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Direct-Fed Microbial Supplementation: Quantifying Growth Performance, Feed Conversion Efficiency, and Economic Impact in Kankrej Calves

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ABSTRACT: This investigation evaluates the influence of Direct-Fed Microbials (DFM) on growth metrics, feed conversion efficiency, and economic parameters in Kankrej calves. Conducted at Gujarat's Animal Nutrition Research Station over 98 days, the study involved 15 calves distributed into three groups, with two experimental groups receiving 2% and 3% DFM in their Total Mixed Ration (TMR). Biweekly assessments revealed a consistent yet non-significant trend towards increased weight in DFM-supplemented groups compared to controls. This pattern suggests a potential for DFM to positively affect growth over time. The DFM cohorts also demonstrated improved feed efficiency, with a marked reduction in Dry Matter Intake (DMI), indicative of enhanced nutrient assimilation and metabolic function. From an economic standpoint, DFM supplementation was associated with decreased feed costs and heightened feed efficiency, suggesting a cost-effective strategy for calf nutrition. Overall, the study provides evidence supporting the integration of DFM into calf-rearing practices, potentially contributing to more economically and environmentally sustainable livestock management.

Keywords: Direct-fed microbials, Kankrej calves, Solid-state fermentation, Growth performance, Feed efficiency, Economic viability, Rumen microflora, Nutritional optimization.

INTRODUCTION

In the domain of animal husbandry, the enhancement of growth performance, feed efficiency, and economic viability is of utmost importance. The application of direct-fed microbials (DFM), which includes a variety of beneficial bacteria and yeasts, is gaining traction for its potential to positively influence these key parameters in livestock (Monteiro *et al*., 2022). DFM is particularly noted for its capacity to bolster gastrointestinal health, which is pivotal for improved growth rates and efficient feed utilization (Alawneh *et al*., 2024).

This research focuses on the Kankrej calf, a breed that is indigenous to the arid regions of Gujarat, India, and is renowned for its resilience and adaptability. Despite its inherent robustness, there is a compelling need to enhance the breed's growth potential and feed efficiency to amplify its economic value in the region's agricultural framework (Patel *et al*., 2014). Our study assesses the impact of DFM supplementation on the growth performance and feed efficiency of Kankrej calves, with a particular emphasis on measurable outcomes such as body weight gain and feed conversion ratios.

The inclusion of DFM in the calves' Total Mixed Ration (TMR) is investigated to determine the degree to which these microbials can elevate growth rates and optimize feed utilization. Additionally, the study examines the economic implications of DFM supplementation, analyzing its cost-effectiveness and feasibility for implementation by local farmers. The anticipated outcomes of this research are expected to make significant contributions to the field of animal nutrition, providing evidence-based insights that could foster more efficient and economically viable rearing practices for Kankrej calves.

MATERIALS AND METHODS

A. Cultivation and Characterization of Direct-Fed Microbials (DFM)

(i) Microbial Isolation and Identification. DFM strains were isolated from ecologically diverse sources, such as raw milk, dairy by-products, and the rumen of healthy ruminants, to ensure a broad representation of microbial efficacy. Advanced selective culturing techniques were employed using media like de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB) and nutrient agar with cycloheximide for *Bacillus* spp. The Hungate roll-tube method was utilized for cultivating strict anaerobes. Metabolic profiling was conducted using Biolog phenotypic microarrays, complemented by HPLC analysis of fermentation end-products, to ascertain the metabolic capabilities and fermentation dynamics of the strains.

(ii) Solid-State Fermentation (SSF) of DFM. The inoculum was prepared by propagating LAB and *Bacillus* spp. strains in their respective liquid media. SSF was performed on pretreated lignocellulosic substrates from vegetable and fruit market waste, optimized for microbial enzymatic activity. A custom-designed fermenter facilitated precise control over environmental parameters, including temperature, humidity, and oxygen levels, with continuous nitrogen flushing to maintain an oxygen-free environment. The fermentation kinetics were monitored, and adjustments were made to optimize microbial growth and metabolite production.

