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ASSOCIATIVE EFFECT OF CEREALS AND OIL CAKES IN CONCENTRATE SUPPLEMENTS AND COMPLETE DIET FOR RUMINANTS ON *In vitro* FERMENTATION KINETICS

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ABSTRACT

Fermentation of grains in dairy ration along with oil seed meals (OSM) and urea-mineral-salt (UMS) fortification was studied using first order kinetic model of *in vitro* gas production (IVGP). Non-reducing sugars in OSM were 6% as compared to 1-3% in grains. Fermentation of oats was steadier than maize or sorghum. Addition of UMS to a dairy cow ration had no impact on IVGP of grains and OSM, and affected the initiation of fermentation but not in groundnut cake. When soya meal was added to grains alongwith UMS, the maize fermentation became steadier and comparable to oats, while spurt of IVGP was observed with sorghum at 7 h. UMS fortification reduced methane in the IVGP only in grains but not in OSM. Study indicated that maize was a good source of grain on urea-based diets compared to sorghum or oats. Addition of natural source of protein as OSM was essential when UMS is added in concentrate supplements.

Keywords: Fermentation kinetics, feedstuffs, fortification, gas production, ruminants

INTRODUCTION

The chemical analysis of feedstuffs although provides idea about nutritive composition does not give information about its fermentation value (Sallam, 2005) that is important for ruminant digestion. *In vitro* gas production (IVGP) technique, a rapid method of analysis, has widely been used during last decades by ruminant nutritionists to study feed degradation, detect small differences in fermentation characteristics between feedstuffs and allow more frequent sampling as compared to *in vitro* digestibility (DePeters *et al*., 2003; Rymer *et al*., 2005). IVGP technique is used to assess biological values of feeds based on the pattern of accumulated gas produced during incubation with rumen fluid under anaerobic conditions (Tedeschi *et al*., 2008). The basic model for rate determination with lag suggested by Krishnamoorthy *et al*. (1991) was simple and its kinetic parameters provide biological interpretations. This technique enables selection of feed or feed constituents for high efficiency of microbial protein (MBP) synthesis in rumen along with high dry matter digestibility and provides a basis for development of feeding strategies to maximize substrate fixation into microbial cells (Makkar, 2004). MBP synthesis in rumen is primarily dependent on carbohydrates as energy source. Starch from maize grains is reported to support more MBP growth than even molasses though later contains more soluble sugars (Luc *et al*., 2009). Choice of grain in the dairy animal ration is conflicted (Gulmez *et al*., 2010) due to the proportion of soluble starch fermented and complexity in their protein (Rooney and Pflugfelder, 1986). This study was undertaken to evaluate fermentation characteristics of three important grains in dairy ration along with common source of oil seed meal protein. In general, urea is used as non-protein nitrogen (NPN) source in dairy rations and hence in the present investigation urea fortification with grains and OSM protein source has been evaluated.

MATERIALS AND METHODS

Grains, oil seed meal and concentrate supplements

Widely used grains in dairy rations viz., maize, sorghum and oats along with oil seed meal (OSM) such as groundnut cake (GNC) and soya meal were selected to evaluate fermentation characteristics. Since straw is an unavoidable choice of roughage in developing countries, finger-millet straw (FMS) was taken as the roughage source. Samples were collected according to AOAC (2005). Concentrate supplements (CS) was prepared with 50 parts of either maize, sorghum or oat as source of grain, 42 parts of soya meal, and 8 parts of urea-minerals-common salt (UMS; ratio 3:3:2) keeping the concentrate to roughage ratio at 70:30.

Chemical analyses

Dry matter (DM), organic matter (OM), total nitrogen (N), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to AOAC (2005). Total soluble sugars were estimated by the method of Yemm and Willis (1954). Reducing sugar was estimated by Somogyi modified method (Somogyi, 1945). The amount of non-reducing sugar was calculated as the difference between total soluble sugars and reducing sugars and starch from the sugar free pellet was estimated by the method of Clegg (1956). Protein bound to NDF and ADF was determined by estimating nitrogen content in the residue of NDF or ADF of the sample (Licitra *et al*., 1996).

