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Research Article

Evaluating the Impact of Polyherbal Formulation, Kolin Plus on Ruminal Fermentation Using Rumen Simulation Technique (RUSITEC) Culture System

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Abstract

Objective: Rumen is a complex ecosystem comprising a wide range of microbial populations which play a vital role in the fermentation process in livestock. However, the fermentation resulted in the production of an excess amount of methane which is responsible for the loss of energy and environmental pollution. The inclusion of feed additives in the diet may modulate the fermentation as well as the performance of the ruminants. The present study was conducted to evaluate the impact of the Polyherbal formulation (PHF), Kolin Plus on the ruminal fermentation using Rumen Simulation Technique (RUSITEC) culture system.

Materials and Methods: Cattle (n = 4) were fistulated as per ethical guideline to collect rumen digesta. The rumen fluid was placed in the fermenters of RUSITEC system for further analysis. Control group was treated with basal diet. Whereas the Kolin Plus treated groups comprised of addition of 40g and 60g Kolin Plus per 500kg animal per day for 21 days along with basal diet. The parameters like *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), *in vitro* crude protein digestibility, *in vitro* crude fiber digestibility, *in vitro* ether extract digestibility, volatile fatty acid (VFA) production, methane production, ammoniacal nitrogen levels and pH change were assessed.

Result: The results indicated that PHF-Kolin Plus intended for the treatment of indigestion in the ruminants showed a significant effect on ruminal pH, ammoniacal nitrogen, IVDMD, IVOMD, *in vitro* crude protein digestibility, *in vitro* crude fiber digestibility, VFA production and methane production.

Conclusion: In conclusion, PHF-Kolin Plus may be considered a promising feed additive that plays an important role in digestion and enhancing the feed efficiency of ruminant livestock.

Keywords: Polyherbal formulation; RUSITEC; Dry Matter; Crude Protein; Crude Fibre; VFA; IVDMD; IVOMD

Abbreviations

PHF: Polyherbal formulation; RUSITEC: Rumen Simulation Technique; DM: Dry Matter; OM: Organic Matter; CP: Crude Protein; CF: Crude Fibre; VFA: Volatile Fatty Acids; IVDMD: *Invitro* Dry Matter Digestibility; IVOMD: *Invitro* Organic Matter Digestibility; TANUVAS: Tamil Nadu University of Veterinary and Animal Sciences; IVCPD: *Invitro* Crude Protein Digestibility; IVCFD: *Invitro* Crude Fat Digestibility; IVEED: *In vitro* Ether Extract Digestibility; AA: Acetic Acid; PA: Propionic Acid; BA: Butyric Acid; GHG: Green House Gases; NH3: Ammonia; CH4: Methane; IAEC: Institutional Animal Ethics Committee; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals

Introduction

The ruminant livestock sector which includes cattle, sheep, goats and buffalo has a significant impact on the food, nutrition as well as livelihoods of mankind globally [30]. They deliver a wide range of products like milk, meat, hide, wool etc which play a crucial role in the world economy. The rumen is the habitation of a complex and highly diverse population of microorganisms comprising bacteria, anaerobic fungi, protozoa, and methanogenic archaea [21]. The rumen microbiome facilitates the ruminants to utilize the low-cost forages i.e. straw, hay, silage, grass etc and transform them into high-quality meat, milk products and energy sources [21]. According to the IFCN dairy research network, 1 out of 3 workers worldwide is engaged in the agri-food industry [38].

The rumen is a complex ecosystem where dietary nutrients like carbohydrates, proteins and lipids are digested by the microbial fermentation process with the help of ruminal microbiota [8]. However, the microbial fermentation event is not fully effective due to the loss of energy caused mainly due to the production of excess methane (CH4) and ammonia (NH3), the greenhouse gases (GHG) contributing to the global warming [36]. The principal biochemical pathway observed in rumen is methanogenesis which involves the removal of the metabolic hydrogen produced by the fermentation process of dietary carbohydrate. The biochemical reaction encompassing the synthesis of methane is as follows: $CO2 + 4H2 \rightarrow CH4 + 2H2O$ [17]. Thus ruminant livestock is responsible for the emission of a substantial amount of GHG especially methane which contributes to the energy loss of up to 12% of gross energy intake [20].

Ruminants are devoid of the enzymes critically required for the digestion and fermentation of feed they consume. The microbial community present in the rumen contributes in the fermentation processes and helps the ruminants to effectively utilize the forages to produce energy [39]. The key factors affecting the growth and activity of the ruminal microbial population are temperature, pH condition, buffering capacity, osmotic pressure and redox potential which may vary due to the altered environmental conditions. The rumen temperature and pH are maintained in the range of 39 to 39.5 °C and 5.5 to 7.0 respectively and may fluctuate immediately after the dietary intake as the fermentation process play a vital role in alteration of the ruminal ecosystem [4]. The ruminal ammoniacal nitrogen (NH3-N) concentration is the limiting factor for the rumen microbiota and interferes with the digestibility of the nutrients present in the diet [14]. Therefore, estimation of the NH3-N level is crucial to evaluate the fermentation efficiency of rumen.

