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THERAPEUTIC EFFICACY OF POLY-HERBAL FORMULATIONS AND HETEROLOGOUS CUD TRANSPLANTATION WITH PROBIOTICS IN AMELIORATING SIMPLE INDIGESTION IN BUFFALOES

G.C.MOHAN[*1], B.R.NAIK[2] AND A.C. KUMAR[3]

[1] *Department of Animal Husbandry, Veterinary Dispensary, Chanugondla, Dhone, Kurnool (A.P)*
[2] *Department of Veterinary Physiology, College of Veterinary Science, Proddatur (AP)*
[3] *Department of Animal Nutrition, College of Veterinary Science, Proddatur (AP)*

ABSTRACT

Therapeutic efficacy of certain poly herbal formulations and probiotics along with heterologous cud transplantation was evaluated in simple indigestion in buffaloes. A total of 18 buffaloes suffering from simple indigestion were treated with Suruchi bolus (10 g twice daily) or Gastricare powder (15g twice daily) or Yeasacc bolus with heterologous cud from healthy sheep. Clinical examination and laboratory analysis of rumen fluid and serum was carried out. Rumen fluid was evaluated for pH, protozoal motility, iodophilic activity, protozoal count, sedimentation activity time, methylene blue reduction time, total protozoa, gas production, ammonia, total volatile fatty acids. In serum, calcium, phosphorus, magnesium, glucose, urea, protein was estimated. All the treatment regimens were successful in ameliorating the changes in rumen liquor and serum biochemical profile. However, treatment with YHC treatment with probiotic Yeasacc bolus + heterologous cud transfer performed better than herbal drugs.

KEYWORDS: **Indigestion, Buffalo, Probiotic, Rumen, Protozoa, Cud, *Sacchromyces cerevisiae.***

*Corresponding author

G.C.MOHAN
Department of Animal Husbandry, Veterinary Dispensary, Chanugondla, Dhone, Kurnool (A.P)

INTRODUCTION

In ruminants, rumen plays an important role in digestion, absorption and assimilation of nutrients. A healthy rumen facilitates increased feed intake and digestion and improves the productivity[1]. However, most commonly ruminal disorders such as anorexia, tympany and indigestion which are characterized by poor appetite and change in pH, decreased rumen motility and decreased protozoal counts is observed[2]. Abrupt change in the feed is the most common reason for indigestion in ruminants. Other factors such as feeding spoiled, moulded feed, use of antibiotics, sudden changes in climatic conditions also cause indigestion[2]. Due to abrupt changes in feed, the ruminal microflora is unable to adapt resulting in indigestion. As rumen microflora plays an important role in ruminant digestion[3], ruminal dysfunction makes the animal susceptible to various metabolic diseases and digestive disorders like simple indigestion, acidic indigestion, alkaline indigestion or post parturient indigestion[4]. Mild simple indigestion is self-limiting and the pH of rumen tends towards acidosis or alkalosis. Decreased appetite is an early sign of indigestion[2] and subsequent prominent symptoms include anorexia, depression, cessation of rumination and eructation, decreased reticulo-ruminal movements and significant reduction in bacterial and protozoal counts[5,6]. Indigestion also leads to hepatic disturbances[7], increases the incidence of Leucocytosis[8] and decreases milk yield in affected animals[9]. Prompt treatment of indigestion is necessary for improving productivity. However, treatment of gastrointestinal diseases of ruminants poses significant challenge as the specific causes are unknown and effective therapies unavailable[10]. Further, the use of conventional treatment strategies is expensive and difficult for the treatment of clinical cases of indigestion in large animals. Hence, traditional remedies are being preferred world-wide[11]. Among the traditional and complementary treatments, herbal drugs forms the most common and important treatment modality[12]. Herbal drugs and poly-herbal formulations are being preferred as they are cheap and safe[13,14,15]. Several studies have also proven the safety of herbal drugs intended to be used for treating various disease conditions[16,17,18]. Several herbal extracts and products have been reported to provide beneficial effects in heavy metal poisoning[19], experimental diabetes[20], pesticide poisoning[21], mycotoxicosis[22] and also for green synthesis of nanoparticles with applications in mastitis and other bacterial infections[23,24] . In this study, two poly-herbal preparations viz., Suruchi bolus and Gastricare powder and probiotic Yeasacc combined with heterologous cud from ovine species were evaluated for clinical efficacy in buffaloes suffering from simple indigestion.

