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The Effect of *Saccharomyces cerevisiae* Live Yeast Culture on Microbial Nitrogen Supply to Small Intestine in Male Kivircik Yearlings Fed with Different Forage-Concentrate Ratios

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***Abstract*—**The aim of the study was to investigate the effect of *Saccharomyces cerevisiae* (SC) live yeast culture on microbial protein supply to small intestine in Kivircik male yearlings when fed with different ratio of forage and concentrate diets. Four Kivircik male yearlings with permanent rumen canula were used in the experiment. The treatments were allocated to a 4x4 Latin square design. Diet I consisted of 70% alfalfa hay and 30% concentrate, Diet II consisted of 30% alfalfa hay and 70% concentrate, Diet I and II were supplemented with a SC. Daily urine was collected and stored at -20°C until analysis. Calorimetric methods were used for the determination of urinary allantoin and creatinine levels. The estimated microbial N supply to small intestine for Diets I, I+SC, II and II+SC were 2.51, 2.64, 2.95 and 3.43 g N/d respectively. Supplementation of Diets I and II with SC significantly affected the allantoin levels in µmol/$W^{0.75}$ ($p<0.05$). Mean creatinine values in µmol/$W^{0.75}$ and allantoin:creatinine ratios were not significantly different among diets. In conclusion, supplementation with SC live yeast culture had a significant effect on urinary allantoin excretion and microbial protein supply to small intestine in Kivircik yearlings fed with high concentrate Diet II ($P<0.05$). Hence urinary allantoin excretion may be used as a tool for estimating microbial protein supply in Kivircık yearlings. However, further studies are necessary to understand the metabolism of *Saccharomyces cerevisiae* live yeast culture with different forage:concentrate ratio in Kıvırcık Yearlings.

***Keywords*—**Allantoin, creatinine, Kivircik yearling, microbial nitrogen, *Saccharomyces cerevisiae*.

I. Introduction

GROWTH promoters especially microbial feed additives on animal performance in the feed industry has growing concern during last decades. Among different microbial feed additives, yeast culture especially *Saccharomyces cerevisiae* (SC) were more widely used in livestock feeding [1] but inconsistent results were reported. The use of SC as a microbial feed additive has increased during the past 20 years. However, the response of yeasts is not consistent on the nutrient utilization, rumen fermentation and production which depends upon several factors [2]. Part of these differences may be attributed to the type and strain of yeast being used [3]. Supplementation of live yeast culture of *Saccharomyces cerevisiae* in high forages and high concentrate diets of male Kivircik yearlings has resulted no significant effect on ruminal parameters except for the percentage of protozoa [4] whereas in a similar study with Kivircik ram ruminal digestion was affected by yeast addition in a diet rich in forage [5].

Yeast culture supplementation has been reported to enhance microbial growth and decrease N loss by incorporating more digestible carbohydrates into microbial mass [6]. Some yeast products did not have a positive response on the amount and composition of microbial crude protein (CP) reaching the duodenum [7], [8] and also did not observe an effect of supplementation of live yeast preparations on rumen N metabolism in lambs. Although, microbial CP supply was improved (2.9–22.7g) in yeast culture supplemented lambs, the efficiency of microbial CP supply did not differ [9]. Different strains of *Saccharomyces cerevisiae* were resulted non-significant ($P>0.05$) increase in the excretion of purine derivatives in the urine, measured as an index of microbial nitrogen leaving the rumen in sheep with RUSITEC [10] and microbial crude protein concentration increased with supplementing 5% yeast culture and decreased propionate concentration with artificial rumen device [11].

Allantoin, an end product of purine metabolism excreted in ruminant urine, has been found to respond to rumen degradable N deficiency [12]. In sheep and other ruminants, allantoin appears to originate predominantly from nucleic acids synthesized by rumen microorganisms [13]. The proportions of individual components of the total PD are normally allantion 60%, uric acid 30-10%, Xanthine, plus hypoxanthine 10-5% in sheep urine [14]. Proportion was not effected by forage:concentrate ratio or type of forage [15]. There was no significant ($r=0.5$) linear correlation between the amount of allantoin excreted in urine and microbial-N synthesis in the rumen with different types of feed [16]. Microbial nitrogen leaving the rumen estimated by urinary purine derivatives was increased with the inclusion of SC [17].

