Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 942-946

Received: 27-07-2018
Accepted: 28-08-2018

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Effect of yeast and herb supplemented paddy straw on haemato: Biochemical and rumen fermentation parameters in sheep

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Abstract
To study the effect of feeding of paddy straw based complete feed with or without supplementation of feed additives on performance and nutrient utilization of crossbred sheep, a growth trial of 60 days was conducted on 20 male crossbred lambs of 8-9 months of age, divided in four groups of five lambs in each group with T_0 (un-supplemented), T_1 (Herb supplemented), T_2 (Yeast supplemented) and T_3 (Combination of herb and yeast). The lambs were fed a complete feed containing paddy straw 60 parts and concentrate mixture 40 parts on a DM basis supplemented with yeast (*Saccharomyces cerevisiae* 2×10^{10}cfu/g) @ 3 g/kg DM and herb (*Urtica dioca*) @ 3% as per the *in vitro* studies carried to arrive at optimum level of incorporation of these feed additives to paddy straw based complete feed. The haemato-biochemical estimates were made periodically as ancillary observations to assess the physiological status of health of lambs. The haemato-biochemical parameters of blood, Hb, PCV, Total serum protein, albumin, globulin, BUN, serum creatinine, and serum enzymes (ALP, AST, ALT) were found to be within the normal range. Significant ($P<0.01$) improvement was observed on Hb, PCV, total serum protein, albumin, globulin, BUN, serum creatinine, among the treatment groups. The rumen fermentation pattern in experimental lambs was studied at 0, 2, 4, 6 and 8 hours post feeding. All the rumen parameters *viz*., pH, TVFA, total nitrogen, NPN and TCA-ppt. N, revealed significant ($P<0.01$) effect of time of sampling as well as feed additives supplementation alone or in combination. However non-significant difference was observed in terms of NH_3-N between different treatments and control.

Keywords: Herb, Haemato-biochemical parameters, rumen fermentation parameters, Yeast

Introduction
Probiotic growth promoters have been used as supplements in animal feeds for more than two decades. These are viable microorganisms that exhibit a beneficial effect on the health of the host by improving the intestinal microbial balance. Yeast also has a positive effect on hematology of the host resulting in general improvement in the health status (Agazzi *et al.*, 2014) [2]. Milewski (2009) [17] recommended the use of *Saccharomyces cerevisiae* culture in lamb feed for its significant effect on haematological parameters Yalcin *et al.* (2011) [24] studied the nutritive value of live yeast culture (*Saccharomyces cerevisiae*) and its effect on milk yield, milk composition and some blood parameters of dairy cows and concluded that the yeast culture supplementation significantly increased the milk yield with also a tendency to increase fat, protein and lactose yields of milk as well. Reports on higher erythrocyte counts and increased hemoglobin concentration following supplementation of probiotics in heifers are well documented (Agazzi *et al.*, 2014 and Ghazanfar *et al.*, 2015) [2, 9]. Reported that while supplementing probiotics in the feed of Kankerj cows, there was a significant improvement in hemoglobin concentration. Hussein (2014) [12] also found significant improvement on the values of Hb, RBC's and WBC's in Najdi male lambs supplemented with prebiotics. No alterations in the concentration of plasma serum aspartate aminotransferase (Ast) and alanine amino transfers (Alt) were observed in lambs fed biologically treated rice straw by El-Marakby (2003) [6].
There has been an improvement in average daily gain, digestibility and rumen fermentation in growing lambs supplemented with microbial feed additives containing *S. cerevisiae* and *Lactobacillus*. Regarding various nitrogen fractions, it was recorded that total nitrogen concentration in incubation medium was similar between treatment and control in all three substrates. Non protein nitrogen and ammonia nitrogen was depressed significantly

