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# Determination Of the Optimum Level of Incorporation of Exogenous Fibrolytic Enzymes, Chicory Root Powder (*Cichorium Intybus*) And Their Combination By *In Vitro* Experiment

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***Abstract***

The present study was carried out with the objectives to determine the optimum level of incorporation of exogenous fibrolytic enzymes, chicory root powder and their combination by *in vitro* experiment. Sixteen healthy Gir calves of around one year of age were randomly selected from herd maintained at cattle breeding farm. Initially 2 Gir calves have been used to collect rumen liquor for *in vitro* study. Experimental calves were randomly divided on body weight basis into 4 groups having 4 calves in each group for *in vivo* study. The experimental feed was prepared using seasonal dry fodder as roughage and BIS type-1 compound cattle feed as concentrate in the ratio of 60:40. As a feed additive five different levels of exogenous fibrolytic enzymes (EFE) (0, 25000, 50000, 100000, 200000 IU per kg of concentrate) and five different levels of chicory root powder (CRP) (0, 0.5, 1, 2, 3 % of concentrate) were used. The *in vitro* study was conducted to determine the *In vitro* Total Gas Production (IVTGP) with basal diet as substrate supplemented with various levels of fibrolytic enzyme, chicory root powder and combination to arrive at the optimum level of incorporation of these supplements for *in vivo* studies. The statistical analysis of data revealed significant effect of level of incorporation of different feed additives alongwith experimental feed on *in vitro* gas production. The result revealed significantly higher ($p < 0.01$) IVTGP at 1 % level of incorporation of CRP and 50,000 IU of EFE per kg of concentrate while in combination 1% CRP + 50000 IU of EFE showed statistically significant value of ($p < 0.05$) IVTGP.

**Keywords: Chicory root powder, Exogenous fibrolytic enzymes, Gir calves, In *vitro* dose optimization.**

## Introduction

Gujarat is an important state in milk production and marketing in India based on co-operative dairy system. In India Gujarat is the 4$^{th}$ largest state in milk production based on co-operative dairy system. It contributed around 13.569 million tons of milk to the total milk pool of India Gir is an

excellent dairy cattle breed, well-known among the milk producers for its milk producing ability, good fertility, heat tolerance, easy maintenance, resistance to diseases and longevity (NDDB, 2017). Calves play an important role in development of the dairy sector of country, as the future of the dairy herd totally depends upon the successful raising of young calves (Anonymous, 2012).

One of the major constraints for the livestock population in India is qualitative and quantitative shortage of feed for livestock. The efficient conversion of energy-rich carbohydrates (cellulose, hemicellulose) of lignocellulosic biomass into accessible sugars is challenging technically, as these materials naturally evolved to resist degradation due to the complex fibrous structure of the materials that have constructed physical barriers to the accessibility of these carbohydrates for enzymatic breakdown (Thammiah *et al*., 2017).

Cellulases and xylanases are the two major enzymes that are specified to break β1-4 linkages joining sugar molecules of cellulose and xylans found in plant cell wall components (Beauchemin *et al*., 2003).

The use of prebiotics is a promising approach for enhancing the role of endogenous beneficial microbiota in the gut (Patel and Goyal, 2012). Inulin (fructo-oligosaccharide) has positive effect in stimulation of the development of the gut microbiota strains, inhibition of the proliferation of pathogenic bacteria and helps in production of Short Chain Fatty Acids from the fermentation of dietary fiber also have significant impact on the immune system by acidification of the colonic environment, acidification of the colon will favors mucin production as well as binding to Short Chain Fatty Acid receptors on immune cells within the gut-associated lymphoid tissues (GALT) (Singh *et al*., 2017).

Keeping all these factors in the mind the *in vitro* experiment was carried out to optimize best level of incorporation of exogenous fibrolytic enzymes, chicory root powder and their combination for further *in vivo* study.

## Materials And Methods

The present study was conducted on Sixteen Gir calves at Cattle Breeding Farm and Department of Animal Nutrition, College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, Gujarat.

