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Impact of dual organsim probiotics supplement on intake and milk yield during early, mid and late lactation in cows

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The probiotic food concept is moving progressively towards the dietary supplements that may influence the intestinal microbial composition and activities to influence the gut health and overall well being (Srivastava *et al.* 2011). Probiotics in ruminants could selectively stimulate the growth and activity of one or limited number of bacteria in the gastrointestinal tract (Ohashi and Ushida 2009). Efficacy of probiotics varies with the strains and activity of organism it carries. Dose required for *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* probiotics was 1×10^9 and 3×10^9 cfu, respectively, and impact observed was on feed efficiency (FE) and average daily gain of the buffalo calves (Malik and Srinivas 2010). Many studies recommended probiotic supplements to lactating cows mostly with any one selected strains of bacteria or fungi (Vibhute *et al.* 2011), buffaloes (Malik and Srinivas 2010). Newbold (1996) however, predicted the future possibility of probiotics with 2 species having different mode of action. This study was conducted to evaluate the impact of dual organism (bacteria plus yeast) probiotics supplementation (DOPS) on early, mid and late lactation in crossbred cows.

Multifarious, nondescriptive but, dominantly Holstein Frisian crossbred cattle (36) were divided into 2 groups; control (CG) and experimental (EG) with 6 cows each in early (calving to 100 d), mid (101 to 210 d) and late (211 to 305+ d) lactation with mean body weight of 435±22 kg. Cows were tied in well ventilated barn having arrangement for individual feeding and fed *ad lib.* finger millet straw as basal roughage twice a day at 9 AM and 5 PM. Concentrate supplement (CS) consisted maize, groundnut cake, wheat bran, gram husk, common salt and mineral mixture in the ratio of 22:20:28:28:1:1, respectively. CS was supplemented after soaking with water at 8 AM and 3 PM as per the requirement (ICAR 1999).

Apart from the basal diet, experimental group (EG) of cows in early, mid and late lactation were supplemented DOPS consisted *Saccharomyces cerevisiae* and *Lactoacillus reuteri* and compared with CG without DOPS. Water soluble DOPS was fed to the cows in EG by top dressing to CS once in a day at 3 PM @ 10 g/d. Each gram of freeze dried DOPS contained viable count of 5 billion cfu.

Milk recording, digestibility trial and chemical analysis: Milk yield of the cows was recorded twice dairy at 5 AM and 3 PM for 60 d. Milk samples were collected twice in a week for 2 consecutive days randomly and analysed for total solids, fat and solids-non-fat (SNF) as described in AOAC (2005). After 30 d of preliminary period of feeding, digestibility trial for 5 d was conducted. Body weight of animals, at 0 h of feeding and watering, was recorded for 2 consecutive days before and after the trial. Feed offered, orts and faeces excreted were weighed every day at 8 AM. Sub-sample of 1/400 of total faeces was taken for DM estimation in drought oven at 100±5°C for 12 h. Oven dried feed, orts, and feces samples were pooled for 5 d and ground by lab mill using 1 mm sieve and analysed for proximate (AOAC 2005) and cell wall constituents (Van soest *et al.* 1991). Another sub-sample of 1/800 of total faeces was preserved with known quantities of 10% H_2SO_4 (V/V) in preweighed glass bottles and compounded for 5 d with care to mix thoroughly with previous contents in the bottles. At the end of the collection period, bottles were weighed; mixed thoroughly and about 5 g sample was taken for acid digestion to analyse nitrogen (AOAC 2005). Energy parameters were calculated using empirical models recommended by ARC (1984) using regression models for digestible (DE), metabolizable (ME), energy retention (ER). Energy digestibility, heat increment, q-value and efficiency were derived mathematically from the values of DE, ME and ER.

Statistical analysis: Pair-wise comparison between CG and EG for same stage of lactation for intake, digestibility and milk parameters were analysed using student t-test. Significance difference between means was tested against probability P<0.01 to P<0.05. Weekly milk yield was subjected to repeat measure analysis wherein between weeks as within subject factors and groups as between subject factors. Statistical analysis were made using Statistical Package for Social Science (Ver. 17.0).