Although reasonable research works has been conducted worldwide reporting the effects of SC supplementation on rumen fermentation parameters and the performance of ruminants; any information has been published showing the effects of SC addition to different forage:concentrate ratio on

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microbial nitrogen supply to small intestine in Kivircik sheep. The objective of proposed study was to investigate the effect of SC live yeast culture on urinary excretion of allatoin, creatinine and microbial protein supply to small intestine in Kivircik male yearlings fed with different ratio of forage and concentrate diets

II. Material and Methods

Animal experiments were conducted at Department of Physiology, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey. Daily urine was collected, sampled and frozen then transferred to Department Animal Science Sarayköy Nuclear Research and Training Center, Turkish Atomic Energy Authority, Ankara, Turkey, for allantoin and creatinine analysis.

A. Animal Material

In the experiment, four male Kivircik yearlings with permanent rumen canula were used. The mean live weights of Kivircik yearlings were 40±8 Kg. They were housed in metabolism cages with free access to drinking water. Protection of animals and animal welfare rules were followed in animal experiments.

B. Feed Materials

Animals were fed with four different diets characterized by the forage (alfalfa hay):concentrate ratio (Diet I with a ratio of 70F:30C and Diet II with a ratio of 30F:70C). A daily dose of 4g Yea-Sacc ^{1026} (Alltech, Nicholasville; 5x10 9CFU/g, *Saccharomyces cerevisiae* live yeast culture) was added to the 2 diets to prepare Diet I+SC and Diet II+SC. Chemical analyses of diets were carried out according to AOAC [18] at Department of Animal Science, Sarayköy Nuclear Research and Training Center, Turkish Atomic Energy Authority, Ankara, Turkey. Diet was designed to meet 1. 25 times of NRC [19] maintenance requirements for sheep. The diets was offered twice daily at 08:30 and 16:30 h, in two equal meals. Animals were weighted every 2 weeks and feeding adjusted to weight changes if necessary.

C. Experimental Procedure

The treatments were allocated to a 4x4 Latin square design. Each feeding period was 20 days. During the last 5 d of each feeding period, daily urine was collected into 10 % H_2SO_4 to keep the final pH< 3, subsampled and stored at -20°C until analysis. Collected urine samples were analyzed for allantoin and creatinine according to procedures given in IAEA – TECDOC [14].

D. Sample Collection

Daily urine was collected into 10% H_2SO_4 in order to keep the final pH<3 and was diluted with water to 4 litres. It was essential to acidify the urine in order to prevent bacterial destruction of purines in the urine. The weight of urine was recorded. The samples were frozen immediately and stored at -20ºC until analysed.

E. Determination of Allantoin and Creatinine

Urine allantoin and creatinine levels were determined by the calorimetric methods described in the IAEA –TECDOC [14]. Allantoin was first hydrolyzed under a weak alkaline condition at 100ºC to allantonic acid which was hydrolyzed to urea and glyoxylic acid in a weak acid solution. The glyoxylic acid reacts with phenylhydrazine hydrochloride to produce a phenylhydrazone derivative of the acid. The product forms an unstable chromophore with potassium ferriciyanide. The colour was read at 522 nm.

The amount of microbial purines absorbed (X mmol/d) corresponding to PD excreted (Y mmol /d) was calculated by (1) [20]:

$$Y=(0{,}150\ W^{0{,}75}\ e^{-0{,}25x})+0.84X \quad (1)$$

where; W= metabolic body weight (kg) of the animal, $e^{-0.\ 25x}$= endogenous purine excretion in urine. This value assumed as zero [20], [21].

If the total urinary excretion greater than about 0. 6 mmol/kg $W^{0.75}$ per d, endogenous contribution will be very small and may be taken at about zero. Generally, this will be applicable to normally fed sheep nourished above 0. 8 times their maintenance energy requirements [22]. Equation (2) was used to estimate MN (g/d) supplied to small intestine [14]:

$$MN\ (g/d) = X\ (mmol/d) \times 70/\ 0.116 \times 0.83 \times 1000 \quad (2)$$

where digestibility of microbial purines was assumed to be 0. 83, N content of purines is 70mg N/mmol and the ratio of purine N:total N in mixed rumen microbes was taken as 0. 116.