In vitro gas production

In vitro gas production (IVGP) studies were conducted using 100 ml plastic syringes. Rumen liquor was collected from 3 cows fitted with flexible rumen cannula (Large cannula #1C, M/s Bar Diamond, USA) and fed FMS and CS (M/s Karnatak Milk Federation, India: 89.7% DM, 15.75% CP, 12.75% total ash, 1.48% EE, 43.01% NDF, 26.1% ADF and 8.98% ADL and 2.4% acid insoluble ash) in the ratio of 70:30. Rumen liquor was drawn before offering feed at 8:00 h with a minimum of 6 h gap from the last offering of drinking water, strained through a double layer muslin cloth and immediately used as incubation media. Oven-dried samples of feedstuffs were weighed and ground by lab mill (M/s Jaico laboratory, India) using 1 mm sieve. Strained rumen liquor (10 ml) was taken along with 200 mg of substrate (Menke and Steingass, 1988) and 20 ml of buffer medium added (Srinivas *et al*., 2008). Piston was lubricated with petroleum jelly and fixed into the cylinder of syringe while removing any excess air. Syringes were kept in BOD incubator at 39°C on a slow moving rotor. Gas production in syringes was measured at 0, $^1/_2$, 1, $1^1/_2$, 2, 4, 6, 8, 12, 24, 36 and 48 h for roughage and only upto 24 h for grains, OSM and CS. At the end of incubation, CO_2 and methane were measured as described by Singh and Rekib (1975) using 25% sodium hydroxide solution (w/v), phenolphthalein indicator and titrated against standard 1 N HCl till pink colour (pH 3.7) appeared. Each ml of 1 N HCl consumed 0.044 ml of CO_2. Simultaneously a blank was also run to make correction in the measurement (Srinivas and Swain, 2009). IVGP values at different intervals were adjusted to blank which contained only buffer and inoculum. Blank corrected IVGP values of different time intervals were fitted to the first order kinetic model for rate determination with lag (Srinivas and Krishnamoorthy, 2005). These mathematical calculations were carried out using MS Excel worksheets (MS Office 2007).

$$Y = Y\propto [1 - e^{-k(t-\lambda)}]$$

Where, Y was total gas production, Y∝ was upper asymptote for total gas production, k represented rate of gas production, λ was lag for initiation of gas production and adjusted to time ‘t’.

Statistical analyses

The effect of IVGP at different time intervals (within subject factors) among different treatment groups (between subject factors) using ANOVA model included the repeat measure (Hirata *et al*., 2009). Multivariate tests included Pillai's Trace. Mauchly's test of sphericity was applied to test homogeneity within subject factors and Greenhouse-Geisser was used when sphericity assumed was significant or null hypothesis was rejected. Significance between estimated marginal means of treatments was estimated using Duncan's multiple range tests at 0.05% level of significance. Significance deference between means was differentiated with alpha superscripts. Variance among homogenous set of samples of grains, OSM, CS and complete ration were analysed using completely randomized block design based on the model: $Y_{ij} = \mu + T_i + B_j + e_{ij}$. Where, Y_{ij} was any observation for which $X_1 = i$ and $X_2 = j$ (X_1 was primary factor and X_2 was block factors), μ was general mean and e_{ij} was random error component (Das and Giri, 1991). Data on chemical analyses, IVGP and kinetics of FMS and FMS fortified with UMS were analyzed for significance using paired student t-test at 0.05% level of significance.

RESULTS AND DISCUSSION

The chemical composition of popularly used 3 grains and 2 OSM in dairy cow rations revealed that CP content in grains was 7.5 to 10.5% and 3 to 5 times lesser than OSM while starch content in grains was 5 to 10 times more compared to OSM (Table 1). Starch content in GNC was 2 times more compared to 4.5% in soya meal. Higher fat content in oats than maize or sorghum grain couples with higher lipase activity has been a disadvantage for longer storage of these grains. Although non-fibrous carbohydrates (NFC) are important for microbial growth in rumen, fermentation heat losses are also more for NFC (Russel, 2007). NFC content in GNC was about 2½ times higher than soya meal. Most of the soluble sugars present in grains or OSM were non-reducing sugars only and present at 5.5 to 6% level in OSM compared to 1 to 3% in grains. According to Bauphan *et al.* (2011), soluble sugar fractions in feedstuffs are important for chemotactic attraction of microbes in rumen for colonization and rapid initiation of fermentation for which OSM are more effective over grains. If soluble sugars help in initial microbial colonization, starch is important as source of energy for bacterial growth (Luc *et al*., 2009) which was 5 to 10 times more in grains than OSM. GNC was superior to soy meal in terms of protein and starch.