MATERIALS AND METHODS

(i) Drugs used: Suruchi bolus (each 5g containing Chitraka 1g;

Pipalli100mg; Sunti100mg; Mareecha 100 mg; Hingu 175 mg; Ajavana 375 mg; Shanhabhasma 750 mg) was obtained from Karnataka Antibiotics & Pharmaceuticals Pvt Ltd., Bangalore, India. Gastricare powder (15 g each containing Gauche 7.5 mg; Shunt 0.6 mg; Heritage 0.54 mg; Lacuna 0.48 mg; Hidaka 0.36 mg; Vedanta 0.15 mg; Mariachi 0.12 mg) was from Himalaya Drug Store Pvt Ltd, Bangalore, India. Yeasacc bolus (contains live yeast cells of *Saccharomyces cerevisiae* 25 x 10^9 cells per bolus) was from Alltech Biotechnology Pvt Ltd, Bangalore, India.

(ii) Animals

A total of eighteen buffaloes with simple indigestion were randomly allotted to three groups and treated as follows: Treatment I was carried out with Suruchi bolus (10 g twice daily); treatment II was carried out with Gastricare powder (15g twice daily) and treatment III was carried out with Yeasacc bolus + 100mL heterologous cud from healthy ovine species (YHC). All the treatments were carried out for three days. Normal healthy buffaloes served as controls.

(iii) Anamnesis

General clinical examination was carried out on all the animals included in the study. Physiological parameters such as rectal temperature, pulse rate, respiratory rate and rumen motility were recorded. The presence of any infectious disease was ruled out prior to

inclusion into the study. Auscultation and percussion was carried out to rule out abomasal displacement. Detailed clinical examination of the digestive system was undertaken as per Rosenberger[25].

(iv) Sample collection

Rumen fluid and serum samples were collected before and after the treatment. About 100-200 mL of rumen fluid was collected using rumen fluid extractor. The collected rumen fluid was immediately examined for colour odour, consistency, pH and methylene blue reduction time (MBRT)[26]. The remaining rumen liquor was preserved in air-tight glass bottles under a thin film of liquid paraffin until laboratory examination. Whole blood was collected from jugular vein into serum vacutainer for sero-biochemical examination.

(v) Laboratory estimations

Rumen liquor was evaluated for qualitative parameters such as protozoal motility[27], iodophilic activity and protozoal count[28]; quantitative parameters such as total protozoal counts[29], sedimentation activity time (SAT)[30], gas production, rumen ammonia nitrogen[31] and total volatile fatty acids (TVFA)[32] were also estimated. In serum, calcium, phosphorus, magnesium, glucose, total protein, blood urea nitrogen (BUN) were estimated using standard kits supplied by Span Diagnostics, Pvt, Ltd, Surat.

(vi) Statistical analysis

The data for qualitative parameters was presented as median (quartile 1 to 3). Wilcoxan signed rank test was used for comparing medians between before and after treatment. For qualitative parameters, the data was presented as mean ± standard deviation (SD). Paired t-test was used for comparing means between before and after treatments. Each treatment group was compared with control group using Students t-test. Statistical Package for Social Science (SPSS) 17.0 v was used for the analysis. The level of significance was set at $p<0.05$.