B. Experimental Design

Fifteen Kankrej calves, aged 6 to 9 months, were selected based on stringent criteria of initial body weight and health status to ensure homogeneity. The study spanned 98 days, with calves assigned to three dietary groups: a control group (T1) fed a basal diet, and two experimental groups (T2 and T3) receiving 2% and 3% DFM supplementation, respectively. Individual housing in ventilated pens was maintained to mitigate environmental stressors and prevent cross-contamination.

(i) Feed Formulation and Management. The Total Mixed Ration (TMR) was meticulously formulated to align with the nutritional guidelines of the Indian Council of Agricultural Research (ICAR) for growing calves. The TMR was dynamically adjusted in response to weekly body weight assessments. Ingredients were sourced locally and analyzed for their proximate composition following AOAC International methods to ensure nutritional consistency and quality.

(ii) Animal Husbandry and Health Surveillance. An acclimatization period of two weeks was implemented, during which calves received health evaluations, vaccinations, and deworming treatments. Additionally, Health surveillance protocol was established, including weekly fecal sampling to monitor gastrointestinal flora and parasitic load, thereby maintaining the health and well-being of the calves throughout the study.

C. Data Collection and Statistical Analysis

To ensure the highest level of data accuracy and reliability, growth parameters such as body weight, body length, withers height, and heart girth were recorded biweekly using measuring instruments. Feed intake was monitored daily, and the proximate analysis of the remaining feed samples provided insights into nutrient utilization efficiency. Fecal samples were analyzed for consistency, pH, and microbial composition, offering a comprehensive view of digestive health. For the statistical analysis, we employed both parametric and non-parametric tests, chosen based on the data's distribution and homogeneity. This approach allowed for a robust examination of the collected data, ensuring that our findings are both statistically significant and relevant to the study's objectives.

RESULTS AND DISCUSSION

A. biweekly body weight (kg) and Body weight gain (g/day)

In this exploration, we documented biweekly body weight (kg) and daily weight gain (g/day) across three treatment cohorts (T1, T2, T3), as delineated in Table 1. Commencing from a baseline of statistical parity, the study unveiled a compelling divergence in growth trajectories, with cohorts receiving DFM supplementation (T2 and T3) demonstrating an accelerated growth rate compared to the control cohort (T1).

Table 1: Average biweekly body weight (kg) and Body weight gain (g/day).

	Body weight (kg)			Body weight gain (g/day)		
	T_1	T_2	T_3	T_1	T_2	T_3
Initial	100.81	100.81	100.01			
P_1	105.78	106.70	105.54	355.32	420.63	395.34
P_2	111.47	114.03	111.18	406.46	523.43	402.36
P_3	118.16	122.25	119.22	477.50	587.57	574.92
P_4	124.47	131.02	128.22	450.56	626.43	642.71
P_5	132.32	139.41	138.15	560.73	598.86	709.00
P_6	141.36	149.02	146.98	645.88	687.00	631.00
P_7	148.09	161.31	158.88	480.61	877.43	849.87
Avg.	**122.81±12.58**	**128.07±11.75**	**126.02±12.35**	**482.44^b±57.55**	**617.34^a±29.23**	**600.74^a±66.06**

*In a row, the superscripts a and b differ significantly (P<0.05)

(i) Nutritional Adequacy of Total Mixed Ration (TMR). The Total Mixed Ration, crafted to align with the ICAR feeding standards (2013), provided a balance of nutrients. This strategic formulation was pivotal in underpinning the calves' developmental journey, as evidenced by the consistent increase in body weight across the cohorts.

(ii) Analytical Dissection of Growth Performance. The study's denouement saw the average weights for T1, T2, and T3 ascend to 148.09 kg (about 326.48 lb.), 161.31 kg (about 355.63 lb.), and 158.88 kg (about 350.27 lb.), respectively. These figures not only signify a robust growth from the inception weights but also suggest an enhanced growth impetus, potentially ascribed to the DFM supplementation.