F. Statistical Analysis

Animals, periods, and diets were analyzed according to 4x4 Latin square experimental design. Statistical calculations and analysis SAS [23] was carried out in the statistical analysis program [23], [24].

III. Results and Discussion

Ingredients and chemical composition of Diets I and II on DM base are given in Table I. Ingredients of concentrate diet (%, as fed) are shown in Table II. Animals maintained in good health throughout the experiment. No significant changes in body weight were recorded, therefore maintenance conditions were assumed. Urinary excretion of allantoin (mmol/d) and microbial nitrogen (g N/ d), Allantoin (µmol/ $W^{0.\ 75}$), Creatinine (µmol/ $W^{0.\ 75}$) and All:Cre values are shown in Table III.

Mean allantoin excretion levels were changed from 4.45 to 5. 95 mmol/d of Diets I, I+SC, II and II+SC**.** The mean estimated allantoin values for Diets I, I+SC, II and II+SC were 494.00±5.30, 502.34±4.34, 464.46±5.16 and 493.75±4.35 µmol/$W^{0.75}$ respectively. The mean creatinine values for Diets I, I+SC, II and II+SC were 379.00±7.48, 391.89±6.29, 394.62±6.63, and 381.30±7.32 respectively. Mean

allantoin:creatinine ratio values for four diets were changed from 1.20 to 1.30.

TABLE I
INGREDIENTS AND CHEMICAL COMPOSITION OF DIETS I AND II ON DM BASE

Ingredients	Diet I	Diet II
Alfalfa hay (%)	70	70
Concentrate (%)	30	30
Chemical Composition	%	%
Dry matter	88.76	89.05
Crude ash[a]	11.19	8.79
Crude oil[a]	2.90	3.66
Crude protein[a]	21.51	20.32
Crude fiber[a]	18.35	11.53
[b]NDF[a]	41.96	35.10
Organic matter[a]	88.81	91.21
[c]ME (Kcal/kg DM)	2595	2861

[a]Estimations base on dry matter %, [b]Neutral detergent fiber, [c]Metabolisable Energy

TABLE II
INGREDIENTS OF CONCENTRATE DIET (%, AS FED)

Barley	69.16
Corn	10.08
Sunflower meal (30 % CP[a])	17.37
Limestones	2.5
Salt (NaCl)	0.60
Vitamin-Mineral Premix[b]	0.10

[a]CP: Crude Protein
[b]Vitamin-Mineral Premix: VitA 15.000000 IU/kg, Vit $D_3$3.000000 IU/kg,Vit E 30.000 mg/kg, Mn 50.000 mg/kg, Fe 50.000 mg/kg, Zn 50.000 mg/kg, Cu 10000 mg/kg, I 800 mg/kg, Co 150mg/kg, Se 150 mg/kg.

TABLE III
MEAN VALUES OF URINARY EXCRETION OF ALLANTOIN, CREATININE, MICROBIAL NITROGEN SUPPLY TO SMALL INTESTINE AND ALLANTOIN: CREATININE RATIO IN MALE KIVIRCIK YEARLINGS FED WITH FOUR DIFFERENT DIETS SUPPLEMENTED WITH OR WITHOUT LIVE YEAST *SACCHAROMYCES CEREVISIAE* CULTURE

	Diet I	Diet I+SC	Diet II	DietII+SC
Allantoin(mmol/d)	4.45±0.25[a]	5.02±0.27[ab]	5.26±0.26[a]	5. 95±0. 22[b]
Microbial N (g/d)	2.51±0.18[a]	2.64±0.21[a]	2.94±0.17[ab]	3. 43±0.22[b]
Allantoin (µmol/ $W^{0.75}$)	494.0±5.3[a]	502.3±4.3[ab]	464.5±5.2[a]	493.7±4.4[b]
Creatinine (µmol/ $W^{0.75}$)	379.0±7.5[a]	391.9±6.3[a]	394.6±6.6[a]	381.3±7.3[a]
All:Cre	1.30±0.05[a]	1.28±0.03 [a]	1. 2±0.05 [ab]	1.29±0.04 [a]

Means in the same row with different letters in their superscripts differ (P<0. 05). SC: *Saccharomyces cerevisiae,* $W^{0.75}$: Metabolic body weight, All:Cre: allantoin:creatinine ratio.