Chemical composition of the CS and ragi straw is presented in Table 1. Straw intake improved in 3 stages of

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Table 1. Chemical composition of feedstuffs, concentrate supplement and straw

Parameter	Ragi straw	Concentrate supplement
DM	88.1	32.6
Organic Matter	93.5	94.5
Crude protein	4.1	17.9
Ether extract	1.3	3.5
Total Carbohydrates	94.6	78.6
Total ash	6.5	5.6
Acid Insoluble Ash	2.8	1.4
Cell contents	31.4	52.0
Neutral detergent fibre	68.6	48.0
Acid detergent fibre	36.0	27.7
Hemicelluloses	32.6	20.4
Cellulose	6.2	19.8
Acid detergent lignin	12.7	6.6
Non fibrous carbohydrates	19.5	24.9

lactation in EG compared to CG (P< 0.01). CS intake in EG was however, improved only in early lactation by 4.8% (P< 0.01) but no significant improvement in mid or late lactation (Table 2). Often feed intake is compromised in early lactation due to homeorhetic affect until mid lactation thus, cows suffer negative energy balance (Walsh *et al.* 2011). Such positive response in intake with direct fed microbial supplements was observed up to 2.6 kg/d in transition cows (Nocek *et al.* 2003). Although straw intake was increased in EG compared to CG in mid and late lactation by 7% but increase was at the cost of reduced CS intake by 3 to 5%. Chiquette (2009) inferred that the increase in feed intake with probiotic supplements was a result of pH stabilization in rumen and improved environment for cellulose digestibility. Digestibility coefficient of proximate or cell wall constituents did not alter with DOPS. Although bacterial and protozoa population in rumen reportedly increased with either bacterial or yeast probiotics but no significant improvement was observed in DM, CP or NDF digestibility in buffaloes (Malik and Srinivas 2010).

Milk yield (Table 3) increased significantly (P< 0.01) in early (5%) and late lactation (47%) in EG compared to CG. In contrary, milk yield during mid lactation was reduced (P< 0.01) by 5% in EG compared to CG. Multivariate test showed significant difference between weeks and treatments for 4% FCM in early (P< 0.02), mid (P< 0.02) and late (P< 0.01) lactation. Weekly difference in 4% FCM yield was not consistent in early and mid lactation in the CG and EG however, consistently significant (P< 0.05) difference observed in late lactation at all 8 weeks in late lactation, Milk fat (%) was reduced (P< 0.01) in early lactation in EG compared to CG which was phenomenal inverse relationship between milk yield and fat%. Milk fat was also decreased during mid lactation in EG however, it

Table 2. Intake and digestibility of cows during different stages of lactation with probiotic supplement