(iii) Statistical Nuances and Growth Trends. Despite the absence of overt statistical disparity in growth rates (P>0.05), a granular inspection of the dataset revealed nuanced yet consistent growth patterns. The T2 cohort, supplemented with 2% DFM, consistently registered higher body weights throughout the study duration, with the T3 cohort displaying a parallel, albeit less pronounced, trend. The percentage differences in mean weights between the treatment cohorts and the control cohort (4.10% for T2 and 2.55% for T3) subtly hint at the positive influence of DFM on growth outcomes. Our findings dovetail with the broader corpus of research, such as the study by Kucukoflaz *et al*. (2022), which reported an insubstantial impact of DFM supplementation on calf weight. Conversely, Ogunade *et al*. (2020); Casper *et al*. (2021); Davies *et al*. (2022); Cappellozza *et al*. (2023) reported a significant increase in weight for DFM-treated calves. These contrasting results illuminate the intricate nature of DFM supplementation and its variable impact on calf growth, thereby punctuating the exigency for continued research to refine DFM application strategies in calf rearing.

(iv) Quantitative Growth Analysis through DFM Supplementation. Table 3 encapsulates the trajectory of body weight gain (g/day) in calves across the experimental cohorts T1, T2, and T3. The data elucidates a consistent pattern of augmented growth in the DFM-supplemented groups (T2 and T3), with daily weight gains outstripping those of the control group (T1). This trend substantiates the hypothesis that DFM supplementation is positively correlated with enhanced growth performance in calves.

(v) Statistical Validation of Growth Enhancement. T The statistical analysis reveals a significant growth trend: groups T2 and T3 not only exceeded the control group in terms of body weight gain, but they did so with statistical significance (P<0.001). This increase in growth indicators likely attests to the effectiveness of Direct-Fed Microbials (DFMs) in improving the Feed Conversion Ratio (FCR), thereby enhancing nutrient assimilation and metabolic efficiency. Recent scholarly work consistently supports the beneficial role of microbial supplementation in animal rearing. For instance, a comprehensive analysis by Nehru *et al*. (2017); Ban and Guan (2021); Król *et al*. (2022) sheds light on the multifaceted benefits of DFM supplementation, highlighting its potential to boost ruminant production and health. Similarly, a study by DeMarco *et al*. (2020); Demarco *et al*. (2021); Casper *et al*. (2022); Biricik *et al*. (2023); Izquierdo *et al*. (2024) explores the impact of bacterial DFM formulations on bovine growth, reporting significant improvements in growth performance and dietary habits. These studies lend credibility to the notable weight gains observed in groups that received microbial interventions. However, the diversity in research findings calls for a more detailed investigation into the factors that influence DFM effectiveness, emphasizing the need for continued scholarly exploration. Emerging research has started to uncover the role of DFMs in mitigating ruminal acidosis, enhancing immune responses, and promoting gut health. These factors collectively contribute to improved growth and milk production and may also potentially reduce methane emissions and the spread of pathogens. Further studies continue to broaden our understanding of the impact of DFMs on livestock growth. For instance, research underscores the potential of DFMs to alleviate ruminal acidosis, bolster immune response and gut health, increase productivity (including growth and milk production), and decrease methane emissions or fecal shedding of pathogen

B. Morphometric Growth Analysis

(i) Body Length Development. The analysis of body length revealed an average increment of 17 cm (about 6.69 in) for group T1, 20 cm (about 7.87 in) for group T2, and 19 cm (about 7.48 in) for group T3. The data suggests that group T2 experienced the most substantial increase, potentially indicating an enhanced growth effect associated with DFM supplementation.

(ii) Height at Withers Advancement. The height at withers exhibited a statistically significant increase in groups T2 and T3 compared to T1 (P<0.05). The increments of 18 cm (about 7.09 in) for T2 and 17 cm (about 6.69 in) for T3, as opposed to 15 cm (about 5.91 in) for T1, reflect a notable response to DFM supplementation, with implications for improved stature development.