RESULTS

The results of clinical examination and physiological data is presented in table 1. The buffaloes in all the three groups showed significantly ($p<0.05$) reduced rumen motility and pH before treatment compared with control animals. After treatment with Suruchi, Gastricare and YHC in respective groups, a significant ($p<0.05$) increase in rumen motility was observed in all groups but was significantly ($p<0.05$) lower than normal animals. All the treatments were successful in normalizing the rumen pH towards normal range. Qualitative examination of the rumen fluid for protozoan motility, iodophilic activity and protozoal concentration showed significant ($p<0.05$) decrease in median scores before treatment compared to control group. After treatment with respective drugs, there was a significant ($p<0.05$) increase in the scores which was comparable to control animals in all parameters (Table 2). Quantitative analysis of rumen liquor, revealed that buffaloes suffering from simple indigestion showed significantly ($p<0.05$) higher SAT, MBRT, ammonia and significantly ($p<0.05$) lower total protozoa, gas production and TVFA compared to control animals. Treatment with Suruchi bolus, Gastricare and YHC significantly ($p<0.05$) reduced SAT, MBRT and rumen ammonia production and significantly ($p<0.05$) increased total protozoa count, gas production and TVFA content in the respective treatment groups. However, treatment with YHC only succeeded in normalizing the mean total protozoal count, gas production and TVFA production comparable to that of control animals (Table 3). Serum biochemical profile is present in table 4. Animals suffering from simple indigestion showed significantly ($p<0.05$) higher glucose, BUN and significantly ($p<0.05$) lower total protein content compared to control animals. All the treatment regimens significantly ($p<0.05$) decreased blood urea nitrogen in respective groups. However, only Gastricare and YHC treatments significantly ($p<0.05$) increased total protein content.YHC treatment alone significantly ($p<0.05$) increased serum calcium, magnesium and significantly ($p<0.05$) decreased blood glucose level after treatment. Further, mean BUN and glucose levels in only YHC treatment were comparable with control animals.

Table 1
Effect of various treatment regimens on clinical parameters in simple indigestion in buffaloes

Parameter		Control group	Treatment I	Treatment II	Treatment III
Temperature	Before	100.00±0.25	100.22±0.52	100.52±0.74	100.25±0.65
(^{o}F)	After		100.63±.0.34	100.45±0.23	100.52±0.34
Pulse	Before	49.83±1.20	50.00±2.90	54.17±4.02	50.83±4.40
(/min)	After		52.33±2.66	52.17±3.87	52.67±3.08
Respiration	Before	20.83±1.01	22.17±2.56	22.83±2.48	20.67±1.21
(/min)	After		22.33±2.16	22.50±2.66	20.67±1.21
Rumen motility	Before	7.35±0..35	1.50±0.55$^{\#}$	1.33±0.52$^{\#}$	1.17±0.41$^{\#}$
(/5min)	After		2.67±0.52$^{*\#}$	2.67±0.52$^{*\#}$	3.00±0.63$^{*\#}$
Rumen pH	Before	7.00±0.52	6.72±0.15$^{\#}$	6.65±0.11$^{\#}$	6.56±0.13$^{\#}$
	After		6.98±0.03*	6.99±0.06*	6.99±0.03*

Treatment I – Suruchi; Treatment II – Gastricare; Treatment III – Yeasacc and cud form ovine species
Values are Mean ± S.D (n=6) Paired t-test and Students t-test using SPSS software 17.0 v
**** Indicates significant difference between before and after means for each parameter (p<0.05)***
 # Indicates means significantly different from control group (p<0.05)

Table 2
Effect of various treatment regimens on qualitative characteristics of rumen liquor in simple indigestion in buffaloes

Parameter		Control group	Treatment I	Treatment II	Treatment III
Protozoan motility	Before	3.00 (3.00-3.00)	2.00(1.00 – 2.00)	1.00(1.00 – 2.00)	2.00(1.75 – 2.00)
	After		3.00*(2.75 – 3.00)	2.50*(2.00 – 3.0)	3.00*(2.75 – 3.00)
Iodophilic activity	Before	3.00 (3.00-3.00)	2.00(1.00 – 2.00)	2.00(1.00 – 2.00)	1.50(1.00 – 2.00)
	After		3.00*(2.75 – 3.00)	3.00*(2.00 – 3.25)	2.50*(2.00 – 3.00)
Protozoal concentration	Before	3.00 (3.00-3.00)	2.00(2.00 – 2.00)	2.00(1.75 – 2.00)	2.00(1.00 – 2.00)
	After		3.00*(2.75 – 3.00)	3.00*(2.75 – 3.00)	3.00*(2.75 – 3.00)