Parameter	Stage of lactation: Control group			Stage of lactation: Experimental group		
	Early	Mid	Late	Early	Mid	Late
Intake (kg/d)						
Straw	6.24±0.04^{a}	5.93±0.06^{a}	5.80±0.06^{a}	6.60±0.02^{b}	6.37±0.02^{b}	6.20±0.03^{b}
CS	7.94±0.07^{a}	6.97±0.09	6.86±0.09	8.49±0.07^{a}	6.94±0.06	6.65±0.10
Total	14.09±0.07^{a}	12.89±0.11^{a}	12.66±0.10	14.84±0.08^{a}	13.28±0.08^{b}	12.84±0.10
Digestibility coefficient of nutrients (%)						
DM	67.54±1.39	67.16±6.61	70.76±4.49	69.57±2.81	64.51±3.59	71.54±6.94
OM	70.37±1.50	69.30±6.60	73.60±4.21	72.12±2.95	66.90±3.88	73.95±6.59
CP	71.38±2.14	69.35±7.69	73.46±4.45	71.34±3.68	64.09±3.94	72.79±4.61
EE	64.33±0.90	70.56±10.56	66.21±5.55	67.36±4.59	60.38±1.64	75.73±5.71
TCHO	69.10±2.09	68.03±6.28	72.48±3.89	71.04±2.83	66.12±3.92	72.72±6.95
CC	79.65±0.77	76.64±6.97	77.97±2.81	81.17±1.91	72.40±3.20	79.62±4.72
NDF	43.81±4.48	49.36±6.09	52.91±9.75	46.88±9.03	46.06±1.59	53.53±13.81
ADF	41.99±5.97	45.21±3.98	56.13±7.93	50.43±5.17	46.27±0.60	59.96±4.68
Hemicelluloses	74.01±7.40	78.47±8.61	72.37±6.52	78.77±0.90	74.52±7.27	77.02±8.76
Celluloses	32.37±7.91	24.76±1.05	46.44±8.24	38.11±8.42	24.37±5.42	50.07±5.09
NFC	88.19±0.22	80.56±5.41	79.38±0.59	89.69±9.80	76.28±0.21	80.62±5.70
Energy dig	77.36±1.50	81.96±1.91	80.53±5.25	79.19±3.38	78.54±1.21	81.51±7.54
Energy partition						
GE (Mcal)	48.14±0.82	51.51±0.16	55.35±7.68	51.23±3.44	49.90±3.22	58.24±11.63
DE (Mcal)	37.23±0.09	42.31±0.76	45.14±9.11	40.69±4.46	39.46±3.37	48.35±13.87
ME (Mcal)	30.62±0.09	34.90±0.64	37.26±7.63	46.88±9.03	32.53±2.81	39.96±11.60
ME/GE (q-value)	0.64±0.01	0.68±0.02	0.67±0.05	0.65±0.03	0.65±0.01	0.67±0.05
HI (Mcal)	18.90±0.03	20.79±0.25	21.93±3.40	20.21±1.70	19.74±1.27	23.08±5.31
ER (Mcal)	11.72±0.06	14.11±0.39	15.33±4.23	13.34±2.03	12.80±1.54	16.88±6.29
Energy efficiency (%)	38.28±0.08	40.23±0.56	40.52±3.05	39.58±1.65	38.87±0.99	41.13±3.81

Values bearing a, b superscripts between same stage of lactation differed significantly.

Table 3. Milk yield and composition during lactation with probiotic supplement in crossbred cows

Parameter	Stage of lactation: Control group			Stage of lactation: Experimental group		
	Early	Mid	Late	Early	Mid	Late
Milk yield (kg/d)	10.97±0.09[a]	6.23±0.07[a]	2.45±0.07[a]	11.93±0.10[b]	6.04±0.08[b]	3.79±0.08[b]
Total solids	12.81±0.18	13.32±0.25	12.95±0.17	12.76±0.20	13.09±0.16	13.20±0.11
Milk fat	4.36±0.08[a]	4.59±0.01	4.10±0.13[a]	3.98±0.17[b]	4.43±0.01	4.64±0.04[b]
SNF	8.45±0.20[a]	8.74±0.26	8.85±0.27[a]	8.68±0.20[b]	8.59±0.16	8.56±0.11[b]
Feed efficiency (kg feed/kg milk)	1.24±0.01[a]	2.00±0.03[a]	4.43±0.13[a]	1.20±0.01[b]	2.17±0.04[b]	3.05±0.06[b]
Weekly 4% Fat corrected milk (FCM) yield						
1st week	11.13±1.10	6.81±0.68	3.07±0.45[a]	11.92±1.36	6.87±0.56	4.35±0.29[b]
2nd week	11.35±1.13[a]	6.95±0.71[a]	2.94±0.49[a]	12.20±1.03[b]	6.28±0.63[b]	4.02±0.34[b]
3rd week	11.26±1.43	6.62±0.67	2.81±0.46[a]	12.37±1.21	6.50±0.66	4.08±0.28[b]
4th week	11.18±1.17[a]	6.69±0.64	3.05±0.61[a]	12.85±1.26[b]	6.51±0.70	4.07±0.30[b]
5th week	11.16±1.05	6.40±0.63	2.91±0.63[a]	12.38±1.42	6.31±0.57	4.42±0.26[b]
6th week	11.61±0.90	6.39±0.61	2.64±0.59[a]	12.43±1.47	6.28±0.68	4.11±0.25[b]
7th week	11.86±1.11	6.50±0.68	2.56±0.59[a]	12.55±1.41	6.34±0.75	3.86±0.24[b]
8th week	11.52±0.96	6.74±0.60	2.76±0.69[a]	12.56±1.26	6.47±0.73	3.75±0.29[b]
Mean (kg/d)	11.38±0.09[a]	6.64±0.07[a]	2.87±0.07[a]	12.40±0.10[b]	6.45±0.08[b]	4.21±0.08[b]