In order to improve the performance of ruminant production systems, various nutritional strategies including addition of phytogenic additives in the animal basal diet are obtained to modulate the ruminal fermentation. The herbal ingredients present in the feed additives alter the ruminal fermentation process and promote the feed efficiency by reducing energy loss [15].

The Rumen Simulation Technique (RUSITEC) is a well-established *in vitro* semi-continuous artificial rumen system which simulate the ruminal environment and helps to investigate the fermentation processes in a standardized condition avoiding the variations caused due to different animals. RUSITEC method is routinely applied to assess the dietary manipulations on the parameters like dry matter digestibility, crude protein & crude fibre digestibility and gas production in ruminant livestock and reduces the use of live animals in nutrition research [37].

The purpose of the present study is to evaluate the effect of PHF-Kolin Plus along with basal diet at a dose level of 40 and 60 g in enhancing the digestibility capacity of nutrients in the RUSITEC culture system.

Materials and Methods

Animals

All animal experiments were conducted under purview of the Institutional Animal Ethics Committee (IAEC). The animals were

housed in the animal facility as per CPCSEA guideline. All protocols of the experiments were reviewed by the IAEC.

Diet and formulation

Four cattle fed with diet containing 60% roughage and 40% concentrate were maintained as per ethical guidelines. Ruminal environment was simulated in fermenters containing rumen digesta of sheep using RUSITEC system. The control group (G1) was basal diet with no feed supplements. Two treatment groups (G2 and G3) of supplemented diet prepared by addition of Kolin Plus in basal diet at a dose range of 40g and 60g per 500kg animal respectively. Kolin Plus™ is a proprietary polyherbal formulation (PHF) developed by M/s. Natural Remedies Private Limited, Bengaluru, India, comprising of *Acacia nilotica* and *Curcuma longa* plant parts.

RUSITEC system apparatus

In brief, healthy cattle (n = 4) were screened and fistulated to collect rumen digesta which was transferred to the laboratory after thorough mixing within 30 min. of collection in a pre-heated vacuum container. In RUSITEC system, each reaction vessel was poured with 500 ml of rumen liquor and 200 ml of artificial saliva (prepared as per protocol laid by McDougall and infused at a constant rate of 0.55 ml/min) [22]. The solid rumen digesta and dry matter (DM) test feed weighing 80g and 20g respectively were added in 2 different nylon bags (130 x 80 mm) with pore size of 20 µm and placed in the perforated container of feed. Finally, the assembly was placed in the reaction vessel and the volume of the vessel was made up to 1 lit with distilled water. The effluent was collected in a flask containing 10 ml of mercuric chloride solution and used for determination of fermentation end products VFA and methane. The samples were incubated at different time points like 12, 24, 36 and 48hrs. and each time the nylon bag containing 10g feed was replaced by new one as described by Thavasiappan., *et al*. [35]. At the end, the bags were drained, squeezed and washed twice with artificial saliva and finally dried at 60°C for 48hrs. The washed saliva was send back to the respective reaction vessel.

Parameters assessed

The parameters like in vitro dry matter digestibility (IVDMD), in vitro organic matter digestibility (IVOMD), in vitro crude protein digestibility, in vitro crude fiber digestibility, in vitro ether extract digestibility, VFA production, methane production, ammoniacal nitrogen levels, pH changes in the rumen at different time points viz.12, 24, 36 and 48 hrs. were determined using Rumen Simulation Technique (TANUVASRUSITECTM Plate-1) as described by Czerkawski and Breckenridge with some modifications [12].

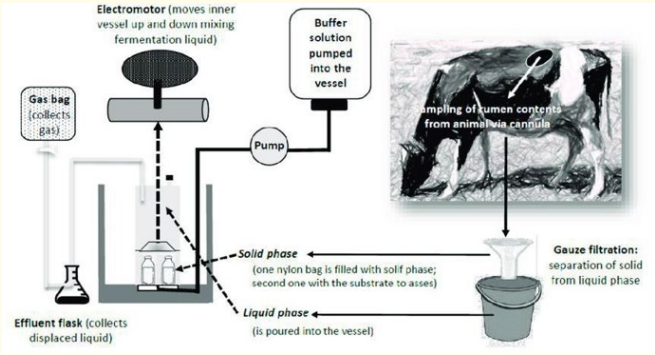


Figure 1: Rumen simulator or RUSITEC.

In vitro dry matter digestibility (IVDMD)

The dry matter disappearance was calculated in RUSITEC by assessing the weight loss of nylon bag, after 12, 24, 36 and 48 hrs. of incubation.