Treatment I – Suruchi; Treatment II – Gastricare; Treatment III – Yeasacc and cud form ovine species
Values are Medians (Q1 to Q3) (n=6) Wilcoxon Signed Rank Test using SPSS software 17.0 v
**** Indicates significant difference between before and after medians for each parameter (p<0.05)***

Table 3
Effect of various treatment regimens on quantitative characteristics of rumen liquor in simple indigestion in buffaloes

Parameter		Control group	Treatment I	Treatment II	Treatment III
SAT	Before	7.5±0.34	12.83±1.83$^{*\#}$	13.00±1.10$^{*\#}$	12.17±1.17$^{*\#}$
(min)	After		8.17±0.75	8.00±0.89	8.00±0.63
MBRT	Before	5.33±0.49	10.50±1.37$^{*\#}$	10.17±1.47$^{*\#}$	9.67±2.88$^{*\#}$
(min)	After		6.17±1.72	5.83±1.47	4.33±1.03
Total Protozoa	Before	3.45±0.20	1.09±0.17$^{\#}$	1.07±0.25$^{\#}$	1.06±0.26$^{\#}$
(x 10^{5})	After		2.02±0.37$^{*\#}$	2.12±0.68$^{*\#}$	3.24±0.49*
Gas production	Before	13.41±0.54	9.58±0.74$^{\#}$	9.50±0.71$^{\#}$	8.67±0.75$^{\#}$
(mL/h)	After		13.50±1.05*	12.33±1.51*	13.08±0.58*
Rumen Ammonia	Before	12.52±1.32	18.89±6.78$^{*\#}$	18.30±2.91$^{*\#}$	15.27±2.63*
(mg%)	After		13.88±3.54	10.18±2.36	9.70±0.81
TVFA	Before	86.00±3.14	61.50±8.56	64.33±7.06$^{\#}$	67.67±5.68$^{\#}$
(mmol/mL)	After		73.17±4.36$^{*\#}$	78.17±8.42$^{*\#}$	82.83±5.67*

Treatment I – Suruchi; Treatment II – Gastricare; Treatment III – Yeasacc and cud form ovine species ;
SAT= Sedimentation activity time; MBRT = Methylene blue reduction time; TVFA = Total volatile fatty acid content;
Values are Mean ± S.D (n=6) Paired t-test and Students t-test using SPSS software 17.0 v
****Indicates significant difference between before and after means for each parameter (p<0.05)***
 # Indicates means significantly different from control group (p<0.05)

Table 4
Effect of various treatment regimens on serum biochemical profile in simple indigestion in buffaloes

Parameter		Control group	Treatment I	Treatment II	Treatment III
Calcium	Before	9.58±0.54	8.50±0.95	8.05±1.40	8.10±1.40
(mg%)	After		8.96±0.71	8.73±1.87	8.52±1.42*
Phosphorus	Before	3.80±0.37	3.74±0.51	3.26±0.89	3.25±0.46
(mg%)	After		3.75±0.53	3.67±0.46	3.40±0.35
Magnesium	Before	2.45±0.13	2.42±0.54	2.27±0.69	2.20±0.29
(mg%)	After		2.60±0.39	2.84±0.78	2.55±0.18*
Glucose	Before	48.70±1.26	58.55±8.53#	61.41±5.24#	63.78±5.47*#
(mg%)	After		54.03±3.67#	58.27±2.41#	52.87±5.73
BUN	Before	10.35±0.54	16.62±2.11*#	15.44±2.05*#	13.32±1.95*#
(mg%)	After		14.27±2.93#	13.65±2.35#	11.27±1.72
Total proteins	Before	8.06±0.53	6.33±0.77#	6.04±0.80#	6.08±0.74#
(g/dL)	After		7.00±0.41	6.88±0.68*	7.88±0.62*