IVGP on oats was steadier than on maize or sorghum from 5 h of fermentation interval (FI). IVGP in maize and sorghum increased at increasing rate from 7 h of FI and was peaked at 12 h (Table 2).

Table 1: Chemical analyses (%) of grains and oil seed meals

Variables	Grains			Oil seed meal	
	Maize	Sorghum	Oats	GNC*	Soya meal
Dry matter	87.44	88.48	93.38	93.63	87.87
Organic matter	98.50	96.12	98.45	93.91	92.41
Crude protein	7.82	10.58	7.58	44.90	45.65
Ether extract	3.48	2.98	3.35	1.74	3.31
Total ash	1.50	3.88	1.55	6.09	7.59
Neutral detergent fibre	27.74	22.48	32.61	26.08	38.74
Acid detergent fibre	5.64	6.63	15.62	14.94	21.49
Cellulose	2.83	1.73	9.90	10.87	16.27
Lignin	1.31	1.90	4.56	2.97	5.29
Non-fibrous carbohydrates	59.47	60.09	52.90	21.19	8.71
Neutral detergent insoluble crude protein	2.13	2.97	3.09	1.63	1.69
Acid detergent insoluble crude protein	0.90	1.13	0.58	0.86	0.53
Reducing sugars	0.00	0.10	0.00	0.00	0.01
Non-reducing sugars	2.59	0.91	1.04	5.51	5.92
Starch	53.86	44.66	49.44	9.57	4.52

All estimates were average of triplicates; *GNC = Groundnut cake

Addition of UMS had no impact on overall fermentation of grains and OSM but affected the initial 1 h of IVGP. However, UMS fortification with GNC not influenced initial IVGP due to more non- reducing sugars. When soya meal was also added to maize, sorghum and oat grains along with UMS, IVGP pattern of maize became steadier and comparable to oat grain, while with sorghum a spurt of IVGP at 7 h of FI was observed. Increase in IVGP on oat grain also shifted from 5 to 7 h in presence of soy meal and UMS. UMS fortification to grains and/or OSM had variable impact on the fermentation pattern of feedstuffs that could be attributed to presence of non-reducing sugars and variation in feedstuff characteristics. Although fortification of UMS to FMS slowdown the fermentation vigour in the initial 12 h, total IVGP after 48 h was comparable (Table 3). On complete ration mixture, IVGP pattern of maize and oat based CS was comparable and extent of IVGP on maize based CS was even better than sorghum based CS. Thus, UMS fortification yielded better improvement in fermentation pattern on maize based CS than either sorghum or oat based CS.

Table 2: *In vitro* gas production (ml g^{-1} substrate) on grains and oil seed meals with and without urea, minerals and salt (UMS) fortification

Feed	*In vitro* Fermentation time intervals (h)								
	0.5	1	2	3	5	7	12	18	24
Maize	0.58	2.00	2.15^{a}	1.29^{a}	6.44^{a}	41.80^{c}	81.70^{b}	66.15^{c}	33.65^{a}
Jowar	2.02	4.00	4.44^{b}	6.30^{b}	17.65^{b}	44.80^{c}	95.20^{c}	66.65^{c}	32.15^{a}
Oat	1.08	4.50	8.80^{c}	9.80^{cd}	36.15^{d}	61.30^{d}	45.70^{a}	41.44^{b}	25.65^{a}
GNC	1.94	2.46	6.79^{bc}	10.33^{d}	23.41^{c}	15.41^{a}	47.35^{a}	13.65^{a}	32.04^{a}
Soya meal	1.36	1.87	6.31^{bc}	7.22^{bc}	21.31^{bc}	23.88^{b}	47.35^{a}	20.29^{a}	54.26^{b}
SEM	0.93^{ns}	1.29	1.23**	1.37**	2.48**	2.98**	6.44**	5.76**	4.62**
Maize + UMS	0.00	0.00^{a}	3.70^{a}	5.50^{ab}	22.71^{c}	44.44^{c}	69.77^{c}	31.40^{c}	35.91^{b}
Jowar + UMS	0.00	0.00^{a}	2.25^{a}	4.80^{a}	18.11^{b}	25.22^{b}	66.65^{c}	39.80^{d}	49.06^{c}
Oat + UMS	0.00	0.00^{a}	2.43^{a}	8.21^{b}	35.90^{d}	42.06^{c}	28.48^{a}	13.77^{a}	26.83^{a}
GNC + UMS	0.45	2.66^{b}	9.85^{b}	7.31^{ab}	15.71^{ab}	19.03^{a}	33.58^{ab}	22.60^{b}	37.27^{b}
Soya meal + UMS	0.00	0.00^{a}	4.55^{a}	6.61^{ab}	12.99^{a}	25.73^{b}	36.28^{b}	33.66^{cd}	31.37^{ab}
SEM	0.28^{ns}	0.46**	2.21**	1.34**	1.73**	2.36**	3.49**	3.34**	3.45**
Maize + soya meal + UMS	0.00	2.89	1.50	3.35^{a}	14.93^{a}	62.78^{a}	61.57^{b}	55.46^{b}	37.50^{b}
Jowar + soya meal + UMS	0.00	1.45	3.00	6.75^{b}	22.51^{c}	96.63^{b}	26.02^{a}	28.50^{a}	15.99^{a}
Oat + soya meal + UMS	0.00	0.49	3.00	4.31^{a}	10.57^{a}	62.92^{a}	68.83^{b}	45.84^{b}	34.34^{b}
SEM	0^{ns}	0.98^{ns}	1.13	0.95**	1.74**	8.60**	4.06**	5.45**	4.34**