In vitro digestibility of sample was calculated using the following formula as mentioned below

$$\textit{In vitro}\text{ digestibility percentage} = \frac{\text{Weight of the bag with samples after incubation}}{\text{Weight of samples before incubation}} \times 100$$

Using the exponential equation of Orskov and Mc Donald (Ørskov and McDonald, 1979) the effective digestibility of dry matter was calculated.

$$P = a + b\,(1-e^{-ct})$$

Where

P = Effective digestibility a = Soluble fraction in percentage b = Insoluble but potentially degradable fraction in percentage a + b = Value of potential digestibility of the material in percentage c = degradation rate, expressed as percentage/h (e is a constant in exponential equation) t = time

In vitro organic matter digestibility (IVOMD)

In vitro ruminal organic matter digestibility was determined according to the method described by Tilley and Terry [10]. Briefly, in a centrifuge tube rumen digesta, McDougall's buffer along with feed sample were incubated for 48hrs. at 39°C in a water bath. Four tubes containing ruminal fluid and buffer were considered as blank. After adding acidified pepsin the tubes were re-incubated in the water bath for another 48hrs. at 39°C. After filtration the residue was dried, weighed and *in vitro* organic matter digestibility was determined by considering the organic matter loss during fermentation and subsequent pepsin digestion.

In-vitro crude protein, crude fibre and ether extract digestibility

The feed materials were ground, passed through a 1 mm sieve and mixed homogeneously. The total ash, crude protein (CP), ether extract and crude fibre (CF) composition of each test feed sample were determined by proximate analysis [26].

Volatile fatty acid (VFA) estimation

Volatile fatty acids (VFA) were determined in rumen fluid using gas chromatography method [11]. Whereas, ammonia (NH3) concentration was analysed based on Conway micro diffusion method [7].

Methane production

Methane production was estimated based on the method described by Blümmel., *et al.* [6]. Rumen fluid (filtered through 100 µm nylon sieve) was mixed with buffer solution and was saturated with CO_2 for 10 minutes. The DM feed sample was inserted into the syringe along with buffered-rumen fluid and closed with a piston to ensure anaerobic condition. The syringe was immediately placed in a water bath at 39°C temperature and gas production were observed at 12 and 24hrs. of incubation.

Finally, the ammoniacal nitrogen level and pH of rumen fluid was assessed according to Slyter *et. al.* with some modifications [32].

Results

In vitro digestibility of dry matter, organic matter and ether extract

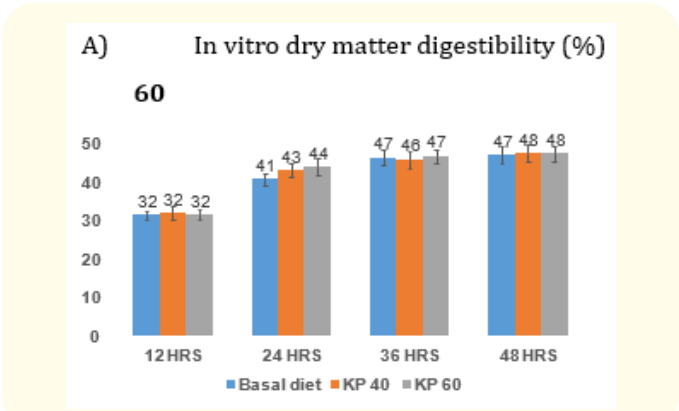
Digestibility of nutrient present in diet reflects the level of nutrient availability for the ruminants [18]. In the present study PHF-Kolin plus was mixed with diet (60% roughage and 40% concentrate) at a dose level of 40g (G2) and 60g (G3) and various parameters for ruminal fermentation were determined and presented in table 1. The PHF supplemented diet groups (G2 and G3) had better IVDMD than the control group (G1) predominantly observed at 24 hrs of incubation. Our current finding suggests that PHF mixed feed (40 g per 500 kg animal) showed approximately 5% better dry matter digestibility in 24 hrs and 1% better digestibility in 48 hrs as compared to control. Diet mixed with Kolin plus (@40g/500kg animal) showed about 75% and 10% better FAT digestibility in 24 and 48 hrs. respectively as compared to control. The organic matter digestibility was also significantly improved ($p < 0.001$) after addition of the PHF in 24 hr of incubation.