Treatment I – Suruchi; Treatment II – Gastricare; Treatment III – Yeasacc and cud form ovine species BUN = Blood urea nitrogen
Values are Mean ± S.D (n=6) Paired t-test and Students t-test using SPSS software 17.0 v
**** Indicates significant difference between before and after means for each parameter (p<0.05)***
 # Indicates means significantly different from control group (p<0.05)

DISCUSSION

In this study, the buffaloes suffering with simple indigestion showed reduced rumen motility, protozoal motility, iodophilic activity, protozoal count, gas production and TVFA production. There was an increase in SAT, MBRT, ammonia production. The rumen pH was slightly acidic. Earlier, Garry[5] stated that in simple indigestion, pH changes were mild and tend towards acidosis or alkalosis. The Protozoan motility decreases whenever there is a reduction in rumen pH[33] and during underfeeding or starvation[26]. Large entodiniomorphs are more sensitive to change in pH. Small trichostomatids (holotrichs) tend to be most tolerant. However, below pH 5.0, all protozoa die[34]. Further, increased SAT indicates decreased microbial activity[5] and suppressed microbial fermentation[35,36]. MBRT is an indirect measure of redox potential and bacterial activity. Increased MBRT and slow gas production time indicated decreased microbial fermentation in rumen due to inactive microflora and fauna[5]. As a consequence for reduced microflora and fauna activity, the TVFA production was decreased in all groups before treatment[37]. The blood glucose content was higher in buffaloes prior to therapy. In order to meet the energy requirement of animals when TVFA production is reduced, glycogenolysis leads to elevated blood glucose[38]. Alternatively, it is also proposed that significantly high blood glucose levels in primary indigestion is a consequence of stress, which releases corticosteroids, catecholamines and thereby promotes glycogenolysis[9]. However, Kasaralikar et al.[39] observed a significant decrease in blood glucose in simple indigestion. Buffaloes prior to therapy revealed higher BUN content. Similar, findings were reported by Kumari et al.[38] which was attributed to the failure of urea recycling process and its neutralization by rumen microbes. Therapy with YHC alone, the BUN content was reduced indicating improved microflora and fauna. The results of the clinical trial in the present study indicated that both of the drugs employed viz., Suruchi, Gastricare were found to be effective in eliciting a favourable response in buffaloes with indigestion. After the completion of therapy, the pH was normal in all the treatment groups. The protozoan motility became vigorous, the concentration per field was optimum and iodophilic activity was improved. Similarly, protozoal count returned

to normal. SAT and MBRT were reduced and gas production, serum protein content was enhanced, rumen ammonia nitrogen content was reduced and TVFA production increased. All these changes indicate the therapeutic efficacy of the drugs used in the present clinical trial in improving the rumen microbial activity. Increase in microflora and fauna results in efficient conversion of ammonia into protein and improved TVFA production [5, 37]. The efficacy of herbal drugs in treating digestive disorders was proved by the studies of many previous workers. Earlier studies have demonstrated the beneficial effects of using herbal and poly herbal formulations in digestive disorders of ruminants. Poly-herbal rumentories such as Ruchmax was used for cows[40], goats[41] and calves[42] with favourable outcome. Another proprietary preparation Pachoplus was used in buffaloes[43] with clinical improvement. In cattle with rumen dysfunctions, 'Himalayan batisa strong' was reported to be beneficial in restoration of rumen function[44]. Singh et al.[9] observed that the use of 'Pachoplus' and 'Ruchamax' as appetite stimulants and digestive tonics gave good results in clinical cases of indigestion. 'Rumbion' an herbal oretic preparation was able to maintain rumen pH, reduce MBRT and increase TVFA in water buffaloes suffering from spontaneous primary and secondary anorexia[45]. Significant improvement in appetite, body condition and rise in milk production was obtained in a clinical trial using 'Himalayan batisa' in digestive disorders of cattle[14]. Probiotics are live microorganisms with a positive effect on the host once ingested. They are used in various gastrointestinal disorders both in human and animals. The use of probiotics in animals was reported to increases weight gain and survival[46]. Probiotics are beneficial in establishing a normal rumen micro environment. These compounds may act by stimulation of appetite, improving fibre digesting bacteria, stimulation of non-specific immunity and production of digestive enzymes and B vitamins that aid indigestion and provide necessary nutrients[47, 48]. In this study, the therapeutic response was comparatively more in animals of treatment group III treated with Yeasacc and ovine rumen liquor. There was increased ruminal microbial activity in all animals of group III after treatment which was not a feature in other groups. In group III animals all the animals after treatment showed improvement in serum calcium with reduction in blood glucose and BUN which was not evident in other treatment groups. The efficacy of cud transplantation is well documented. Several authors have earlier reported that the use of cud transplantation brought about early restoration of floral activity, which was reflected by improvement in appetite, rumination, rumen motility[49, 50]. It was suggested that trans-faunation should be performed whenever the viability or activity of microflora is in doubt[5]. The essentiality of rumen liquor transplantation in treating digestive dysfunction of rumen is well documented[2]. Rumen liquor transplantation is economical in treating with ruminal dysfunctions. Heterologous or homologous rumen liquor transplantation can achieve the re-establishment of rumen ecosystem with complete clinical recovery.