Values bearing different superscripts within a column for same set of variables differ significantly. *P < 0.05 and **P < 0.01; UMS = urea-mineral-salt mix; ns = non significant

Table 3: *In vitro* gas production (ml g^{-1} substrate) on diets with different grain sources in concentrate supplement

Feed	*In vitro* Fermentation intervals (h)										
	0.5	1	2	3	5	7	12	18	24	36	48
Finger millet straw	0	0	6.82	0.52	5.69	10.86	13.16	21.25	27.97	23.23	22.80
Finger millet straw + UMS	0	1.00	3.50	2.00	4.50	4.00	9.50	15.00	20.00	26.50	27.00
SEM	0	0.67^{ns}	1.40^{ns}	1.31^{ns}	1.11^{ns}	0.81^{ns}	2.49^{ns}	1.50^{ns}	3.68^{ns}	4.25^{ns}	2.18^{ns}
Diet 1: Straw + CS-M	0	2.60	3.10	2.60	8.80^{b}	11.20^{b}	27.60^{b}	24.30^{b}	33.10	33.80^{b}	18.80^{b}
Diet 2: Straw + CS-J	0	1.23	3.78	1.89	5.00^{a}	8.11$_{a}$	30.66^{c}	23.11^{ab}	34.34	33.78^{b}	15.66^{a}
Diet 3: Straw + CS-O	0	2.50	3.13	2.50	10.00^{b}	11.88^{b}	23.75^{a}	19.38^{a}	32.50	24.38^{a}	20.00^{b}
SEM	0	1.01^{ns}	0.99^{ns}	1.05^{ns}	1.01**	1.57*	1.25**	1.91*	2.39	2.10**	1.63*

Values bearing different superscripts within a column for same set of variables differ significantly. *P < 0.05 and **P < 0.01; UMS = urea-mineral-salt mix. Concentrate supplement (CS) consisted of soyabean meal + urea + molasses + salt and, maize (CS-M) or jowar (CS-J) or oat (CS-O) grain; NS = Non significant