Due to treatment with probiotic cultures in high, medium and low concentrate substrate, resulting in higher TCA-perceptible nitrogen due to yeast supplementation in all three substrates. From these results, it was concluded that yeast supplementation had a significant and positive effect on rumen fermentation. Pandey and Agrawal (2001b) [19] observed the influence of dietary supplementation of probiotic on rumen fermentation in rumen fistulated cross bred bullocks and concluded that the probiotic altered the rumen microbial population to improve fermentation efficiency. Yeast supplementation significantly improved rumen fermentation by stabilizing rumen pH, decreased concentration of lactic acid, ammonia nitrogen, NPN and increased TVFA, total protozoal counts, total nitrogen and TCA perceptible nitrogen. Herbal preparations help in the digestion process, being a component of nature, these preparations are considered safe, cost effective and environment friendly having no side effects. Hence, their inclusion in the diet is encouraged to enhance animals' performance, improve feed utilization, maintain health and alleviate adverse effect of environmental stress (Bhatt, 2000) [4]. Many of the naturally occurring secondary plant metabolites have shown potential to improve rumen fermentation to increase feed efficiency and live body weight gain. For use in organic farming, herbal preparations pass many of the regulatory hurdles easily unlike chemical feed additives which lack such qualities. Although the potential benefits of nettle are still not entirely defined, yet many studies have strengthened its claim as an effective traditional medicine. *In-vitro* and *in-vivo* tests studies have approved many of the nettle's pharmacological effects besides recognizing its richness in protein, minerals and vitamins and proven great nutritional value. In the perspective of a large medical use, several clinical trials conducted in humans have confirmed its pharmacological and nutritional properties.

3. Materials and Methods

Twenty male polled cross lambs of 8-9 months age, having a uniform weight and body conformation, were selected and divided into 4 groups of five animals in each group to study the effect of paddy straw based complete feed with and without supplementation of feed additives. The lambs were fed a complete feed containing paddy straw 60 parts and concentrate mixture 40 parts on DM basis, supplemented with yeast (*Saccharomyces cerevisiae* 2×10^{10}cfu/g) @ 3 g/kg DM (T_2), herb (*Urtica diocia*) @ 3% (T_1). In T_3 group combination of yeast @3 g/kg DM and herb @ 3% DM was supplemented to complete feed while complete feed without supplementation served as control (T_0). Various haemato-biochemical parameters were investigated at the start of the experiment and at monthly intervals to judge the physiological health conditions of the experimental animals. Hb and PCV were estimated by automatic hematology analyzer (SB-21 Vet). Total protein, albumin, BUN, creatinine and serum enzymes (ALP, AST, and ALT) were estimated by using commercial diagnostic kits. Globulin was determined by subtracting albumin from total protein.

Rumen fermentation parameters were studied in polled cross bred lambs using same dietary regimes Various ferementation parameters like. PH, TVFA and nitrogen fractions such as total nitrogen, NH3-N, TCA-ppt. N and NPN were estimated at the different hours post feeding to ascertain the effect of supplementation of feed additives. Rumen liquor of animals, strained through four layers of muslin cloth and designated as SRL was brought to the laboratory in a pre-warmed (39 ^{o}C) thermos flask for further analysis of total volatile fatty acid, rumen ammonia nitrogen, total nitrogen, TCA – perceptible nitrogen and non- protein nitrogen. Rumen fluid pH was measured immediately after collection using portable digital pH meter (pen type) at the site of collection. Total volatile fatty acids were determined according to the method of Barnett and Reid (1957) [3] using Markham still distillation apparatus. Rumen ammonia nitrogen was estimated by spectrophotometer as per the method of Chaney and Marbach (1962) [5]. Total nitrogen was estimated by Kjeldahl technique. Non – protein nitrogen was estimated by precipitating rumen liquor with trichloroacetic acid.5 ml of rumen liquor was precipitated with 5 ml of 20 percent trichloroacetic acid and kept overnight under refrigerated conditions. Next day, after centrifugation at 2000 rpm for 10 minutes, the whole supernatant was transferred into digestion tubes for estimation of nitrogen. The value thus obtained was reported as non-protein nitrogen and the difference of above two was reported as TCA perceptible nitrogen.