CONCLUSION

Poly-herbal formulations provide adequate treatment modality for treating clinical cases of rumen dysfunction. However, homologous or heterologous cud transfer along with probiotic treatment provides superior efficacy over herbal treatments.

REFERENCES

1. eauchemin KA, Rode LM, Yang WZ, Applying nutritional management to rumen health Agriculture & Agri-Food Canada, Research Centre, Lethbridge, Canada. Can J Anim Sci, 80: 107-114, (2000).
2. Radostits OM, Gay CC, Hinchcliff KW and Constable, PD Ed., Veterinary Medicine: A textbook of cattle, horses, sheep, pigs and goats, 10th Edition, Saunders Publishers: (2006).
3. Yokoyama MT and Johnson KA.The ruminant animal: Digestive physiology and nutrition, D.C. Church ed. Prentice-Hall: 125 (1998).

4. Allen MS, Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J Dairy Sci, 80:1447-1462 (1997).
5. Garry FB. Indigestion in ruminants. In: B. P. Smith (ed.): Large animal internal medicine 2nd ed. Mosby, St. Louis and Baltimore: 722-747, (2002).
6. Irmak K, Sen I, Mahmut OK and Turgut K, Effect of simple indigestion and pneumonia on plasma thiamine concentration in cattle. Kafkas Univ Vet Fak Derg 4:63-67, (1998).
7. Padmaja K and Rao DST, Biochemical and therapeutic studies on post-parturient indigestion (ppi) with particular reference to hepatic insufficiency in buffaloes. International Journal of Pharma and Bio Sciences, 3: 40-45, (2012).
8. Stocker H, Lutz H and Rusch P, Clinical, haematological and biochemical findings in milk-fed calves with chronic indigestion. Vet Rec, 145:307-11, (1999).
9. Singh N, Akbar MA, Kumari R and Khanna BM, Effect of some treatment on ruminal environment and milk production in clinical cases of indigestion in buffaloes. Indian Vet Med J, 20: 115-118, (1996).
10. Berschneider HM, Complementary and alternative veterinary medicine and gastrointestinal disease. Clin Tech Small Anim Pract 17:19-24, (2002).
11. Maphosa V and Masika PJ, Ethnoveterinary uses of medicinal plants: a survey of plants used in the ethnoveterinary control of gastro-intestinal parasites of goats in the Eastern Cape Province, South Africa. Pharm Biol, 48:697–702, (2010).
12. Ke F, Yadav PK and Ju LZ, Herbal medicine in the treatment of ulcerative colitis. Saudi J Gastroenterol, 18: 3–10, (2012).
13. Arora SP, Thakur SS, Tripathi AN and Chhabra A, Influence of Galog on digestibility and milk production of Karan Swiss cows. Indian Veterinary Journal, 60: 46-50, (1983).
14. Nooruddin M, Clinical trial of Himalayan Batisa in loss of appetite of cattle. Indian J Anim Sci, 8:69-70, (1983).
15. Singh N, Rajesh, K and Akbar, MA, Biochemical and microbial changes in rumen of anorexic buffaloes. J Vet Physiol Allied Sci, 8:36-44 (1989).
16. Sakuntala Devi PR, Adilaxmamma K, Srinivasa Rao G, Srilatha Ch and Alpha Raj M, Safety Evaluation of alcohoic extract of *Boswelliaovalifoliolata* stem-bark In Rats. Toxicology International, 19 (2): 115-120, (2012).
17. Pradhan SC and Girish C, Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Indian J Med Res, 124:491-504, (2006).
18. Atole SK, Jangde CR, Philip Preety, Rekhe DS, Aghav DV, Waghode HJ and Chougule AM, Safety evaluation studies of Citrulluscolocynthis for diabetes in rats. Vet World, 2:423-25, (2009).
19. Pavan Kumar Y, Adilaxmamma K, Venkateswarlu U, Chandrasekhara Rao TS and Alpha Raj M, Protective effect of *Trianthemaportulacastrum*on cadmium induced toxicity in rats. Journal of Veterinary Pharmacology & Toxicology, 11(1-2):80-84, (2013).
20. Rao VKV, Adilaxmamma K, Prasad PE and Alpha Raj M, Hypoglycaemic and hypolipidemic effects of *Cassia auriculata* Linn seed extract in alloxan induced diabetes mellitus. Journal of Veterinary Pharmacology & Toxicology, 12(1-2):82-86 (2013).
21. Bharathi P, Gopala Reddy A, Rajasekhara Reddy A and Alpha Raj M, A Study of Certain Herbs Against Chlorpyrifos-induced Changes in Lipid and Protein Profile in Poultry. Toxicology International, 18 (1) 44 – 46, (2011).
22. Usha Rani M, Gopala Reddy A, Dilip Reddy G and Alpha Raj M, Oxidative stress due to ochratoxin and T-2 toxin either alone or in combination and evaluation of protective role of *Curcuma longa*, *Zingiber officinale*, toxichek and activated charcoal. Toxicology International, 16 (1) 63-68, (2009).
23. Chaitanya Kumar TV, Muralidhar Y, Prasad PE, Prasad TNVKV and Alpha Raj M, Evaluation of therapeutic potential of nanosilver particles synthesised using aloin in experimental murine mastitis