Table 4: *In vitro* gas production kinetics in grains and oilseed cakes

Feed	Y-asymptote (ml g^{-1})	λ (h)	Y_{α} (ml g^{-1})	$T_{½}$ (h)	k ($x10^2$)	CO_2 (%)	Methane (%)
Maize	236.76^{c}	0.70^{a}	212.09^{b}	7.04^{a}	9.88^{c}	82.42^{c}	17.58^{a}
Jowar	273.21^{d}	0.68^{a}	247.80^{c}	6.80^{a}	10.19^{c}	82.38^{c}	17.62^{a}
Oat	234.42^{c}	0.68^{a}	212.95^{b}	6.76^{a}	10.27^{c}	81.88^{c}	18.12^{a}
GNC	153.38^{a}	0.76^{b}	134.86^{a}	7.56^{b}	9.21^{b}	75.25^{b}	24.75^{b}
Soyameal	183.85^{b}	0.86^{c}	155.58^{a}	8.61^{c}	8.11^{a}	72.64^{a}	27.36^{c}
SEM	14.55^{**}	0.02^{**}	13.37^{**}	0.21^{**}	0.26^{**}	0.99^{**}	0.99^{**}
Maize + UMS	213.44^{b}	0.72^{a}	190.57^{d}	7.21^{a}	9.62^{d}	83.02^{c}	16.98^{b}
Jowar + UMS	205.89^{b}	0.79^{cd}	179.07^{c}	7.89^{cd}	8.80^{ab}	85.19^{d}	14.81^{a}
Oat + UMS	158.25^{a}	0.73^{ab}	140.84^{b}	7.30^{ab}	9.51^{cd}	81.28^{c}	18.72^{b}
Soya meal + UMS	151.18^{a}	0.76^{bc}	132.96^{ab}	7.61^{bc}	9.16^{bc}	71.42^{a}	28.58^{d}
GNC + UMS	148.45^{a}	0.80^{d}	128.44^{a}	8.00^{d}	8.67^{a}	74.48^{b}	25.52^{c}
SEM	5.76^{**}	0.01^{**}	4.87^{**}	0.17^{**}	0.20^{**}	1.03^{**}	1.03^{**}
Maize + soya meal + UMS	239.97^{b}	0.71^{b}	214.93^{b}	7.11^{b}	9.78^{a}	76.50^{b}	23.50^{a}
Jowar + soya meal + UMS	230.29^{b}	0.71^{b}	206.88^{b}	7.08^{b}	9.80^{a}	75.65^{b}	24.35^{a}
Oat + soya meal + UMS	200.84^{a}	0.65^{a}	183.99^{a}	6.53^{a}	10.63^{b}	71.67^{a}	28.33^{b}
SEM	11.36^{**}	0.01^{**}	10.34^{**}	0.15^{*}	0.23^{**}	1.50^{**}	1.50^{**}
Finger millet straw	86.27	0.90^{a}	71.79	8.97^{a}	7.81^{a}	75.73	24.27
Finger millet straw + UMS	59.50	0.91^{b}	49.13	9.09^{b}	7.76^{b}	74.28	25.72
SEM	6.02^{ns}	0.01^{*}	5.36^{ns}	0.08^{*}	0.10^{*}	0.30^{ns}	0.30^{ns}
Finger millet straw + CS-M	165.63^{c}	1.88^{b}	96.39^{b}	17.67^{b}	3.93^{a}	78.52	21.48
Finger millet straw + CS-J	157.50^{b}	1.68^{a}	90.74^{a}	17.88^{b}	3.88^{a}	76.92	23.08
Finger millet straw + CS-O	150.00^{a}	1.96^{b}	89.80^{a}	16.93^{a}	4.11^{b}	76.65	23.35
SEM	3.52^{**}	0.06^{**}	2.34^{*}	0.33^{*}	0.08^{*}	0.88	0.88

Values bearing different superscripts within a column for same set of variables differ significantly. $^{*}P < 0.05$ and $^{**}P < 0.01$; UMS = urea-mineral-salt mix. Concentrate supplement (CS) consisted of soyabean meal + urea + molasses + salt and, maize (CS-M) or jowar (CS-J) or oat (CS-O) grain; ns = non significant;

Y∝ was significantly higher on sorghum grain and comparable between maize and oats while λ was similar in three grains (Table 4). According to Srinivas and Krishnamoorthy (2005) both the kinetic parameters are related to microbial characteristics in the inoculum. Though initiation of fermentation among three grains was similar, extent of fermentation was higher in sorghum thus, explaining quantitative difference in fermentation as influenced by microbial activity. Hence, it could be presumed that sorghum may support more microbial activity in the rumen. Similarly, between GNC and soya meal, Y∝ was more on later and contrary was true for λ. Since reducing sugars were about similar in both the OSM, only solubility of nutrients, particularly starch, might make the difference. UMS fortification to grains alone had adverse impact on the IVGP kinetics and more conspicuous with sorghum followed by oats grain while it was lesser with maize grain. Though UMS fortification affected IVGP kinetics of OSM, decrease in Y∝ was lesser than for the grains. UMS fortification however, decreased k and extended T½ in grain or OSM. Both the kinetic parameters were related to the physical characteristics of feedstuffs, hence, any stress of rapid release of ammonia in the inoculum from urea may not have impended the impact on substrate unlike on microbial activity (Srinivas and Swain, 2009). Moreover, ammonia released from urea even softens the substrate, particularly fibrous feedstuffs as evident from the reduced rate constant on FMS + UMS. UMS fortification reduced methane proportion in the IVGP only in grains but not in OSM. This study indicated that maize is a good source of grain on urea based diets compared to either sorghum or oats and addition of natural source of protein as OSM appears to be essential when UMS is added in CS.

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