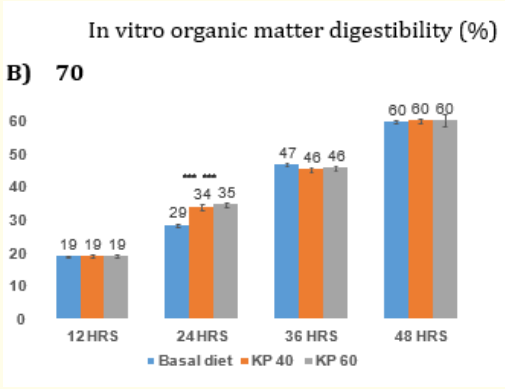
Parameters	Hours	Experimental Groups		
		G1: Basal Diet	G2: Basal Diet + KP 40	G3: Basal Diet + KP 60
In vitro dry matter Digestibility (IVDMD)	12	31.54 ± 1.18	32.06 ± 1.75	31.67 ± 1.49
	24	40.91 ± 1.53	43.15 ± 1.74	44.17 ± 2.28
	36	46.53 ± 1.97	46.01 ± 2.15	46.93 ± 1.87
	48	47.26 ± 2.25	47.71 ± 2.10	47.52 ± 2.08
In vitro organic matter Digestibility (IVOMD)	12	19.12 ± 0.29	19.07 ± 0.35	19.08 ± 0.37
	24	28.62 ± 0.49	34.23 ± 0.95***	34.75 ± 0.78***
	36	47.05 ± 0.61	45.59 ± 0.71	45.95 ± 0.77
	48	60.00 ± 0.69	60.34 ± 0.59	60.26 ± 1.87

In vitro crude Protein Digestibility (IVCPD)	12	14.65 ± 0.82	12.79 ± 0.38	14.61 ± 0.40
	24	34.59 ± 1.25	44.93 ± 0.97***	48.42 ± 0.81***
	36	55.83 ± 0.92	58.03 ± 1.37	64.30 ± 0.79***
	48	65.09 ± 0.85	64.83 ± 1.03	64.74 ± 1.73
In vitro crude fiber Digestibility (IVCFD)	12	12.61 ± 0.28	13.04 ± 0.43	14.61 ± 0.35***
	24	24.69 ± 0.99	28.12 ± 1.06*	30.66 ± 0.85***
	36	35.73 ± 0.80	36.43 ± 0.82	36.62 ± 1.10
	48	39.04 ± 1.04	43.18 ± 1.00*	44.08 ± 1.16**
In vitro ether extract Digestibility (IVEED)	12	0.26 ± 0.05	0.29 ± 0.04	0.27 ± 0.05
	24	1.54 ± 0.07	2.70 ± 0.08***	2.86 ± 0.16***
	36	2.76 ± 0.08	3.00 ± 0.16	3.64 ± 0.12***
	48	4.71 ± 0.11	5.16 ± 0.22	4.80 ± 0.16
Overall Volatile fatty acid Production	12	77.16 ± 0.64	79.32 ± 0.51	75.93 ± 1.07
	24	96.21 ± 0.78	91.41 ± 0.97***	90.41 ± 0.57***
Critical Volatile fatty acid Production (AA_PA+BA)	12	81.18 ± 1.16	85.83 ± 0.96*	98.02 ± 1.65***
	24	94.20 ± 1.79	107.05 ± 1.35***	108.87 ± 1.10***
Methane Production (CH4)	12	202.20 ± 20.56	205.00 ± 4.28	204.00 ± 4.52
	24	625.00 ± 6.87	496.00 ± 5.21***	485.00 ± 4.28***
Ruminal pH	12	6.80 ± 0.02	6.75 ± 0.02	6.74 ± 0.01
	24	6.68 ± 0.01	6.70 ± 0.02	6.81 ± 0.02
	36	6.80 ± 0.02	6.79 ± 0.02	6.80 ± 0.02
	48	6.79 ± 0.02	6.77 ± 0.01	6.80 ± 0.02
Ammoniacal Nitrogen (NH4-N)	12	5.62 ± 0.10	5.47 ± 0.11	5.44 ± 0.14
	24	6.48 ± 0.09	6.88 ± 0.11	6.93 ± 0.15
	36	12.48 ± 0.17	12.87 ± 0.12	13.71 ± 0.10
	48	11.83 ± 0.16	13.03 ± 0.17	12.70 ± 0.12

Table 1: Effect of PHF on ruminal fermentation associated parameters.

*p < 0.05, **p < 0.01, ***p < 0.001 significantly different between the column one-way ANOVA followed by Dunnett's multiple comparison test.





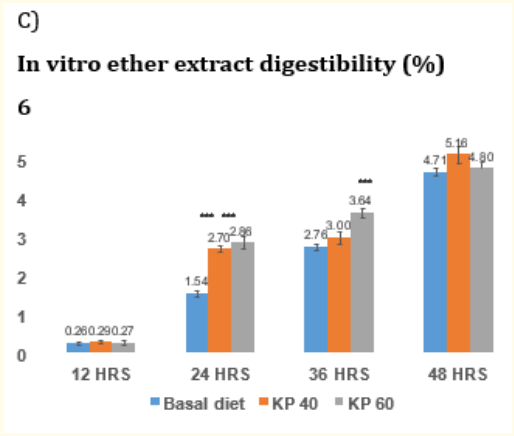
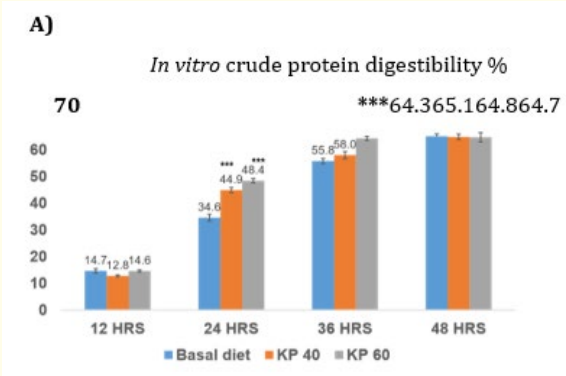


