Effect of Dietary Addition of Fennel (*Foeniculum vulgare*) Seed on Growth Performance, Haemato-Biochemical Profile and Faecal Microbiota of Kankrej Calves

M. M. Pawar1, S. S. Patil1, Y. M. Gami2, S. S. Patel3, S. H. Raval4, C. P. Modi1 and J. R. Patel1

1Dept. of Animal Nutrition, 2Livestock Research Station, 3Dept. of Veterinary Microbiology, 4Dept. of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, Gujarat (385 506), India

ABSTRACT

The experiment was conducted during the month of February and March, 2023 at Livestock Research Station, Kamdhenu University, Sardarkrushinagar, Gujarat, India to evaluate the effect of dietary addition of fennel (*Foeniculum vulgare*) seed on growth performance, haemato-biochemical profile and faecal microbiota of Kankrej calves. Eighteen Kankrej calves were randomly divided into three treatment groups, viz. Basal diet (control), Basal diet+5 g animal−1 day−1 of fennel seed powder supplementation and Basal diet+10 g animal−1 day−1 of fennel seed powder supplementation for a period of 60 days. Results revealed that there was numerically higher body weight gains were observed in groups fed 5 and 10 g of fennel seed daily than the control, the difference was statistically not significant (p>0.05) among the treatment groups. The mean faecal counts (log10 cfu g−1 in fresh faeces) of lactobacillus were increased, while coliform were reduced in groups fed 5 and 10 g of fennel seed daily as compared to control group. There was no effect (p>0.05) on the haemoglobin, haematocrit, total erythrocytes count and total leucocytes count among the treatment groups. No difference (p>0.05) was found in serum levels of total proteins, albumin, globulin, urea, triglycerides, cholesterol, ALT and AST concentrations among the treatment groups. It may be concluded that dietary fennel seed powder supplementation in calves improved growth performance and had beneficial effect on faecal microbiota with higher faecal lactobacillus count and lower faecal coliform count. Fennel seed supplementation did not affect haemato-biochemical profile of calves.

KEYWORDS: Blood biochemistry, calves, faecal microbes, fennel seed, growth


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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.
1. INTRODUCTION

India is an agriculture-based country and livestock are the most important and essential sub-division of our economy contributing 4.11% Gross Domestic Product and 25.6% of total Agriculture Gross Domestic Product (Anonymous, 2020). Livestock is playing pivotal role in accelerating the rural economy and ultimate sustainable livelihood for 70% population in rural areas. As per 20th Livestock Census, cattle population in India was 192.49 millions (Anonymous, 2019). With the total milk production of 230.58 million tonnes during 2022–23, India ranks 1st in the world in terms of total milk production (Anonymous, 2023). Indigenous cattle in India are robust, resilient and are particularly suitable to the climate and environment of their respective breeding tracts (Srivastava et al., 2019). In Indigenous cows, calves are necessary to keep cows in milk production. Moreover, calves are the future replacement stock of the herd. The calves have to be nourished well in their early phases of life as the development rate is accomplished right from the day of conception (Solanki et al., 2022; Sorang et al., 2023). In calves, morbidity and mortality rates are high between day one and six months of life. The addition of antibiotics to animal feeds has been widely used to promote growth performance, the feed conversion rate, product quality and animal health (Wu et al., 2020). However, injudicious use of antibiotics results in microbial resistance to drugs in calves (Credille et al., 2024; Fossen et al., 2024). Recently, use of various phytogenic feed additives as alternative to feed antibiotics to improve animal performance and health has been tried in livestock animals (Pawar et al., 2021; Saini et al., 2022). The beneficial effects of phytogenic feed additives in livestock animals’ performance may be associated with their positive effect on intake (Devi et al., 2022; Modi et al., 2022) and nutrient digestion (Fahim et al., 2022) or their characteristics as antibacterial, antioxidative, and immunity stimulation (Pawar et al., 2019; Hashemzadeh et al., 2023).