(iii) Heart Girth Expansion. The heart girth measurements showed an expansion of 19 cm (about 7.48 in) for T1, 22 cm (about 8.66 in) for T2, and 20 cm (about 7.87 in) for T3. Despite the apparent growth, the differences did not reach statistical significance, indicating a consistent growth trend across all treatment groups. The observed morphometric changes align with previous studies that have documented the beneficial effects of microbial supplementation on calf development. The significant increase in height at withers is particularly consistent with findings from similar research, reinforcing the potential of DFM supplementation in promoting structural growth. Nonetheless, the variability in results across different studies underscores the complexity of biological responses to DFM supplementation, necessitating further investigation. These findings are consistent with the research conducted by Kucukoflaz *et al*. (2022); Lopez *et al*. (2024) which reported significantly greater (P<0.05) height at withers in groups supplemented with DFMs compared to the control group. Similarly, Lesmeister *et al*. (2004) documented significantly higher height at withers in calves receiving 2% supplemental yeast culture compared to those receiving 1% supplemental yeast culture. Moreover, Nehru *et al*. (2017) demonstrated a significant increase in height at withers in the treatment group fed *Saccharomyces cerevisiae* compared to the control group.

Table 2: Increase in body length (cm) of calves under experiment.

Animal	Initial (cm)			Final (cm)			Increase (cm)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1	88	90	88	107	114	107	19	24	19
2	105	102	105	123	118	126	18	16	21
3	94	97	94	113	118	114	19	21	20
4	116	95	116	130	117	133	14	22	17
5	110	112	110	127	130	126	17	18	16
Avg.	103	99	103	120	119	121	17	20	19
C.V%	13.36								
C.D	NS								

Table 3: Increase in height at the withers (cm) of calves under experiment.

Animal	Initial (cm)			Final (cm)			Increase (cm)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1	93	90	84	110	108	101	17	18	17
2	110	100	97	125	117	115	15	18	18
3	99	95	94	116	115	111	17	20	17
4	94	95	102	107	112	118	13	18	16
5	105	107	101	118	123	119	13	16	18
Avg.	100	97	96	115	115	113	15^b	18^a	17^a
C.V%	8.93								
C.D	2.060								

*In a row, the superscripts a and b differ significantly (P<0.05)

Table 4: Increase in heart girth (cm) of calves under experiment.

Animal	Initial (cm)			Final (cm)			Increase (cm)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1	104	102	101	124	128	124	20	26	23
2	131	116	111	151	137	131	20	21	20
3	111	107	104	131	130	125	20	23	21
4	109	110	131	125	133	149	16	23	18
5	127	127	120	144	145	140	17	18	20
Avg.	116	112	113	135	135	134	19	22	20
C.V%	11.25								
C.D	NS								

C. Nutrient Retention

(i) Biweekly Average Dry Matter Intake (DMI) in kg/day. The data delineated in Table 5 elucidates the biweekly average DMI across seven distinct periods, comparing the control group (T1) with the DFM-supplemented cohorts (T2 and T3). The initial DMI for T1 was recorded at 3.51 kg/day, with T2 and T3 commencing at 3.08 and 3.26 kg/day, respectively. A progressive increment was observed, culminating in T1 reaching a peak intake of 5.43 kg/day, juxtaposed with T2 and T3, which exhibited intakes of 5.28 and 5.18 kg/day. Despite a discernible ascension in T1's intake, statistical analysis revealed no significant divergence (P>0.05) among the groups, suggesting that DFM supplementation did not substantially alter the aggregate DMI. In contrast, when evaluating DMI relative to body weight (kg/100 kg BW) and metabolic weight (g/kg W0.75), the statistical landscape shifts, revealing significant variances (P<0.05). T2 and T3 demonstrated a reduction in feed consumption by 10.72% and 7.83% respectively, compared to T1. These findings echo the narrative of enhanced feed efficiency through DFM supplementation, aligning with the precedent set by Erasmus *et al*. (1992); Alshaikh *et al*. (2002); Jia *et al*. (2022); Maamouri *et al*. (2022) who reported a decrement in DMI post yeast supplementation. The exposition of crude protein intake (CPI) further substantiates the efficacy of DFM supplementation. The CPI for T2 and T3 was markedly lower than T1, with decrements of 10.5% and 9.50% in g/100 kg BW, and 7.72% and 7.71% in g/kg W 0.75, respectively. These statistically significant contrasts (P<0.05) accentuate the potential of DFM in bolstering protein utilization efficiency in calves.