Figure 2: Histogram representing A) In vitro dry matter digestibility %, B) *In vitro* organic matter digestibility % and C) In vitro ether extract digestibility %. (*p < 0.05, **p < 0.01, ***p < 0.001 significantly different).

Crude protein, crude fibre digestibility

As shown in figure 3, Kolin plus (40g/500kg animal) mixed diet revealed 30% better CP digestibility in 24 hrs. There was significant difference ($p < 0.001$) in CP digestibility in both G2 and G3 experimental groups as compared to G1 in 24 hr of incubation. After 36 hr. G3 showed significantly enhanced digestibility as compared to G1. Inclusion of Kolin plus at a dose range of 40g per 500kg animal improved the CF digestibility approximately by 14% and 11% in 24 and 48 hrs respectively as compared to control. There were significant ($p < 0.001$) improvement in CF digestibility after 24 and 48 hr of incubations in G3 experimental group. However, the p value was <0.05 when CF digestibility of G12 was compared with G1 in 24 and 48 hr.



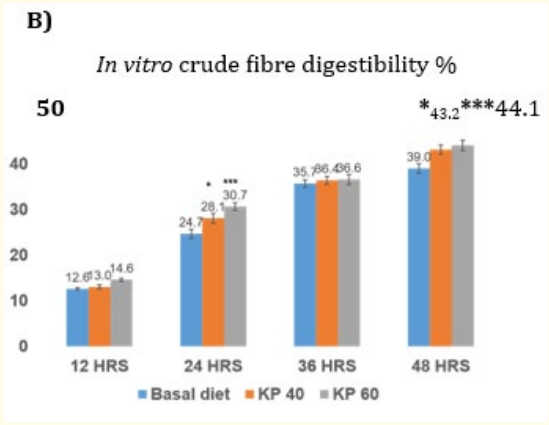


Figure 3: Histogram representing A) *In vitro* crude protein digestibility % and B) In vitro crude fibre digestibility %. (*p < 0.05, **p < 0.01, ***p < 0.001 significantly different).

Methane production

Our findings revealed that, PHF mixed diet at a dose range of 40 and 60 g showed anti-methanogenic effect at 24 hrs of incubation. Kolin plus showed reduced methane production by 20% at 24hrs. of incubation when compared with control group. Both G2 and G3 showed significant difference ($p < 0.001$) in methane production as compared to G1 in 24hr.

Methane production (ml)

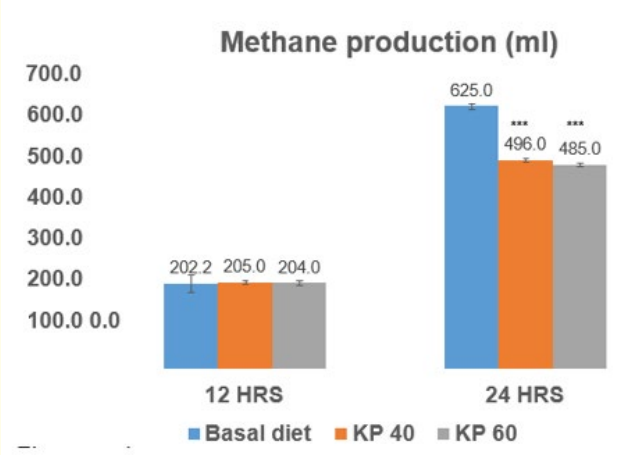


Figure 4: Histogram representing methane production (ml) (*p < 0.05, **p < 0.01, ***p < 0.001 significantly different).

VFA

Production

Herbal feed additive supplementation has enhanced the benefit in VFA production. The present study revealed that addition of Kolin plus in diet increased the critical VFA production up to 6% and 13% at 12 and 24 hrs. of incubation respectively in comparison of that observed in control group. However, Kolin plus did not improve overall VFA production throughout the experiment. In the present experiment, the amount of acetic, propionic and butyric acids, was higher during the fermentation of Kolin plus added diet as compared with the normal basal diet. The G3 experimental group showed significant ($p < 0.001$) improvement in critical VFA production in case of both 12 and 24 hr of incubation as compared to control. G2 also showed significant enhancement in critical VFA production in 12 ($p < 0.05$) and 24 hr ($p < 0.001$) of incubations.