Rumen liquor was collected from two donor calves at 2 h post-feeding through a stomach tube against negative pressure created by a suction pump (Lane *et al*., 1968). The collected rumen liquor was strained through four layered muslin cloth and called as Strained Rumen Liquor (SRL). The SRL was brought to the laboratory in a pre-warmed (39±1°C) thermos flask. Carbon dioxide gas was passed

through the SRL for one minute and was maintained at 39±1°C temperature for further analysis.

The medium was prewarmed to 39°C and bubbled with $CO_2$ till the blue color of the medium disappeared. After the medium becomes colorless strained rumen liquor (SRL) was added. Then 30 ml of incubation medium was injected to each calibrated glass syringe containing different levels of finely ground experimental feed along with fibrolytic enzymes (cellulase and xylanase), chicory root powder and combination as described by Menke *et al*. (1979). The experimental feed was prepared using seasonal dry fodder as roughage and BIS type-1 compound cattle feed as concentrate in the ratio of 60:40. As a feed additive five different levels of exogenous fibrolytic enzymes (EFE) (0, 25000, 50000, 100000, 200000 IU per kg of concentrate) and five different levels of chicory root powder (CRP) (0, 0.5, 1, 2, 3 % of concentrate) was used.

**Table 1: Composition of media for *in vitro* study**

| **Particulars** | **Quantity of different solutions and SRL (ml)** |
|---|---|
| Micro mineral solution | 0.10 |
| Buffer solution | 190.23 |
| Macro mineral solution | 190.23 |
| Resazurin solution | 0.95 |
| Reducing solution | 38.05 |
| Distilled water | 380.45 |
| **Total media** | **800** |
| Rumen liquor | 400 |
| **Total mixture** | **1200** |

The syringes were shaken gently, residual air or air bubble was removed and outlet was closed. The level of piston was recorded and the syringes were placed in an incubator (39±1°C) with periodic manual shaking (every 2 hour). Gas produced during fermentation was measured after 24 hrs. After incubation of 24 h, the content of each syringe was filtered through pre-weighed Gooch crucible. Composition if media for *in vitro* study is presented in Table 1.

The *in vitro* study was conducted to determine the *In vitro* Total Gas Production (IVTGP) with basal diet as substrate supplemented with various levels of fibrolytic enzyme, chicory root powder and combination to arrive at the optimum level of incorporation of these supplements for *in vivo* studies.

## Statistical Analysis

The collected data was statistically analysed by two-way analysis of variance for *in vitro* study as per procedures suggested by Snedecor and Cochran (1994). The significance of mean differences was tested by Duncan's multiple range test (DMRT).

## Results And Discussion

*In vitro* experiment was carried out using exogenous fibrolytic enzymes (cellulase and xylanase) and chicory root powder alone and in combination were used as feed supplement and added at different levels in the experimental feed which was prepared by using groundnut haulm as roughage and compound cattle feed type-1 as concentrate in the ratio of 60:40 to arrive optimum level of its incorporation by conducting *in vitro* experiment. Proximate composition for experimental feed is given in Table 2. The *in vitro* total gas production was studied to determine the optimum level of incorporation of exogenous fibrolytic enzymes (cellulase and xylanase) and chicory root powder as feed supplement.

**Table 2: Average proximate composition of feed/fodder for *in vitro* trial**

| Proximate Composition (% DM basis) | Ingredients (Feed/Fodder) | |
|---|---|---|
| | Groundnut haulms % | Compound cattle feed Type-1 % |
| DM | 94 | 92 |
| OM | 91.72 | 90.42 |
| CP | 10.36 | 21.73 |
| EE | 1.54 | 3.93 |
| CF | 21.83 | 10.81 |
| NFE | 57.99 | 53.95 |
| TA | 8.28 | 9.58 |

## *In vitro* total gas production (IVTGP) at 24 h incubation

The effect of individual feed additive as well as in combination at different levels of incorporation were assessed by *in vitro* gas production technique (Menke *et al*., 1979). The various levels used for exogenous fibrolytic enzymes and chicory root powder for *in vitro* study were (0, 25000, 50000, 100000, 200000 IU per kg of concentrate) and (0, 0.5, 1, 2, 3 % of concentrate) respectively and the treatments were designated as $EC_1$ to $EC_{25}$ (total 25 replications) for determination of optimum level of incorporation of exogenous fibrolytic enzyme and chicory root powder based on *in vitro* total gas production (TGP) profile.