The data obtained from the experiment was processed and analyzed statistically using the Statistical Package for the Social Sciences, Base 14.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA

4. Results and Discussion

4.1. Haemato-Biochemical Studies

To ascertain normal physiological status of experimental animals, different haemato-biochemical parameters were studied (Table 1). Significant effect of the treatments i.e. supplementation of yeast in combination with herb in complete feed on haemoglobin and PCV was observed. However, non-significant effect was observed on supplementing yeast and herb alone. The values of Hb reported in the present study are in close agreement with observations of Agazzi *et al.* (2014) [2], and Ghazanfar *et al.* (2015) [9] who reported that probiotic mix supplementation increased ($P<0.05$) the hemoglobin levels and erythrocytes counts in heifers. Milewski's (2009) [17] findings support the use of *Saccharomyces cerevisiae* in lamb feed had a significant effect on haematological indicators, resulting in higher red blood cell counts and white blood cell counts, increased haemoglobin concentrations and higher lymphocyte percentages, indicating that the preparation actively stimulated the immune system of lambs. Lee *et al.* (2015) [14] reported that substitution of TMR with medicinal plant by-products in the diet of Hanwoo steers at late fattening period improved TLC, TEC, Hb and PCV while reduced the blood platelet in treated groups over control. In contrast, Mahgoub *et al.* (2008a) [15] reported lower lymphocyte, monocyte and eosinophil counts in animals consuming diets rich in phenols and tannins. Gupta *et al.* (2006) [10] studied the effect of herbs as feed additive on haemato-biochemical constituents in growing crossbred heifers fed paddy straw based ration and concluded that the inclusion of herbs at 1% level in concentrate mixture as feed additive pose no stress and had no harmful effect on crossbred heifers as assessed from haemato-biochemical constituents.

Total serum protein, albumin and globulin was statistically higher in yeast supplemented animals in combination with herb (T_3), Which may be due to the increase of total serum protein by yeast supplementation may be due to the stimulation of rumen microbes that cause changes in microbial protein synthesis and increased protein passage (Table 1). Hussein (2014) [12] reported that lambs supplemented with probiotic mix shows significant improved values of plasma total protien, albumin, and globulin concentrations than control group. Lee *et al.* (2015) [14] has reported significant improvements in serum total proteins and its fraction concentrations in animals supplemented with different herbs as feed additives.

Significant differences among the treatment groups were observed in blood biochemical variables. A positive correlation exists between level of protein (N) intake and BUN concentration (Kamezos *et al.*, 1994) [13]. Lambs offered un-supplemented diet recorded significantly lower BUN values than feed additives supplemented diets, probably as a result of lower intake of protein and energy intake in un-supplemented group compared to feed additives supplemented groups. Serum creatinine levels in present study in different treatment groups were statistically different being lower in yeast + herb supplemented groups than control, however yeast alone (T2) could not significantly reduced serum creatinine levels confirming earlier reports of Milewski and Sobiech (2009) [17] and Hussein (2014) [12] on yeast feeding in sheep..

In this study plasma enzyme activities of AKP, ALT, AST and CK were not altered by feed additive supplementation. These results are also in line with those of Mahrous *et al.* (2011) [16] found that the addition of biologically treated sugarcane bagasse did not cause significant changes in GOT and GPT that in accordance with the present results. Other workers, EL-moghazy *et al.* (2015) [7], and El-Marakby (2003) [6] pointed out that no significant differences were found among treatments in plasma serum (Ast) and (Alt) for lambs fed biologically treated rice straw compared with control ration. Similarly, Hosoda *et al.* (2006) [11] and Gautam *et al.* (2014) reported no difference in the levels of ALT by the dietary herb inclusion.

4.2. Rumen Studies

The overall mean pH of feed additive supplemented groups was significantly higher in comparison to control group. In all the treatment groups a significant fall in pH was observed at 4 h after feeding, possibly due to greater production of VFA's obtained at same stage of fermentation. While at 6 h post feeding pH tended to increase and could be explained on the basis of greater inflow of bicarbonate rich alkaline saliva buffering the ruminal contents. The fall in pH at 4 h post feeding was also less in supplemented groups in comparison to control. The elevated rumen pH to a certain extentappears to be the result of the increase in number of total and cellulolytic bacteria in rumen (Table 2).

Regarding non-significant differences were recorded for rumen pH in urtica herb supplemented group in comparison to control group. These findings are consistent with the earlier reports of Hosoda *et al.* (2005, 2006) [11] and Afzal (2017) [1].

