

Supplementary Information (ppt)-different phases of the experiment with images



**EFFECTIVENESS OF PROBIOTIC AND COMBINATIONS OF PROBIOTIC
WITH PREBIOTIC AND PROBIOTIC WITH RUMENOTORIC FOR
EXPERIMENTALLY INDUCED RUMINAL ACIDOSIS IN SHEEP**

BY

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of Science in Veterinary clinical medicine**

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OUTLINES

❑ INTRODUCTION

- ❖ Statements of the problem
- ❖ Objective

❑ MATERIALS AND METHODS

- ❖ Study animals and Study Area
- ❖ Experimental Design And Period
- ❖ Therapeutically Interventions
- ❖ Data Management

❖ RESULTS

❖ CONCLUSION AND RECOMMENDATIONS

INTRODUCTION

- ❑ Small ruminants have been providing socio-economic development in our country.
- ❑ However, the performance of the sheep industry has been poor due to different constraints.
- ❑ Major constraints in sheep involve ruminal acidosis.
- ❑ Therefore, in order to solve this problem;
- ❑ the promising alternative was found by using probiotic and its combinations.

Statements of the problem

- ❑ Ruminants are important livestock species in developing countries such as Ethiopia.
- ❑ Small ruminants industry constitutes a one of major part of animal production in Ethiopia
- ❑ However; feed deficiency, poor managements and rumen disorders had a great economical loss

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- ❑ The most common rumen disorder is usually ruminal acidosis that arises from ingestion of a large quantity of carbohydrate-rich diet.
- ❑ Generally, dysfunctional rumen results in
 - ❖ impaired digestion
 - ❖ Reduction of production
 - ❖ increased susceptibility digestive and
 - ❖ metabolic diseases

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- ❑ Even though there is traditional buffering agents such as;
 - ❖ Magnesium hydroxide
 - ❖ sodium bicarbonate but;
- ❑ There is recent trends to improve the ruminal acidosis.
- ❑ Probiotics, rumenototics, prebiotics and sybiotics
- ❑ In Ethiopia has not done any clinical trial about these agents and not use clinically.
- ❑ Keeping this in view, the study was planned with the following objectives

General Objective

- ❑ To compare the therapeutic effectiveness probiotics, a combination of probiotics with prebiotics, probiotics with rumenototics and standard treatments of experimentally induced acidosis in sheep

Specific objective:

- ❖ Evaluate and compare therapeutic effectiveness of alternative treatments.
- ❖ Compare the therapeutic effectiveness of the alternative ruminal acidosis treatments with the standard treatment.

MATERIALS AND METHODS

❑ **Study animals:**

- ❖ Local breed sheep were bought from the same origin and as much as possible equivalent age, body condition and size.

❑ **Sample Size:**

- ❖ The total number of animals used for the study was 25 sheep.
 - In each group 5 sheep of equivalent age, body condition and body size were randomly assigned.

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- **Experimental Design And Period;**
- Experiment was conducted from December 2019 to may 2019
- to evaluate and compare the therapeutic effectiveness of Probiotic and its combination comparing with standard treatment.
- The experimental design was a completely randomized design

Experimental Data Collection

- ❑ General physical examination was taken before and after treatment (HR,RR,To)
- ❑ Evaluation of haematological, serum and ruminal Changes
 - ❖ Determination of blood parameters (pH,PCV,Hgb)
 - ❖ rumen fluid analysis (pH,protozoal motility,MBRT and SAT)
 - ❖ Serum analysis(total protein albumin, AST and ALP)



Bloodcollection



Measure heart rate

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Body temperature



Ruminal fluid collection



Therapeutical interventions

- ❑ For experimental therapeutic study 25 sheep was randomly divided into five groups(GC, PT,PPT, PRT AND ST).
- ❑ Each group comprises 5 animals
- ❑ Then, oral dose of 50g/kg with wheat flour after 24 hour fasting was allowed.
- ❑ Subsequently, ruminal acidosis was induced successfully

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Induction phase



Ruminal pH



Serum analysis

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- ❑ Then four different types of treatments were allocated randomly to four groups.
- ❑ To ascertain the comparative efficacy of various regimens
- ❑ by comparing with positive control group and within group.
- ❑ For assessing ameliorative potential of probiotics, prebiotics and their combinations.