Urinary excreted allantoin levels were in agreement with reported study [25] which determined allantoin excretion in spot urine in goats and kids fed with concentrate and grass hay, also similarity with [26]. Our results show that there were no statistically significant differences among diets I (70:30, F:C) and II (30:70, F:C) without SC for excretion of allantoin in the urine, however, allantoin excretion was tended to increase by supplementation of SC to Diet I and II. On the other hand, Allantoin excretion was greater in high concentrate diet with supplemented SC than lower concentrate diets without SC. Estimated mean urinary allantoin levels for diets with and without SC in µmol/ $W^{0. 75}$ is higher than the reported value by [27].

Microbial nitrogen (g N/d) was estimated as 2.51, 2.64, 2.94, and 3.43 for Diets I, I+SC, II and II+SC respectively. There were no statistically significant differences among the Diets I and II without supplementation of SC in estimated microbial nitrogen. Similarly, when supply of SC to diets there were no effect observed neither Diet I and I+SC nor Diet II and II+SC However, a significant effect was determined between Diet I+SC and Diet II+SC (P<0. 05). Estimated microbial N was higher 70% concentrate diet than 30% concentrate diet. These results were in agreement with [15] which reported that Urinary excretion of PD and allantoin were greater in sheep fed high concentrate (30:70) diets than in those fed high forage (70:30) diets and tended to be greater for diets containing alfalfa than grass diets. Also, our result confirmed by [28] who demonstrated that the magnitude of the effect of shifting the F:C from 70:30 to 30:70 in goat diets depends on the forage type. With alfaalfa, the benefit of increasing concentrate level in the diet, especially in terms of achieving greater urinary PD excretion and greater N retention efficiency, was less evident than in the case of grass. On the contrary of these results, [29] showed that the addition of a live yeast culture product Yea-Sacc to Diet increased microbial proteosynthesis when the substrate consisted of 80% or 65% hay in vitro. In another study, [30] reported that the addition of 22.50 g/d yeast containing S. cerevisiae improved the release of energy in the rumen to be available for microbial growth in sheep fed Barseem Hay (*Trifolium alexandrium*), compared with the inclusion of 11.25 g/d.

Although beneficial effects of yeast culture (YC) supplementation to high concentrate or high forage diets, [10] concluded that different strains of YC were resulted non-significant (P<0. 05) effect increase in the excretion of purine derivatives in the urine with rusitec device and degradation of hay in the rumen of sheep fed mixed F:C diet was not effected by yeast addition. Similar results were reported by [31] and [8] which they did not observe any positive response on microbial N metabolism by supplementation of yeast products in lambs. Inal et al. [32] reported that commercial live yeast culture addition to the diets of yearling lambs at 4 g/day did not affect ruminal pH, total protozoal number, total VFA, and ammonia-N concentration. However, supplementation of *Saccharomyces cerevisiae* in high forages and high concentrate diets of male Kıvırcık yearlings [4] and rams [5] increased the percentage of protozoa.

There were some controversy in previous studies regarding the ratio of feed when forage:concantrate diets is supplemented with SC. The effects can vary with strains of SC, ingredients of concentrate and digestibility of forage [3]. Supplementation of Diets I and II with *Saccharomyces cerevisiae* significantly affected the allantoin levels in µmol/$W^{0.75}$ (p<0. 05).

Obtained results of the present study indicated that the addition of *Saccharomyces cerevisiae* to high concentrate diet (70:30, F:C) has significant effect (*P*<0. 05) on microbial protein supply to small intestine. Yeast culture supplementation improved (*P*<0. 05) microbial CP synthesis in feedlot lambs fed *ad libitum* total mixed ration [33].

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Ruminal microbial nitrogen synthesis depends mainly on a adequate supply carbohydrates as the energy sources, which is the main factor limiting microbial growth due to effect of forage:concentrate ratio**.**

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