The ammonia nitrogen concentration in rumen was observed at peak level 4 h post feeding in all the experimental groups. The concentration of ammonia was varying less at 0 h but showed variation at 4 h post feeding among the groups. The peak concentration of ammonia at 4 h was possibly due to maximum proteolytic deaminase activity at this hour, while decrease in concentration at 6h post feeding onwards may be due to simultaneous absorption or its utilization by the microbes in synthetic activity of rumen. There was no significant effect of feed additives on concentration of ammonia nitrogen alone as well as in their combinations. These results are in accordance with several reports Galip, 2006 and Brossard *et al.*, 2006 indicated either no change in NH_3-N on yeast supplementation. Similarly, the results of the present study regarding herb supplementation corroborate well with the earlier reports of Wanapat *et al.* (2008, 2013) [23].

Regarding nitrogen and nitrogen fractions *viz.*, total nitrogen, TCA-ppt N and NPN also showed similar effect of time of sampling as shown in case of ammonia nitrogen i.e. values increased initially, reached to peak at 4 h post feeding and then declined continuously up to 12 h post feeding but there were some variations individually. The peak concentration at 4 h post feeding might be due to more rumen microbial activity during this period. The peak concentration of total-N, NPN, NH_3-N in SRL has also been reported during this period by many workers (Gupta *et al.*, 2006a) [10]

Table 1: Effect of probiotic and herb supplementation on haemato-biochemical parameters and enzyme profile

Particulars	Treatment groups			
	Control group	T1	T2	T3
Haemato- Biochemical parameters				
Hb (g %) **	9.53±0.21^{a}	10.13±0.42^{ab}	10.23±0.28^{ab}	10.70±0.43^{b}
PCV%**	28.80±0.57^{a}	30.60±1.28^{ab}	30.70±0.86^{ab}	32.10±1.31^{b}
Total serum protein (g/dl) **	7.19±0.03^{a}	7.35±0.07^{ab}	7.28±0.05^{a}	7.51±0.11^{b}
Serum albumin (g/dl) **	2.69±0.04^{a}	2.76±0.05^{a}	2.94±0.05^{b}	2.78±0.06^{ab}
Serum globulin (g/dl) **	4.50±0.06^{ab}	4.59±0.07^{bc}	4.34±0.03^{a}	4.73±0.10 ^{c}
Blood urea nitrogen level (mg/dl)	15.10±0.09^{a}	15.48±0.05^{b}	15.15±0.06^{a}	15.80±0.08^{c}
Serum creatinine (mg/dl)	1.83±0.47^{b}	1.45±0.05^{a}	1.79±0.05^{b}	1.58±0.05^{a}
Serum enzyme profile				
ALP (IU/l)	200.76 ±0.34	200.47 ±0.29	200.04 ±0.18	200.08 ±0.18
ALT (IU/l)	20.54 ±0.43	20.81 ±0.29	20.40 ±0.24	21.17 ±0.34
AST (IU/l)	98.21 ±0.97	98.70 ±0.69	97.89 ±0.87	98.91 ±0.89

[ABCD] Means superscripted with different letters in a row for a particular data differ significantly from each other *(P<0.05), ** (P<0.01

Table 2: Effect of probiotic and herb supplementation on Rumen fermentation parameters

	Treatment groups			
	T1	T2	T3	T4
pH**				
0	6.80 ±0.007^{a}	6.802 ±0.013^{a}	6.85 ±0.010^{b}	6.86 ±0.010^{b}
2	6.56 ±0.02^{a}	6.60 ±0.01^{b}	6.67 ±0.010^{c}	6.64 ±0.007^{bc}
4	6.43 ±0.01^{a}	6.48 ±0.03^{a}	6.57 ±0.01^{b}	6.57 ±0.01^{b}
6	6.75 ±0.011^{a}	6.74 ±0.012^{a}	6.74 ±0.014^{a}	6.87 ±0.019^{b}