*In vitro* gas production technique helps to determine nutrient utilization in a scientific way and its accuracy in describing digestibility in animals has been validated in numerous trials. This technique

can be used to predict animal performance at much lower cost. Total gas production (ml) at 24 hours of incubation by *in vitro* gas production technique for Exogenous fibrolytic enzymes and Chicory root powder for different doses (total 25 combinations) using Gir calf rumen liquor to ascertain best level of exogenous fibrolytic enzymes, chicory root powder and best combination for further *in vivo* study have been presented in tabular form in Table 3.

**Table 3: *In vitro* total gas production (ml/200mg experimental feed at 24 hrs.) under *In vitro* trial.**

| Level of EFE (IU/kg of Concentrate) \ Level of Chicory root powder (CRP) (% of Concentrate) | 0 | 0.5 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| 0 | EC 1 **15** | EC 2 **18.5** | EC 3 **19**** | EC 4 **17** | EC 5 **14** |
| 25,000 | EC 6 **16.5** | EC 7 **20** | EC 8 **17** | EC 9 **16.5** | EC 10 **17** |
| 50,000 | EC 11 **20.5**** | EC 12 **24** | EC 13 **23*** | EC 14 **18.5** | EC 15 **18.5** |
| 1,00,000 | EC 16 **19.5** | EC 17 **22.5** | EC 18 **20** | EC 19 **19** | EC 20 **17** |
| 2,00,000 | EC 21 **18.5** | EC 22 **19.5** | EC 23 **17.5** | EC 24 **20** | EC 25 **15** |

**= Significant at 1 per cent (P < 0.01), *= Significant at 5 per cent (P < 0.05)

The statistical analysis of data presented in table 4 revealed significant effect of level of incorporation of different feed additives along with experimental feed on *in vitro* gas production. The result revealed significantly higher (p <0.001) IVTGP at 1 % level of incorporation of CRP and 50,000 IU of EFE per kg of concentrate while in combination 1% CRP + 50000 IU of EFE showed statistically significant value of (p <0.005) IVTGP. However, data of *in vitro* trial indicates that beyond this level of incorporation resulted into reduction in IVTGP and no further beneficial effect were found.

**Table 4: ANOVA of *in vitro* total gas production**

| Sources of variation | d.f. | MSS | F Value | P Value | Level of Significant |
|---|---|---|---|---|---|
| EFE | 4 | 28.930 | 18.310 | <0.001 | ** |
| CRP | 4 | 28.930 | 18.310 | <0.001 | ** |
| EFE × CRP | 16 | 3.343 | 2.116 | 0.045 | * |
| Residual | 25 | 1.580 | | | |
| Total | 49 | 6.621 | | | |

**= Significant at 1% (P < 0.01), *= Significant at 5% (P < 0.05)

The increase in total gas production appeared to be associated with higher DM and OM degradability in supplemented groups due to increase in bacterial number and activity. The rate of fermentation might have also increased in EFE and CRP supplemented groups and hence IVTGP was increased in present study.

Similar findings were also reported for EFE by many researchers, where higher IVTGP was observed due to enzyme supplementation. Elghndour *et al.* (2013), Sipai *et al*. (2013), Dey *et al*. (2014) and Lunagariya *et al*. (2017) who reported significant (p<0.05) increase in IVTGP by enzyme supplementation while Turner *et al*. (1999) and Salman *et al.* (2017) were also noticed similar trend of *in vitro* DM and OM degradability by incorporation of CRP/ inulin.

## Conclusions

Supplementation of exogenous fibrolytic enzymes (EFE) (50,000 IU/kg of concentrate), chicory root powder (CRP) (1% of concentrate) alone and their combination (EFE 50,000 IU/kg of concentrate + CRP 1% of concentrate) revealed Significantly higher (p <0.001) *In vitro* total gas production (IVTGP).

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