model. IET Nanobiotechnology, 7(3):78-82, (2013).
24. Chaitanya Kumar,TV, Prasad TNVKV, Adilaxmamma K, Alpha Raj M, Muralidhar Y and Prasad PE, Novel synthesis of nanosilver particles using plant active principle aloin and evaluation of their cytotoxic effect against *Staphylococcus aureus*. Asian Pacific Journal of Tropical Disease, 4(Suppl. 1): S92-S96, (2014).
25. Rosenberger G, Clinical examination of cattle. 3rd Edition Verlag Paul Parey, Berlin and Hamburg: (1979).
26. Dirksen G, Is the "methylen blue-reduction-probe" usable as quick-test for clinical examination of rumen fluid? Dtsch Tierärztl Wschr, *76,* 305-309, (1969).
27. Misra SK and Singh U, Studies on the clinic-pathological and therapeutic aspects of indigestion in cattle. Indian Veterinary Journal, 51:698-704, (1974).
28. Misra SK, Dash PK, and Mohanty GP, The protozoan fauna of the rumen and reticulum of Indian cattle. Indian Veterinary Journal, 49:464-469, (1972).
29. Naga MA and El- Shalzy K, Activities of rumen microorganisms in water buffalo (*Bos bubalis* L.) and in zebu cattle. J Dairy Res, 36: 1-10, (1969).
30. Nicholus RE and Katherinee P, Simple methods for the detection of unfavourable changes in rumen ingesta. Journal of American Veterinary Medical Association, 133: 275-277, (1958).
31. Conway EJ, Microdiffusion analysis and volumetric error. 4th Edition, Crosby-Lockwood and Son Ltd, London: (1957).
32. Barnett AJG and Reid RL, Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. J Agric Sci 48:315-321, (1957).
33. Steen A, Field study of dairy cows with reduced appetite in early lactation: clinical examinations, blood and rumen fluid analyses. Acta Vet Scand, 42, 219-228, (2001).
34. Belknap EB and Navarre CB, "Differentiation of gastrointestinal diseases in adult cattle", Vet Clin North Am Food Anim Pract, 16:59, (2000).
35. Nicholas RE and Penn KE, Simple methods for determination of unfavourable changes in ruminal ingesta. Journal of American Veterinary Medical Association 133: 275-77, (1958).
36. Misra SK and Tripathy RC, Studies on the rumen liquor from cattle feed exclusively on paddy straw. Indian Veterinary Journal, 40:496-499, (1963).
37. Singh N, Akbar MA and Kumari R, Effect of tranquilizers on biogenic amines, volatile fatty acid and milk production in clinical cases of primary indigestion in buffaloes. Indian Veterinary Journal, 74:590-93, (1997).