Treatments groups	Composition	Dose	Company	Country	Route	Duration
Probiotic alone	Saccharomyces spp	1gram	IndiaMART	India	Oral	3 days
	Bifidobacterium spp					
Probiotic & Prebiotic	Saccharomyces spp	1gram	IndiaMART	India	Oral	3 days
	Bifidobacterium spp					
	FructoOligoSacchride	100 miligarm				
Probiotic & Rumenotonic	Saccharomyces spp	1gram	IndiaMART	India	Oral	3 days
	Bifidobacterium spp					
	AntimonyPotassiumtartarate ferrous sulphate, Copper sulphate, cobalt chloride	1 bolib.i.d				
Sodium bicarbonate	NaHco ₃	1g/kg	----	China	Oral	3 day

Statistical Analysis

- ❖ The data was analyzed statistically
- ❖ MANOVA
- ❖ protozoan motility was measured median (Q_0 - Q_3) and Kruskal-Wallis equality-of-populations rank test.
- ❖ For statistical inference with $P\text{-value} < 0.05$ (at 5% level of significance)

RESULTS

❑ **Health Status of the Animals**

- ❖ acidosis was successfully induced and showed common symptoms as usual.
 - nervous depression
 - Watery and acidic smelling diarrhea
 - cessation of feed intake
 - Tooth grinding was showed

Comparison of mortality rate

- ❑ The results of the present study revealed that most of the therapeutic regimens were effective in eliciting a favorable response in acidosis sheep.
- ❑ Except one sheep died from five (20%) from the treatment I (probiotics alone) (PT)
- ❑ no death due to induced acidosis in the treatment groups as compared to the death of two sheep (40%) in the control group(CG).
- ❑ Effect of various treatment regimens on different parameters are summarized depicted in the next table.

Parameter	Time of Rx	Control group	Treatment I (DT)	Treatment II (DDT)	Treatment III (DDT)	Treatment IV (ST)	Total mean
Heart rate	Before	134.40±9.21	142.40±10.88	140.80±14.45	142.80±8.67	138.8±7.16	139.84±9.29
	After (R _x)						
	Day1	138.00±8.72	127.20±7.16	123.20±8.67	128.20±126 [#]	116.40±4.6	127±0.20
	Day2	124.00±6.93	116.00±8.64	104.80±5.23 [#]	108.8±7.64 [#]	102.40±4.6 [#]	109±9.540
	Day3	112.67±3.05	107.00±8.87 [#]	97.20±6.88 [#]	91.20±7.69 [#]	98.00±8.00 [#]	98.27±8.49
Temperature	Before	38.40±8.764	38.00±.707	37.60±548	37.40±.548	38.20±.447	37.9± 0.612
	(To)	After (R _x)					
	Day1	38.60±.89	38.20±.837	38.00±.707	38.80±.447 [#]	39.20±1.05 [#]	38.36±0.97
	Day2	38.60±.000	39.00±.000	38.40±548	39.40±.548	39.20±.837 [#]	39.00±0.67
	Day3	38.80±000	39.00±39.00	39.00±.000	40.00±.000 [#]	39.80±0.55 [#]	40±0.00
Respiratory rate((min)	Before	40.40±8.764	35.2±3.347	33.60±8.295	44.00±.5.66	31.60±8.52	36.96±8.05
	After(Rx)						
	Day1	33.60±7.266	30.40±4.561	25.60±4.561	26.00±4.472 [#]	24.40±2.966 [#]	28±5.745
	Day2	28.67±1.16	24.00±3.266	23.20±3.347 [#]	20.80±3.37 [#]	20.40±2.96	26.00±0.18
	Day3	22.67±5.03	22.00±2.309	19.60±2.966 [#]	16.80±1.05 [#]	17.20±2.63 [#]	19.27 ±3.147

From this table we can deduce that

parameter	Date of improvement	Treatment group
HR	1 st ,2 nd , 3 rd	III and IV(PRT and ST)
	1 st ,2 nd ,	II(PPT)
	2 nd ,&3 rd	I(PT)
T0	2 nd & 3 rd	III(PRT)
	1 st ,2 nd , 3 rd	IV(PRT)
RR	1 st ,2 nd , 3 rd	III(PPT)
	1 st ,&3 rd	IV(ST)
	2 nd ,&3 rd	II

Values expressed as (#)superscript are significantly significant between control groups at p<0.05. Values were expressed by means ±SD paired t-test before and after treatment by STATA version14

One way ANOVA followed by Bonferroni post hoc test was used to compare between groups