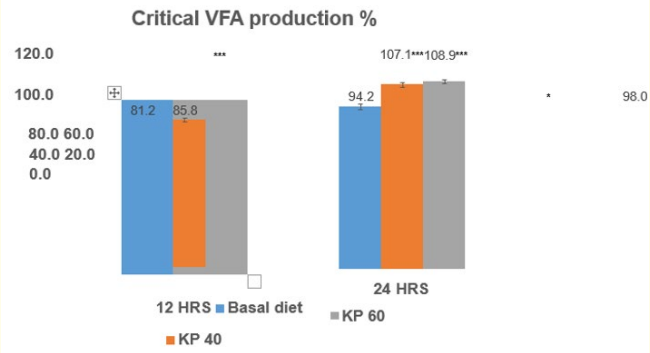


Figure 5: Histogram representing critical VFA production % (*p < 0.05, **p < 0.01, ***p < 0.001 significantly different).

Ammoniacal-nitrogen

Our data reveals that using PHF, Kolin Plus along with basal diet can increase the rumen ammonia level and support the fibre digestion (Table 1).

Ruminal pH

In the present results, the ruminal pH was observed as 6.8 which is the normal pH range of rumen to regulate the fermentation process. However, there is no noticeable difference in the pH value of rumen between the groups.

Discussion

Use of antibiotic feed supplements as growth promoters was practiced for a long period of time in ruminant livestock production sectors. However, the rumen fermentation process is altered due to the extensive use of antibiotics in the ruminant diet affecting the quality of products such as milk and meat [21]. Moreover, the residue of antibiotics presents in the food producing animals develops antibiotic resistance among the consumers. Therefore, the European Union (EU) has banned the usage of antibiotics in ruminant nutrition as feed additive [2]. In recent time, scientists are investigating the feed additives from natural sources that may improve the performance of the ruminants by modulating the rumen fermentation.

Polyherbal formulation (PHF) can be defined as a source of natural feed supplement which helps to improve the performance of the animals. It can act as a natural rumen modifier and play beneficial role on rumen microbial populations by improving ruminal fermentation efficiency and reducing methane gas production. Kolin Plus is a PHF manufactured by M/s. Natural Remedies Private Limited, Bengaluru, India. It is developed by proportional mixture of the phytopharmaceuticals derived from the plant parts, *Acacia nilotica* and *Curcuma longa*. The present study data revealed that addition of Kolin Plus in ruminant diet might enhance the digestibility by altering the rumen fermentation. Maneewan., *et al*. reported that basal diet supplemented with dietary turmeric powder might promote DM, CP and CF digestibility in nursery pigs [19]. and lambs [2]. It was also reported that high content of tannin present in *Acacia nilotica* plant part might offer a suitable environment to the ruminal microbiota for the fermentation process. Previous reports exhibited that the leaves of *Acacia* spp. contain high CP that improves the productivity of the ruminants [23]. Further, it was observed that low level of lignin in the leaves of *Acasia* spp. increased the feed palatability and the digestibility of DM and CP in the ruminants [24]. The value of DM, OM and CF digestibility is negatively correlated to the lignin content of feed as the rumen microbiota can rapidly degrade the nutrient with low lignin content present in the ration. Similarly, scientists identified that total lignin loss and improved *in vitro* digestibility of nutrients were interrelated [40]. Moreover, previous reports exerted that acacia seed pods might be used as an energy source by improving the efficiency of energy utilization [29]. Therefore, the

present product Kolin plus comprising of *Acacia spp.* and *Curcuma longa* is efficient in improving the feed efficiency of the ruminants. Our present study data were in agreement with this statement.

Ruminal fermentation is the largest source of methane which is one of the important greenhouse gas contributing to the climate change and global warming [5]. A scientific study reported that ruminants could generate 250-500 l/day methane gas depending on dry matter intake (DMI) and contributed significantly in environmental pollution [41]. Myriads of literatures corroborated that reduced ruminal methane production may improve the efficiency of nutrient utilization by preventing the feed energy loss in ruminants [34]. The phytopharmaceutical, curcumin present in *C. longa* has the suppressing effect on gram positive methanogenic bacteria like *Methanobacterium ruminatium*, *Methanobacterium formicum* etc and reduces the methane production in ruminant livestock [9]. Moreover, the diet rich in tannin was also reported to have beneficial role in diminishing excessive methane formation in ruminants [9]. *A. nilotica* is the rich source of tannin and shows evidences to provide potential effect in methane mitigation and *in vitro* digestibility of nutrients [16]. According to the study reported by Nawab *et. al., Acacia* spp. possesses anti-parasitic and anti-bloat characteristics and helps to inhibit the enteric methane emission during fermentation process promoting the nutrient digestibility, milk and meat quality, and reducing energy loss in the rumen [25]. This was in agreement with our findings where Kolin Plus containing both plant parts of *C. longa* and *A. nilotica* showed beneficial effect on the fermentation process and modulated the activity of methane producing bacteria. Thus, Kolin Plus supplemented diet may efficiently reduce methanogenesis in ruminants.