The phytogenic feed additives, fennel (*Foeniculum vulgare*) seed supplementation in livestock shown to promote the growth performance and health of young animals (Kargar et al., 2021; Nowroozinia et al., 2022; Ansari et al., 2024). Fennel is an aromatic plant belonging to the Apiaceae family. Anethole and limonene are the main phytochemicals of fennel (Noreen et al., 2023). The fennel seeds have aroma (due to essential oils such as fenchone, trans-anethole, estragole, and limonene), antioxidant and anti-inflammatory (due to flavonoids, essential oils, phenolic acids, hydroxycinnamic acids, coumarin, and tannin), estrogenic (due to photoanethole and dianethole) and antimicrobial (due to 1,3-benzenediols, oleic acid, undecanol, linoleic acid, 5-hydroxy-furanocoumarin, and 2,4-undecadienal) properties (Barrahi et al., 2020; Jadid et al., 2023; Kaveh et al., 2023). Mitra and Mukherjee (2010) previously showed that fennel seed cures gastrointestinal disorders such as indigestion, flatulence, and diarrhea. Also, it was reported that fennel seed regulate the appetite and improve hyperlipidemia, mainly by influencing the expression of insulin and leptin receptors (Zakernezhad et al., 2021). Moreover, recently the encapsulated essential oil and aqueous extract from fennel seeds had shown considerable reduction in the blood glucose content (Zolkepli et al., 2022). Recent studies have shown that dietary supplementation of fennel seed in growing animals improved feed intake, growth performance, lipid profile and gut health (Kargar et al., 2021; Ansari et al., 2022; Ansari et al., 2024) We hypothesized that feeding of fennel seed powder would improve growth performance and beneficial faecal microbiota in calves. Therefore, the present study was carried out to investigate the effect of dietary addition of fennel (*Foeniculum vulgare*) seed on growth performance, haemato-biochemical profile and faecal microbiota of Kankrej calves.

2. MATERIALS AND METHODS

The present study was conducted at Livestock Research Station, Sardarkrushinagar, Gujarat, India which is located in semi-arid region of North Gujarat, India having latitude of 24.32' North and longitude of 72.31' East and at an elevation of 189 meters above the mean sea level. This experiment was carried out during the month of February and March, 2023. The experimental protocol was approved (No. VETCOLL/IAEC/2021/17/PROTOCOL-03) by the Institutional Animal Ethics Committee. Eighteen Kankrej calves were randomly divided into three treatment groups, viz. T1: Basal diet (control), T2: Basal diet+5 g animal-1 day-1 of fennel seed powder supplementation and T3: Basal diet+10 g animal-1 day-1 of fennel seed powder supplementation. The experiment was conducted in a completely randomized design and consisted of 10 days of adaptation period and 60 days of experimental feeding. Representative samples of feeds and fennel seed powder were analyzed for chemical composition (Anonymous, 2007).

The body weight of each calf was recorded at the beginning (0d) and thereafter at fortnightly intervals during the experimental feeding. Rectal faecal samples were collected at the beginning (0d), 30th and 60th day of experimental feeding to determine the faecal microbial populations of *Lactobacilli* and *coli*forms. Bacterial populations were enumerated by serial 10-fold dilutions (10^-1 to 10^-9) with the total volume of 10 ml including 1 g homogenized faeces and 9 ml normal saline (0.9% NaCl) and plated in triplicate onto selective media—MRS agar for *Lactobacilli* (Himedia); EMB agar,
Levine (Himedia), for coliforms (Sharma et al., 2017). The agar plates were incubated aerobically at 37°C for 24 hr. Colonies on the agar plates were counted as colony-forming units (CFU) per g faeces and then converted to log_{10} cfu g⁻¹. At the end of experimental feeding (60th day) blood samples from jugular vein were collected from each calf in the sterilized vials with and without anticoagulant. The fresh blood samples were analyzed for haemoglobin, haematocrit, total erythrocytes and leucocytes count using automatic analyzer. The serum samples were analyzed for glucose, total proteins, albumin, urea, triglycerides, cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using commercial diagnostic kits.

The experimental data generated were analysed by ANOVA using the statistical software program SPSS. Data of the growth performance and haemato-biochemical parameters were analysed using one-way ANOVA. Data for faecal Lactobacilli and coliforms were analysed using two-way ANOVA. The difference between the means was declared significant at p<0.05.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The chemical composition of feeds and fennel seed used in experimental feeding of Kankrej calves is given in Table 1. The content of crude protein was 20.56, 6.78 and 3.56% in concentrate feed, hybrid Napier fodder and jowar hay, respectively. The contents of dry matter, crude protein, ether extract, crude fiber, nitrogen free extract and ash in fennel seed powder were 93.76, 9.38, 9.76, 18.21, 49.68 and 12.97%, respectively. Similarly, Noreen et al. (2023) reported content of dry matter, protein, fat and fiber in fennel seed was 93.7, 9.5, 10.0 and 18.5% respectively.