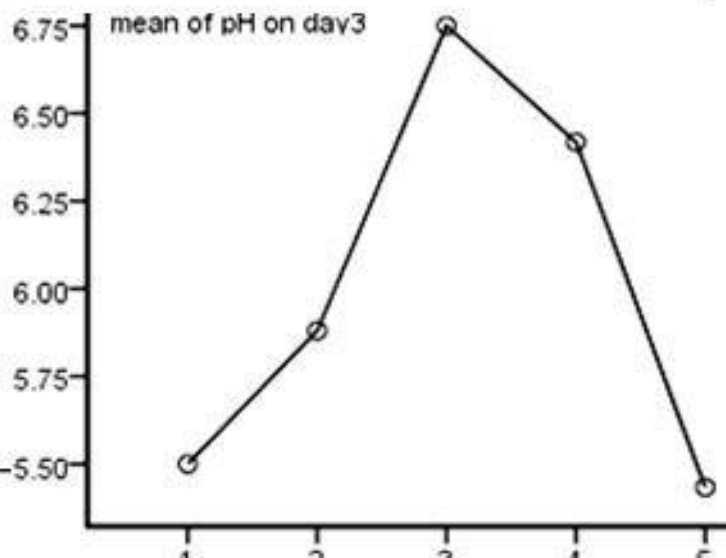
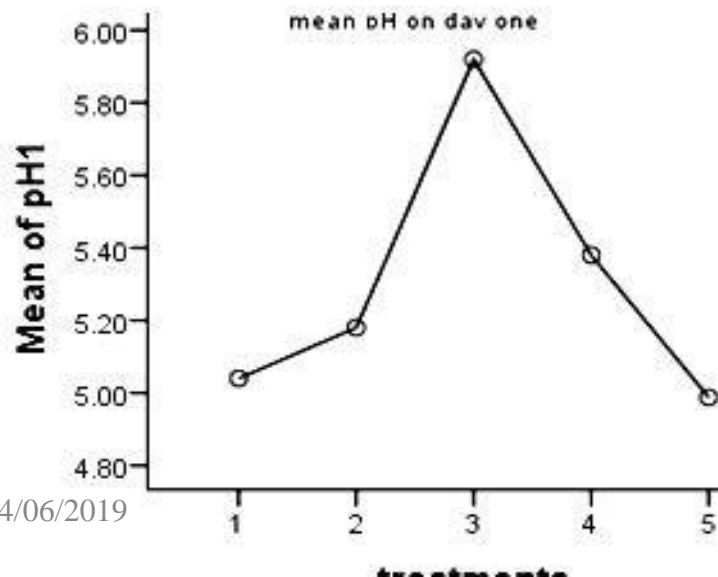
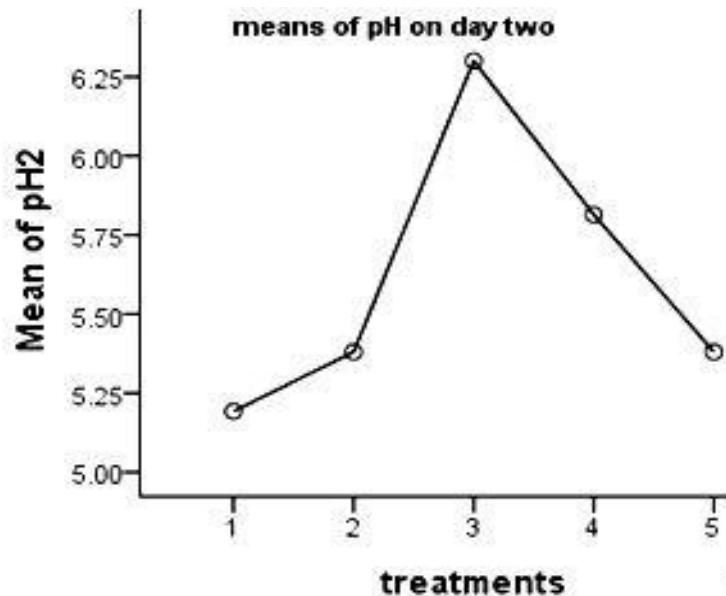
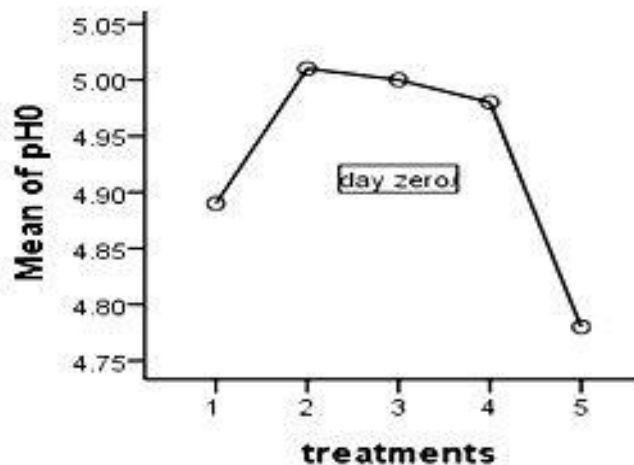
Effect of various treatment regimens on hematological parameters on acidotic sheep (mean±SD)