38. Kumari R, Akbar MA, Singh N and Sangwan ML, Status of certain blood metabolites, hormones, biogenic amines and their impact on production in healthy and indigestion cases of lactating murrah buffaloes. Indian Journal of Animal Nutrition, 12: 147-150, (1995).
39. Kasaralikar VR, Patil NA, Ravikanth K, Thakur A and Maini S, Clinico-therapeutic evaluation of Ruchamax and Ruchamax-N in various digestive disorders and restoration of normal ruminal function. International Journal of Phytopharmacy 4 : 67-69, (2014).
40. Walia R, Ravikanth K and Maini S, Efficacy of Ruchamax N in treatment of Digestive Disorders in Cow. Vet. World, 4:126-127, (2011).
41. Handekar PB, Kolte AY, Mendhe HC, Puri RM, Ravikanth K, Maini S and Rekhe DS, Effect of polyherbal formulations on ruminal digestion in Goat. Vet World 3:230-233, (2010).
42. Kolte A, Ravikanth K, Rekhe D and Maini S, Role of Poly-herbal formulation in modulating rumen biochemical and growth performance parameters in Calves. The Internet Journal of Veterinary Medicine 6 (2), (2008).
43. Kumari R and Akbar MA, Clinical efficacy of some herbal drugs during indigestion in buffaloes. Buffalo Bulletin, 25:3, (2006).
44. Sardar P, Kewalramani N and Kaur H, Effect of herbal bio-stimulator strong powder supplementation on rumen fermentation and *in vitro* dry matter digestibility (IVDMD). Indian Journal of Animal Sciences, 75:656-658, (2005).
45. Mandial RK, Prasad B and Pal B, Efficacy of Rumbion in experimental

acid indigestion in calves. Indian Journal of Indigenous Medicines, 11: 19-23, (1994).
46. Yanbo W and Zirong X, Effect of probiotics for common carp (*Cyprinuscarpio*) based on growth performance and digestive enzyme activities. Anim. Feed Sci. Technol., 127:283–292, (2006).
47. Tuohy KM, Probert HM, Smejkal CW and Gibson GR, Using probiotics and prebiotics to improve gut health. Drug Discov Today, 8:692-700, (2003).
48. Denev SA, Peeva Tz, Radulova P, Stancheva N, Staykova G, Beev G, Todorova P and Tchobanova S, Yeast cultures in ruminant nutrition. Bulgarian Journal of Agricultural Science, 13:357-374, (2007).
49. Choudhari PC, Randhava SS and Misra SK, Effect of lactic acidosis in electrolyte changes in blood and rumen liquor in buffalo-calves. Zentralbl Veterinarmed A, 27:358-63, (1980).
50. Tufani NA, Makhdoomi DM and Hafiz A, Rumen acidosis in small ruminants and its therapeutic management. Iranian Journal of Applied Science, 3:19-24, (2013).