In previous experiment, diet supplemented with *Acacia spp.* showed higher levels of acetate, propionate and butyrate as compared to the normal diet [1]. In addition, VFAs contribute in the 70% of the caloric requirement of the animals. A higher concentration of propionic acid in the rumen during fermentation helps in reducing the energy loss [31]. Our study results also showed that, Kolin Plus inclusion enhanced the critical VFA production during fermentation process.

Deficiency in ammoniacal-nitrogen level resulted in the reduction of rumen microbiota [3]. The rumen microbes have potential impact on the digestion of degradable protein and converting into amino acid and finally ammonia. Ammonia is an important source of nitrogen for the growth of rumen microbial population. Diminished growth of microbiota down regulates the digestibility of the fibrous feed. The present study data corroborated that Kolin Plus might improve the rumen ammonia concentration which positively effects the nutrient digestion of the ruminants.

When the ruminants consume diet containing fibre or structural carbohydrates, the pH tends towards 7.5. However, the diet containing lots of soluble carbohydrates leads to the pH range of 5.0 [33]. The alterations in diet may result in a shift in the population of cellulolytic and amylolytic microbes in the rumen resulting in generation of a favourable pH in the rumen microenvironment to facilitate the microbial fermentation procedure [13]. Our result showed that Kolin Plus was effective in developing favourable ruminal pH for nutrient digestion.

Conclusion

In depth analysis of the present study exhibited that Kolin Plus modulated the ruminal fermentation processes and promisingly enhanced the digestibility dietary nutrients digestibility as well as diminished the methane emission. It efficiently altered the growth of ruminal microbiota and the nutrient digestion. Thus, Kolin Plus addition in ruminant diet may improve the overall performance of the animals, prevent energy loss and enhance digestion among the ruminants. Therefore, Kolin Plus may serve as an excellent natural feed supplement and it has positive impact in improving the feed efficiency of the animals.

Conflict of Interest

None.

Acknowledgement

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Bibliography

1. Aderao Ganesh N., *et al*. "Effect of complete feed block with tree leaves rich in hydrolysable and condensed tannins on nutrient utilization, rumen fermentation and growth performance of lambs". *Journal of Animal Physiology and Animal Nutrition* 104.1 (2020): 101-108.

2. Anadón A. "WS14 The EU ban of antibiotics as feed additives (2006): alternatives and consumer safety". *Journal of Veterinary Pharmacology and Therapeutics* 29 (2006): 41-44.

3. Belanche Alejandro., *et al*. "Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation". *The Journal of Nutrition* 142.9 (2012): 1684-1692.

4. Bhatta R., *et al*. "Influence of temperature and pH on fermentation pattern and methane production in the rumen simulating fermenter (RUSITEC)". *Asian-Australasian Journal of Animal Sciences* 19.3 (2006): 376-380.

5. Black John L., *et al*. "Methane emissions from ruminants in Australia: Mitigation potential and applicability of mitigation strategies". *Animals* 11.4 (2021): 951.

6. Blümmel M., *et al*. "*In vitro* gas production: a technique revisited". *Journal of Animal Physiology and Animal Nutrition* 77.1-5 (1997): 24-34.

7. Broderick GA and JH Kang. "Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media". *Journal of Dairy Science* 63.1 (1980): 64-75.

8. Castillo-González AR., *et al*. "Rumen microorganisms and fermentation". *Archivos de Medicina Veterinaria* 46.3 (2014): 349-361.

9. Chaudhry Abdul Shakoor and Mohammad Mehedi Hasan Khan. "Impacts of different spices on in vitro rumen dry matter disappearance, fermentation and methane of wheat or ryegrass hay based substrates". *Livestock Science* 146.1 (2012): 84-90.

10. Cone JW., *et al*. "Different techniques to study rumen fermentation characteristics of maturing grass and grass silage". *Journal of Dairy Science* 82.5 (1999): 957-966.

11. Cottyn Bernard G and Charles V Boucque. "Rapid method for the gas-chromatographic determination of volatile fatty acids in rumen fluid". *Journal of Agricultural and Food Chemistry* 16.1 (1968): 105-107.

12. Czerkawski JW and Grace Breckenridge. "Design and development of a long-term rumen simulation technique (Rusitec)". *British Journal of Nutrition* 38.3 (1977): 371-384.

13. Faniyi TO., *et al*. "Role of diverse fermentative factors towards microbial community shift in ruminants". *Journal of Applied Microbiology* 127.1 (2019): 2-11.

14. Firkins JL., *et al*. "Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy". *Journal of Dairy Science* 90 (2007): E1-E16.

15. Hassan Faiz-ul., *et al*. "Phytogenic additives can modulate rumen microbiome to mediate fermentation kinetics and methanogenesis through exploiting diet–microbe interaction". *Frontiers in Veterinary Science* 7 (2020): 575801.