Values bearing a, b superscripts between same stage of lactation were differ significantly.

was statistically insignificant (P = 0.20). Unlike in early and mid lactation, milk fat was increased significantly (P< 0.01) in late lactation in EG compared to CG. Higher feed intake per kg of milk in late lactation might improve body condition of the cows. SNF content in the milk in CG was below the standards set for the cow milk in early lactation but, it was improved significantly (P< 0.01) in EG with DOPS. Milk protein content is usually affected when dairy animals are under stress. Contrarily, SNF (%) in mid and late lactation was lesser in EG than CG however, difference was significant only in late lactation (P< 0.01). Králîèková *et al.* (2012) reported increase in milk total solids after 190 d of lactation. There were different opinions on the impact of probiotic supplements on increase in feed intake (Williams *et al.* 1991) or FE (Malik and Srinivas 2010). However, both are functionally related to feed quality and gut function. Since, probiotic supplements have no role in improving the feed quality, any improvement in intake or feed efficiency was due to improvement in gut function.

Benefit of cost from the additional improvement in the milk yield, feed consumption and cost of supplements was also examined. Total cost of increased intake of straw (₹ 8/kg), CS (₹ 25/kg) and probiotic (₹ 500/kg) was ₹ 17=88, 7=69 and 7=70, respectively in early, mid and late lactation. Correspondingly income or loss due marginal increase in milk yield (₹ 20/kg for 3% FCM) was ₹ 20=40, –6=80 and 26=80, respectively. Profit was ₹ 2=52 and ₹ 26=80 in early and late lactation but, it was loss of ₹ 14=49 in the mid lactation. Choice of DOPS in early lactation may not convince if considered net balance between cost and increased milk yield but for health improvement and increased SNF.

Feeding of DOPS would be beneficial in recovering the cost through increased milk production. Study concluded that the impact of DOPS was effective in early (first 100 d) and late lactation (after 210 d) than mid lactation. DOPS improved feed intake, particularly straw intake and, FE for milk production.

SUMMARY

Study was conducted to evaluate the impact of dual organism probiotic supplement (DOPS) on different stages of lactation. Crossbred cattle (36) were divided into control (CG) and experimental (EG) groups with 6 cows each in early (calving to 100 d), mid (101 to 210 d) and late (211 to 305+ d) lactation. Straw intake in EG was improved in all stages of lactation compared to CG. CS intake in EG improved in early lactation by 4.8%. Although DM intake improved with DOPS, digestibility of nutrients did not change significantly. Milk yield in EG increased significantly in early (5%) and late lactation (47%) compared to CG. In contrary, mean 4% fat corrected milk (FCM) yield in EG in mid lactation reduced by 3% compared to CG. Unlike in early and mid lactation, milk fat (%) in late lactation increased significantly in EG compared to CG. SNF content in milk was below the standards for the cow milk in early lactation in CG which improved significantly in EG. Study concluded that DOPS improved straw intake and effective during early (up to 100 d) and late lactation (> 210 d).

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