the nicotinic acid infusions, cattle were maintained on their diets for 4 d before they were euthanized. Total liver fatty acids tended ($P=0.16$) to be elevated for steers that had received the nicotinic acid, with C16:0 and C18:1cis-9 demonstrating the largest increases. It appears that nicotinic acid (16 g/d) can inhibit ISOP-stimulated lipolysis, but the large negative effect on feed intake suggests that caution should be exerted when providing pharmacological doses of nicotinic acid to cattle.

Key Words: nicotinic acid, cattle

583 Effects of niacin infusion on transcript and protein abundance of the niacin receptor GPR109A in bovine tissues. B. J. Bradford*, L. K. Mamedova, K. S. Spivey, and E. C. Titgemeyer, *Kansas State University*, *Manhattan*.

Pharmacological effects of niacin (nicotinic acid) are likely mediated by GPR109A, a G protein coupled receptor with affinity for niacin. In most species, GPR109A is expressed primarily in adipocytes and macrophages, accounting for antilipolytic and cutaneous flushing responses to niacin. Tissue distribution of GPR109A has not been thoroughly investigated in cattle. We determined where GPR109A is expressed in cattle and assessed effects of niacin infusion on abundance. Six ruminally-cannulated steers (225 kg) were fed a grain-based diet (with 60 mg/d zilpaterol-HCl) near expected intake. Three steers received continuous abomasal infusion of 16 g/d nicotinic acid in 2 L water, and 3 received water alone. Steers provided 16 g/d niacin had dramatic reductions in feed intake, requiring discontinuation of niacin infusion after 3 d. Thereafter, steers were maintained with no niacin treatment until the end of the planned 7-d period. Steers were then euthanized and tissue samples were collected from tailhead fat, backfat, kidney fat, longissimus muscle, and liver for analysis of GPR109A mRNA by quantitative real-time PCR and protein by Western blotting. The mRNA for GPR109A was found in all tissues analyzed, and was most abundant in liver tissue, despite the fact that it has not been observed in liver of other species. Tailhead and kidney fat contained more mRNA for GPR109A than did longissimus muscle, as expected. Protein analysis by Western blot demonstrated presence of GPR109A protein in all tissues, except 3 of 6 backfat samples. The greatest abundance of GPR109A protein was in tailhead fat, kidney fat, and liver. Niacin treatment had no impact on mRNA or protein abundance of GPR109A in any analyzed tissue ($P>0.17$), but this is confounded by reduced feed intake by niacin and removal of treatments 4 d before euthanasia. The novel identification of the niacin receptor in bovine liver may provide