8	6.81 ±0.013^{a}	6.88 ±0.017^{b}	6.94 ±0.010^{c}	6.95 ±0.009^{c}
TVFA (mEq/l)**				
0	74.86 ±0.25^{a}	79.34 ±0.76^{c}	80.67 ±0.43^{c}	77.65 ±0.35^{b}
2	80.64 ±0.29^{a}	93.91 ±0.49^{c}	90.05 ±0.37^{b}	89.73 ±1.62^{b}
4	96.40 ±0.43^{a}	111.01 ±0.530^{c}	106.50 ±0.40^{b}	107.87 ±0.654^{b}
6	85.74 ±0.72^{a}	95.90 ±0.31^{bc}	97.37 ±0.710^{c}	94.21 ±0.59^{b}
8	77.43 ±0.71^{a}	89.39 ±0.50^{c}	86.72 ±0.98^{b}	85.21 ±0.76^{b}
Ammonia nitrogen (mg/dl)				
0	20.91±0.18	20.96±0.34	20.88±0.30	20.97±0.36
2	22.26±0.36	22.53±0.44	22.45±0.24	22.35±0.44
4	23.70±0.19^{a}	25.60±0.39^{b}	25.27±0.28^{b}	27.29±0.82^{c}
6	21.63±0.36	22.05±0.43	21.86±0.31	22.17±0.46
8	20.10±0.12	20.25±0.32	20.20±0.17	20.44±0.31
Total-N (mg/l)**				
0	73.50±0.75^{a}	94.92±0.98^{b}	93.91±0.85^{b}	95.15±0.36^{b}
2	101.90±0.33^{a}	112.39±0.73^{b}	112.27±0.55^{b}	114.85±0.26^{c}
4	116.71±0.51^{a}	118.77±0.42^{b}	122.06±0.59^{b}	129.98±0.83^{c}
6	106.31±0.61^{a}	116.62±0.98^{b}	117.39±0.87^{b}	116.23±0.33^{b}
8	72.86±0.68^{a}	82.19±0.84^{b}	80.91±0.86^{b}	82.13±0.52^{b}
TCA-perceptible nitrogen (mg/dl)**				
0	40.85±0.11^{a}	48.73±0.72^{b}	50.73±0.83^{c}	56.28±0.35^{d}
2	56.78±0.41	67.04±0.48	66.28±0.55	58.81±12.20
4	62.06±0.88^{a}	78.13±0.31^{b}	78.24±0.66^{b}	87.56±0.82^{c}
6	56.08±0.84^{a}	65.73±0.52^{b}	65.47±0.52^{b}	78.47±1.19^{c}
8	41.36±0.30^{a}	48.94±0.44^{c}	45.24±0.42^{b}	45.22±0.69^{b}
Non-protein nitrogen (mg/dl)**				
0	37.14±2.14^{ab}	42.34±2.07^{b}	37.99±2.49^{ab}	34.55±1.33^{a}
2	48.47±1.12	45.11±0.53	45.29±1.55	46.68±1.15
4	51.02±2.29^{b}	49.98±0.63^{b}	48.96±0.36^{b}	42.79±0.52^{a}
6	43.39±3.01^{b}	52.13±0.98^{c}	51.52±0.48^{c}	36.46±0.80^{a}
8	33.10±0.91^{ab}	33.16±0.98^{ab}	31.84±0.31^{a}	34.74±0.78^{b}

^{ABCD} Means superscripted with different letters in a row for a particular data differ significantly from each other *(P<0.05), ** (P<0.01)

5. Conclusion

All haemato-biochemical parameters of blood *viz.*, Hb, PCV and biochemical parameters (total serum protein, albumin, globulin, BUN, serum creatinine, and serum enzymes (AKP, AST, ALT) were well within the normal range. Significant improvement was observed in terms of Hb, PCV, total serum protein, albumin, globulin, BUN, serum creatinine, among the treatment groups. A significant improvement across periods was observed which could be attributed to adequate balanced nutrition. All the rumen parameters *viz.*, pH, TVFA, lactic acid concentration, NPN, total nitrogen, and TCA-ppt.N revealed highly significant effect of time of sampling as well as feed additives supplementation alone or in combination. However non-significant difference was observed in terms of NH3-N between different treatments and control groups. The value of rumen pH was found to be minimum at 4 h post feeding with simultaneous maximum values of TVFA, NH_3-N, total nitrogen, TCA-ppt. N and NPN recorded at the same hour.

From present study application of herb (*Urtica dioica*) at 3% level and probiotic (*Saccharomyces cerevisiae* 2×10^{10}cfu/g at 3 g/kg level preferably in combination is a promising method to improve the nutrient utilization of poor quality paddy straw by sheep. Thus, farmers can adopt this method of bio-fortification of poor quality crop residues for better nutrient utilization, growth performance, health status and economical animal performance.

6. Acknowledgement

I place on records my thanks to Directorate of Research, SKUAST-K, for financial help and Mountain Research station for Sheep and Goat, Shuhama for cooperation in this endeavour.

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