Table 5: Average biweekly dry matter intake (kg/d).

Periods	T_1	T_2	T_3
P_1	3.51	3.08	3.26
P_2	3.97	3.72	3.82
P_3	4.59	4.06	4.36
P_4	5.16	4.88	4.92
P_5	5.29	4.97	4.81
P_6	5.27	5.17	5.03
P_7	5.43	5.28	5.18
Average	**4.75±0.37**	**4.45±0.26**	**4.48±0.31**
Source of variation	**SE.m**	**CD**	**CV%**
T	0.13	NS	16.88

(ii) Sequential Biweekly Crude Protein Intake (CPI) in g/day. Table 6 encapsulates the biweekly CPI trajectory for the control (T1) and DFM-supplemented groups (T2 and T3) over seven periods. The initial CPI for T1 was established at 382.83 g/day, with T2 and T3 initiating at 336.27 and 355.74 g/day, respectively. A consistent upward trend was observed, with T1's intake peaking at 597.36 g/day, contrasted with T2 and T3, which peaked at 580.88 and 570.11 g/day. The mean CPI was computed as 516.42 g/day for T1, 484.19 g/day for T2, and 487.63 g/day for T3, with the standard deviation reflecting the variability within each treatment group. The absence of significant differences in CPI between treatments, as indicated by the standard error of the mean (SE.m), aligns with the findings of Raeth-Knight *et al*. (2007), who discerned no impact on CPI when Holstein cows were administered a blend of *Propionibacterium freudenreichii* and *Lactobacillus acidophilus*.

Table 6: Average biweekly crude protein intake (g/d).

Periods	T_1	T_2	T_3
P_1	382.83	336.27	355.74
P_2	415.43	388.40	399.35
P_3	505.12	446.68	479.90
P_4	542.05	512.60	516.63
P_5	592.34	556.58	539.27
P_6	579.81	567.90	552.41
P_7	597.36	580.88	570.11
Average	**516.42±40.61**	**484.19±28.74**	**487.63±33.25**
Source of variation	**SE.m**	**CD**	**CV%**
T	14.99	NS	**17.76**

D. Feed Efficiency

The feed conversion efficiency (FCE) of calves was measured in terms of crude protein (CP), total digestible nutrients (TDN), and dry matter (DM) for groups T1, T2, and T3. The results indicated that the periodical FCE of DMI, CPI, and TNDI in the DFM-fed groups (T2 and T3) was statistically better ($P<0.05$) compared to the control group (T1). The FCE of DMI, CPI, and TDNI were 30.89% and 25.52%, 27.65% and 21.76%, and 30.94% and 25.65% better in the T2 and T3 groups, respectively, when compared to T1.The study suggests that DFM supplementation enhances overall feed efficiency, likely due to its beneficial effects on DM digestibility and the modified nutrient turnover by rumen microbes, as well as the kinetics of nutrient transport through the intestinal bacteria. Lower FCE values indicate more efficient feed conversions. Despite similar feed consumption, the live weight gains in the T2 and T3 groups were significantly higher than the control group, demonstrating improved FCE with DFM supplementation. The findings are supported by Direkvandi *et al*. (2020), who reported significant effects of supplemented DFM on feed efficiency. Conversely, Raeth-Knight *et al*. (2007) found no significant difference in feed efficiency between DFM-supplemented groups and the control group. As referenced in Tables 7-9, DFM-supplemented groups T2 and T3 consumed almost the same quantity of feed as the control group (the difference was 6.52 and 5.85%, but not significant), but the difference in live weights at the end of the seventh period was 24.53 and 21.84% in T2 and T3 groups compared to the control group. Such values indicated that DFM supplementation improved feed conversion efficiency in these groups.

Table 7: Periodical feed conversion efficiency of DMI (kg/kg gain).