3.2. Growth performance

The data of effect of fennel seed supplementation on growth performance of Kankrej calves is given in Table 2. The final body weights of Kankrej calves were 66.92, 68.55 and 67.80 kg in T₁, T₂ and T₃ groups, respectively. No significant difference was observed in final body weights among the dietary treatment groups. The overall body weight gain was 25.86, 27.77 and 26.90 kg in T₁, T₂ and T₃ groups, respectively. Though, numerically higher body weight gain was observed in T₁ and T₃ than the control, the difference was statistically not significant (p>0.05) among the treatment groups. Earlier studies also reported improved weight gain in dairy calves supplemented with fennel seeds (Saeedi et al., 2017; Kargar et al., 2021; Nowroozinia et al., 2022; Ansari et al., 2022; Ansari et al., 2024). Phytochemical compound with estrogenic activity play important roles in skeletal muscle and bone growth (Nowroozinia et al., 2022), improve glucose uptake into muscle cells and provide long-lasting anabolic conditions (Saeedi et al., 2017; Hajalizadeh et al., 2019), modulate the immune responses, and are anti-inflammatory (Nowroozinia et al., 2022).

3.3. Faecal microbiota

The data pertaining to faecal microbial counts are shown in Table 3. The faecal lactobacillus counts (log_{10} cfu g⁻¹ in fresh faeces) at 60th day and overall average were increased in T₁ group followed by T₂ group as compared to the control group, while the faecal coliform counts (log_{10} cfu g⁻¹ in fresh faeces) at 60th day and overall average were numerically reduced in T₁ and T₂ groups as compared to T₃ group. However, the faecal lactobacillus and coliform counts were statistically not significant (p>0.05) among the dietary groups. Anwar et al. (2009) found that fennel essential oils had extensive antibacterial activity against particularly E. coli. Ghiasvand et al. (2021) found that essential oil of fennel reduced E. coli population in the intestines of broiler chickens.

Moreover, Gende et al. (2009) reported that fennel essential oil exhibits considerable antibacterial action due to its active component, anethole, which was shown to be particularly abundant in the oil of fennel (92.7%). Barrahi et al. (2020) found that fennel (Foeniculum vulgare) seed essential oil was efficient against a variety of microbes, including bacteria, yeast, and fungal strains. The presence of soluble fibre in fennel seeds acts as prebiotics in caecum and colon. Prebiotics in the caecum and colon are readily fermented by beneficial bacteria such as lactobacillus which produce

![Table 1: Proximate composition of feeds and fennel seed](image)

![Table 2: Effect of fennel seed supplementation on growth performance (kg) of Kankrej calves](image)
Table 3: Effect of fennel seed supplementation on faecal microbiota of Kankrej calves

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Dietary groups</th>
<th>Period average</th>
<th>SEm</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus (log_{10} cfu g^{-1} of fresh faces)</td>
<td>T₁</td>
<td>T₂</td>
<td>T₃</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>7.75</td>
<td>7.77</td>
<td>7.76</td>
<td>7.76</td>
</tr>
<tr>
<td>30 day</td>
<td>7.91</td>
<td>7.94</td>
<td>7.98</td>
<td>7.94</td>
</tr>
<tr>
<td>60 day</td>
<td>7.78</td>
<td>7.84</td>
<td>8.11</td>
<td>7.91</td>
</tr>
<tr>
<td>Average</td>
<td>7.81</td>
<td>7.85</td>
<td>7.95</td>
<td>7.87</td>
</tr>
<tr>
<td>SEm±</td>
<td>0.086</td>
<td>0.089</td>
<td>0.232</td>
<td></td>
</tr>
</tbody>
</table>

| Coliform (log_{10} cfu g^{-1} of fresh faces) |          |          |          | 7.94<sup>bc</sup> | 0.107 | 0.725 | 0.011 | 0.950 |
| Initial                            | 7.91 | 7.94 | 7.98 |          |          |       |       |       |
| 30 day                             | 7.81 | 7.59 | 7.61 |          |          |       |       |       |
| 60 day                             | 7.54 | 7.36 | 7.39 |          |          |       |       |       |
| Average                            | 7.75 | 7.63 | 7.66 |          |          |       |       |       |
| SEm±                               | 0.149 | 0.206 | 0.255 |     |         |

<sup>ABC</sup>Means with different superscripts in a column differed significantly (p<0.05)

antimicrobial agent and metabolic compound, which may have bactericidal action against gram negative bacteria (Ohland and MacNaughton, 2010).