16. Jayanegara A., *et al*. "Reduction in methane emissions from ruminants by plant secondary metabolites: Effects of polyphenols and saponins". *Sustainable Improvement of Animal Production and Health; Odongo, NE, Garcia, M., Viljoen, GJ, Eds* (2010): 151-157.

17. Ku-Vera Juan Carlos., *et al*. "Role of secondary plant metabolites on enteric methane mitigation in ruminants". *Frontiers in Veterinary Science* 7 (2020): 584.

18. Leng RA. "Factors affecting the utilization of 'poor-quality'forages by ruminants particularly under tropical conditions". *Nutrition Research Reviews* 3.1 (1990): 277-303.

19. Maneewan C., *et al*. "Effect of turmeric on growth performance, nutrient digestibility, hematological value and intestinal histology in nursery pig". *Journal of Swine Health and Production* 20.5 (2012): 231-240.

20. Martin Cecile Diego P Morgavi and Michel Doreau. "Methane mitigation in ruminants: from microbe to the farm scale". *Animal* 4.3 (2010): 351-365.

21. Matthews Chloe., *et al*. "The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency". *Gut Microbes* 10.2 (2019): 115-132.

22. McDougall EI. "Studies on ruminant saliva. 1. The composition and output of sheep's saliva". *Biochemical Journal* 43.1 (1948): 99.

23. Mokoboki HK., *et al*. "Intake and relative palatability indices of acacia species fed to sheep and goats". *Agroforestry Systems* 81 (2011): 31-35.

24. Moore Kenneth J and Hans-Joachim G Jung. "Lignin and fiber digestion". (2001).

25. Nawab Aamir., *et al*. "The potential effect of dietary tannins on enteric methane emission and ruminant production, as an alternative to antibiotic feed additives–a review". *Annals of Animal Science* 20.2 (2020): 355-388.

26. Odebunmi EO., *et al*. "Comparative proximate analysis of some food condiments". *Journal of Applied Sciences Research* 6.3 (2010): 272-274.

27. Odhaib Kifah J., *et al*. "Influence of graded levels of turmeric (Curcuma longa) as feed additives alternatives to promote growth and enhance health status in lambs". *Biochemical and Cellular Archives* 21 (2021): 3025-3032.

28. Ørskov EGIL-ROBERT and I McDonald. "The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage". *The Journal of Agricultural Science* 92.2 (1979): 499-503.

29. Paswan Jitendra Kumar., *et al*. "Effect of feeding Acacia nilotica pod meal on hematobiochemical profile and fecal egg count in goats". *Veterinary World* 9.12 (2016): 1400.

30. Pawlak Karolina and Małgorzata Kołodziejczak. "The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production". *Sustainability* 12.13 (2020): 5488.

31. Russell James B and Robert B Hespell. "Microbial rumen fermentation". *Journal of Dairy Science* 64.6 (1981): 1153-1169.

32. Slyter LL., *et al*. "Effect of ruminal ammonia concentration on nitrogen utilization by steers". *Journal of Animal Science* 48.4 (1979): 906-912.

33. Staples CR., *et al*. "Effects of intake of a mixed diet by dairy steers on digestion events". *Journal of Dairy Science* 67.5 (1984): 995-1006.

34. Tapio Ilma., *et al*. "The ruminal microbiome associated with methane emissions from ruminant livestock". *Journal of Animal Science and Biotechnology* 8 (2017): 1-11.

35. Thavasiappan V., *et al*. "*In vitro* rumen degradability study of wet brewer's spent grain by modified rumen simulation technique (TANUVAS-RUSITEC TM)". *International Journal of Current Microbiology and Applied Sciences* 9.5 (2020): 1118-1127.

36. Ungerfeld Emilio M. "Metabolic hydrogen flows in rumen fermentation: principles and possibilities of interventions". *Frontiers in Microbiology* 11 (2020): 589.

37. Wetzels Stefanie U., *et al*. "The application of rumen simulation technique (RUSITEC) for studying dynamics of the bacterial community and metabolome in rumen fluid and the effects of a challenge with *Clostridium perfringens*". *PLoS One* 13.2 (2018): e0192256.

38. Wyrzykowski L., *et al*. "IFCN long-term dairy outlook". *The IFCN Vision of the Dairy World in* 2030 (2018).

39. Xu Qingbiao., *et al*. "Gut microbiota and their role in health and metabolic disease of dairy cow". *Frontiers in Nutrition* 8 (2021): 701511.

40. Zhong Huimin., *et al*. "The effect of lignin composition on ruminal fiber fractions degradation from different roughage sources in water buffalo (*Bubalus bubalis*)". *Agriculture* 11.10 (2021): 1015.

41. Zicarelli Luigi. "The role of ruminants on environmental pollution and possible solution to reduce global warming". *Journal of Agricultural Science and Technology* 8 (2018): 239-252.