Periods	T_1	T_2	T_3
P_1	9.87	7.32	8.24
P_2	9.76	7.10	9.49
P_3	9.62	6.91	7.59
P_4	11.46	7.79	7.66
P_5	9.43	8.30	6.79
P_6	8.17	7.52	7.97
P_7	11.30	6.02	6.10
Average	**9.94^a ±0.40**	**7.28^b±0.19**	**7.69^b±0.40**
	SE	**CD**	**CV %**
T	0.34	1.11	11.96

*In a row, the superscripts a and b differ significantly ($P<0.05$)

Table 8: Periodical feed conversion efficiency of CPI (kg/kg gain).

Periods	T_1	T_2	T_3
P_1	1.05	0.82	0.89
P_2	1.02	0.76	1.03
P_3	1.06	0.82	0.88
P_4	1.20	0.85	0.85
P_5	1.06	0.98	0.82
P_6	0.90	0.83	0.92
P_7	1.24	0.66	0.69
Average	**1.07^a±0.04**	**0.81^b±0.03**	**0.86^b±0.04**
	SE	**CD**	**CV %**
T	0.03	0.11	11.37

*In a row, the superscripts a and b differ significantly ($P<0.05$)

Table 9: Periodical feed conversion efficiency of TNDI (kg/kg gain).

Periods	T_1	T_2	T_3
P_1	5.37	3.99	4.49
P_2	5.31	3.86	5.17
P_3	5.24	3.76	4.13
P_4	6.24	4.24	4.17
P_5	5.14	4.52	3.70
P_6	4.45	4.10	4.34
P_7	6.16	3.28	3.32
Average	**5.41^a±0.22**	**3.96^b±0.10**	**4.18^b±0.22**
	SE	**CD**	**CV %**
T	0.18	0.60	11.98

*In a row, the superscripts a and b differ significantly (P<0.05)

E. Economics of feeding

The investigation into the economic implications of feed intake revealed a discernible variance among the treatment groups. The mean total mixed ration (TMR) intake per day (kg/ day) was observed as follows: T1 at 504.70 kg, T2 at 473.25 kg, and T3 at 476.76 kg. When examining the financial aspects, the total feed cost and daily feed cost per day (₹/day) were calculated to be ₹8829.27 and ₹90.09 for T1, ₹8406.98 and ₹85.79 for T2, and ₹8533.86 and ₹87.08 for T3, respectively. These figures indicate that both the total and daily feed costs were marginally reduced in the DFM-supplemented groups (T2 and T3) compared to the control group (T1), with daily feed costs being 4.88% and 3.39% lower, respectively. Furthermore, the feed cost per kg of gain was 29.16% and 22.52% lower in T2 and T3 than in T1, highlighting the cost-effectiveness of DFM supplementation.

Table 10: Economics of feeding.

Attributes	T_1	T_2	T_3
Cumulative total mixed ration intake (kg/day)	504.70±38.54	473.25±24.99	476.76±27.47
Total DFM cost (₹)	0.00	127.97±6.76	193.38±11.14
Total feed cost (₹)	8829.27±674.38	8406.98±443.99	8533.86±491.70
Daily feed cost (₹/day)	90.09±6.88	85.79±4.53	87.08±5.02
Feed cost (₹/kg gain)	186.68±12.56	139.17±7.60	148.89±13.90

CONCLUSIONS

Our study demonstrates that direct-fed microbials (DFMs) at 2% and 3% concentrations significantly improve feed conversion efficiency and growth metrics in cattle calves, while also offering economic benefits through reduced feed costs. These findings suggest DFMs as a viable strategy for enhancing livestock productivity and profitability. Future research should further investigate the long-term effects of DFMs to solidify their role in sustainable cattle farming.

PROSPECTS

The promising outcomes of this study on direct-fed microbials (DFMs) in Kankrej calves pave the way for future research to delve deeper into the nuances of microbial influence on livestock. Subsequent investigations should aim to dissect the long-term physiological and economic impacts of DFM supplementation, explore the scalability of these benefits across varied breeds and environmental conditions, and assess the potential for DFMs to contribute to sustainable agricultural practices. By focusing on these strategic areas, future research can build upon the current findings to foster a more efficient, cost-effective, and environmentally conscious cattle industry.

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Conflict of Interest. None.

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