3.4. Haematology–biochemical profile

The effect of fennel seed supplementation on haematobiochemical parameters of Kankrej calves is shown in Table 4. There was no effect (p>0.05) on the haemoglobin, haematocrit, total erythrocytes count and total leucocytes count among the treatment groups. No difference (p>0.05) was found in serum levels of total proteins, albumin, globulin, urea, triglycerides, cholesterol, ALT and AST concentrations among the treatment groups. In line with the present findings, no effect on blood glucose concentrations was observed in previous study (Fahim et al., 2022). On the contrary, the increase in plasma glucose concentration was reported by other studies (Mahmoud et al., 2020; Moosavi-Zadeh et al., 2023). In the present study, the lack of fennel seed effect on blood concentrations of total proteins, albumin, globulin and urea may imply that no alterations in the demand and competition for amino acids occurred and, in turn, shows no challenge to hepatic synthesis of albumin or globulin, required to sustain nutrient transportation and normal immune function (Kargar et al., 2018).

Cholesterol and triglycerides are 2 main indicators of lipid metabolism, and their concentrations are highly associated with health and diminished early mortality in calves (Renaud et al., 2018). In our study, there was no effect of fennel seed supplementation on blood cholesterol and triglycerides in calves, however, other studies reported increased (Ansari et al., 2022) and decreased blood triglycerides in dairy calves (Lakhani et al., 2019). The ALT

Table 4: Effect of fennel seed supplementation on haematobiochemical profile of Kankrej calves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary groups</th>
<th>SEm</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g dl⁻¹)</td>
<td>T₁</td>
<td>T₂</td>
<td>T₃</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>21.23</td>
<td>19.80</td>
<td>18.93</td>
</tr>
<tr>
<td>TEC (10⁶ µl⁻¹)</td>
<td>12.83</td>
<td>11.67</td>
<td>11.97</td>
</tr>
<tr>
<td>TLC (10⁹ µl⁻¹)</td>
<td>13.08</td>
<td>14.27</td>
<td>13.03</td>
</tr>
<tr>
<td>Blood biochemical parameters</td>
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</tr>
<tr>
<td>Glucose (mg dl⁻¹)</td>
<td>60.72</td>
<td>62.06</td>
<td>62.95</td>
</tr>
<tr>
<td>Total protein (g dl⁻¹)</td>
<td>5.39</td>
<td>5.50</td>
<td>5.51</td>
</tr>
<tr>
<td>Albumin (g dl⁻¹)</td>
<td>2.78</td>
<td>2.83</td>
<td>2.85</td>
</tr>
<tr>
<td>Globulin (g dl⁻¹)</td>
<td>2.60</td>
<td>2.67</td>
<td>2.66</td>
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<tr>
<td>Urea (mg dl⁻¹)</td>
<td>8.45</td>
<td>8.47</td>
<td>8.30</td>
</tr>
<tr>
<td>Triglycerides (mg dl⁻¹)</td>
<td>19.84</td>
<td>16.31</td>
<td>17.30</td>
</tr>
<tr>
<td>Cholesterol (mg dl⁻¹)</td>
<td>87.84</td>
<td>85.26</td>
<td>84.81</td>
</tr>
<tr>
<td>ALT (U l⁻¹)</td>
<td>26.59</td>
<td>23.76</td>
<td>23.38</td>
</tr>
<tr>
<td>AST (U l⁻¹)</td>
<td>107.45</td>
<td>106.20</td>
<td>110.31</td>
</tr>
</tbody>
</table>

TEC: Total erythrocytes count; TLC: Total leucocytes count; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

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and AST in liver and other tissues such as skeletal muscles are main indicators of tissue damage or dysfunction. In the present study, blood concentration of ALT and AST was not affected by treatment indicating no adverse effect of fennel seed supplementation in calves. These variations in findings among the experiments might be due to variations in experimental design, species, diet compositions, doses supplemented, and physiological stage of experimental animals.

4. CONCLUSION

Dietary fennel (*Foeniculum vulgare*) seed powder supplementation in calves improved growth performance and had beneficial effect on faecal microbiota with higher faecal lactobacillus count and lower faecal coliform count. Fennel seed supplementation did not affect haemato-biochemical profile of calves.

5. REFERENCES