Parameter			Control group	Treatment I(PT)	Treatment II(PPT)	Treatment III(PRT)	Treatment IV(ST)
Blood pH		Before	6.20±0.447	6.60±.548	6.20±.447	6.60±.548	6.40±.48
		After (R _x)					
		Day1	6.40±0.548	6.59±.648	6.60±.548	6.80±.447	7.40±.548[#]
		Day2	6.00±0.00	7.00±.00[#]	6.68±.547	7.100±.100[#]	7.480±.447[#]
		Day3	6.67±.577	7.00±00	7.00±00[#]	7.50±00[#]	7.57±.577[#]
PCV		Before	44.00±4.183	42.00±5.7	41.80±3.768	38.00±6.819	44.20±2.387
(%)		After (R _x)					
		Day1	43.20±3.70	39.00±3.742	37.40±2.88[#]	34.60±5.367[#]	42.00±2.345
		Day2	39.00±1.00	37.25±4.272	35.60±1.881	29.80±6.261[#]	39.80±1.483[#]
		Day3	35.55±7.12	33.00±3.559	29.40± 2.99[#]	24.80±10.134[#]	37.80±1.789[#]
hemoglobin		Before	15.40±1.67	14.00±2.000	15.80±.447	12.80 ±1.92	14.40±1.517
(gm/dl)		After (R _x)					
		Day1	14.80±2.168	13.20±2.168	14.5±.707	12.10 ± 2.19	13.80±1.78
		Day2	13.00±1.000	12.00±2.168	11.40±.548	11.40±1.817[#]	13.20±1.095
		Day3	12.67±.577	11.50±1.732	11.10±0.89[#]	11.00±2.121[#]	13.00±1.000

Effect of various treatment regimens on of rumen liquor experimental induced acidosis in Sheep

Parameter	Time of Rx	Control group(GC)	Treatment I(PT)	Treatment II (PPT)	Treatment III (PRT)	Treatment IV (ST)
Ruminal (pH)	Before	4.40±.54	4.80±.46188	5.00. ±447	4.96±.837	4.97±.637
	After (R _x)					
	Day1	4.96±.43	5.04±.27	5.18±.31	5.92±.54[#]	5.38±.540
	Day2	5.31±.53	5.19±.33	5.380±.23	6.30±.41[#]	5.85±.540[#]
	Day3	5.4333±.4934	5.50±.216	5.880±.19[#]	6.75±0.34[#]	6.42±.570[#]
protozoan	Before	0.00*(0.-00)	0.00*(0-00)	0.00*(0-1.00)	0.00*(0-00)	0.0*(0 -00)
Motility test	After (R _x)					
	Day1	0.00*(0 -1.00)	0.00*(0 -2.00)	1.00*(0-2.00)	2.0*(0-2.00)	1.00*(0 -1.00)
	Day2	1.00(1.0-1.00)	1.00*(0-1.00)	1.00*(1-2.00)	2.0*(2-3.00) [#]	1.00*(1-1.00)
	Day3	1.00*(1 -2.00)	1.50*(1.00-3.00)	1.00*(1 -300)[#]	3.0*(2-3.0) [#]	2.00*(2-3.00) [#]
MBRT	Before	10.33±1.528	9.25±1.708	9.40±2.966	9.40±2.302	10.20±1.643
(min)	After (R _x)	9.67±1.528	8.25±1.708	7.60±1.51	4.60±3.507[#]	6.40±1.673[#]
SAT	Before	1.33±.577	1.50±.577	1.40±.548	1.60±.894)	1.20±.447

mean ruminal pH between treatment groups for three consecutive days



CONCLUSION

- ❑ Ruminant acidosis is the long standing problem in our country
- ❑ For resolving this problem many traditional buffers have been used.
- ❑ More importantly, in this study the better promising alternative was studied.
- ❑ To start with, experiments were conducted on the proper model, sheep, for inducing ruminant acidosis
- ❑ the adopted model was successfully induced acute rumen acidosis

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- ❑ For this treatment probiotics and standard treatment were provided
- ❑ Therefore, from the present experimental study it has been found that probiotics with rumenototics and standard treatment has provided better therapeutic modality.
- ❑ The present work could be a guide to veterinary professionals for amelioration of rumen acidosis

RECOMMENDATIONS

- The following recommendations were forwarded
 - ❖ To treat acidosis in ruminant animals bacterial probiotics and yeast probiotics along with rumenototics should be recommended
 - ❖ During treatment of acidosis with common standard antacid treatment, other supplements should be recommended
 - ❖ Probiotics should be recommended preferably for long therapeutic periods
 - ❖ Further, study should be conducted particularly about bacterial

Thank you

