DEVELOPMENT, STANDARDISATION AND VALIDATION OF PURINE EXCRETION TECHNIQUE FOR MEASURING MICROBIAL PROTEIN SUPPLY IN YERLİ KARA CROSS BREED

N. Cetinkaya[(1)], S. Yaman[(2)], H. Ozdemir[(1)], A. I. Gucus[(1)], H. Ozcan[(1)], A. Sogut[(1)]

[(1)] Turkish Atomic Energy Authority, Ankara Nuclear Research Center in Agriculture and Animal Sciences, Saray, Ankara, Turkey
[(2)] MARA Lalahan Livestock Central Research Institute, Lalahan, Ankara,Turkey

Abstract

Three experiments were conducted to evaluate of the developed techniques for uric acid, allantoin and creatinine in Yerli Kara cross-breed cattle on farm at different feeding level locally available feed resources and linking the observed information to feed intake and to assess of protein nutrition status of Yerli Kara cross-breed dairy cattle using urinary PD and creatinine excretion. In Experiment I. Response of daily PD excretion to feed intake in Yerli Kara cross-breed on state farm was measured. Animals were fed a mixed diet containing 30% wheat straw and 70% compounded feed. The diet contained 90% DM, its N and OM contents were 124 and 950 g/kg DM, respectively. In Experiment II. Spot urine sampling techniques was applied at state farm. Four Yerli Kara cross-breed bulls live weight with a mean of 211±41.3 kg were used. Experimental design, feeding and diet were the same as in Experiment I. The treatments were allocated according to a 4x4 Latin Square design. In Experiment III. Spot urine sampling techniques was applied at smallholder farms. Compound feed containing 65% barley, 25% bran, 6% sunflower seed meal, 3% marmer dust and 1% mineral and vitamin mixture (120 g/kg DM-Crude Protein and 950 g/kg DM-Organic Matter)-was offered total in between 2 to 3 kg in two parts one in the morning (07:30 h) and one in the afternoon (17:00 h). Compound feed ingredients were similar given to all animals but Groups I, II and III animals were receiving 1 to 2 kg/d of straw (30 g CP/kg DM, 930g OM/kg DM), grass hay (70g CP/kg DM ,915 g OM/kg DM), straw and grass hay respectively.

There were significant correlations (R^2=0.99) between PD excretion (mmol/d) and DOMI (kg/d) for YK-C cattle. PD exceretion (mmol/d) was plotted against PD: Creatinin $W^{0.75}$ to obtain slope and use as constant for the estimation of daily PD exceretion from spot sampling from animals held by small holders. The equation could be expressed as: PD (mmol/d)= -2.3+0.953 ([PD]:[C]x$W^{0.75}$). The constant (C)-the slope as mmol/kg $W^{0.75}$ was 0.953.

The coefficient of variation (CV) for the uric acid, allantion, PD, creatinine, total-N, PD:CxW$^{0.75}$ (PDC Index) in spot urine samples of four treatments were less than 5%.

Developed banding system can be used for YK and YK-C cattle for the spot urine sample measurement.There were not significant difference for each parameter between the spot urine sampling times of Groups I, II and III. The corresponding microbial-N values to PDC Index of groups I. II and III were 15-25 g/d. Experimentaly estimated DOMI was 2.21±0.15 kg/d. By using the equation (DOMI= 344+48.7xPDC Index) estimated DOMI (g/d) of groups I, II, and III were 2.8±0.6, 2.6±0.7 and 2.7±0.7 respectively. CV% of Groups I, II and III were 22, 27 and 26% respectively.

In conclusion, the PDC Index in spot urine samples could be used under farm condition as an indicator of microbial protein supply in YK-C cattle, and also DOMI can be estimated from PDC Index in spot urine samples under field conditions.

1. Introduction

Previous studies in sheep and cattle have demonstrated that the urinary excretion of purine derivatives (PD) provides an index of the intestinal flow of microbial protein [2]. The purine derivatives technique is a simple and non-invasive alternative to measure microbial-N flow to intestine in ruminants. There are information and quantitative models for cattle and sheep and, colorimetric methods which allow the use of this technique. The purine derivatives technique has the advantaje of using intact animal but, the disadvantage of requiring total urine collection. The use of urine spot-samples together with the purine derivatives:creatinine ratio index can be an alternative to replace total urine collection. Since the total urine collection makes the developed models impractical, spot urine PD and creatinine measurement and banding system was suggested by Chen et all [3]. Yerli Kara Cross (YK-C) breeds are common cattle in Turkey. It was of interest to establish whether the approach of PD excretion to estimate microbial protein supply is applicable in Yerli Kara Cross-breeds cattle.

The model based on urinary purine derivatives (PD) excretion for estimating rumen microbial protein production was previously developed in Yerli Kara Cross-breeds [4, 5].

The aim of the present study is to achieve the following objectives:

a) Evaluation of the developed colorimetric techniques for uric acid, allantoin and creatinine in Yerli Kara cross-breed cattle on farm at different feeding level locally available feed resources and linking the observed information to feed intake
b) Assessment of protein nutrition status of Yerli Kara cross-breed dairy cattle using urinary PD and creatinine excretion

2. Materials and methods

2.1. Experiment I. Response of daily PD excretion to feed intake in Yerli Kara cross-breed on state farm.

Four Yerli Kara cross-breed bulls live weight with a mean of 209±39.4 kg were used. The bulls were housed in metabolism cages and fitted with urine collection aprones. Animals were fed a mixed diet containing 30% wheat straw and 70% compounded feed. The diet contained 90% DM, its N and OM contents were 124 and 950 g/kg DM, respectively. The diet was offered twice daily at 09:30 and 17:30 h, in two equal meals. Fresh water was available freely. The compound feed was consisted of 67% barley, 10% wheat bran, 20% sunflower cake, 1% salt, 1.5% mermer dust and 0.5% mineral and vitamin mixture. During the preliminary period, the diet was given at *ad libitum* level of intake. Voluntary intake was measured for each animal over a period of 2 weeks. After the preliminary period, animals were fed at 4 fixed levels as 95, 80, 60 and 40% of the voluntary intake. Voluntary intakes were 6kg DM/d for four bulls. The treatments were allocated according to a 4x4 Latin Square design. Each feeding period lasted for 3 weeks.

During the last 3 days of last week of each feeding period daily total urine and faeces were collected. Collected urine samples were analyzed for uric acid, allantoin [6], creatinine [7] and total-N [1]. Feaces samples were analyzed for OM and DM.

2.2.Experiment II. Spot urine sampling at state farm.

Four Yerli Kara cross-breed bulls live weight with a mean of 211±41.3kg were used. Experimental design, feeding and diet were the same as in Experiment I. The treatments were allocated according to a 4x4 Latin Square design.

At each feeding period spot samples were collected in two days. The sampling times were at 09:30 and 11:30 in the morning and 14:30 in the afternoon. Collected urine samples were analyzed for uric acid, allatoin [6], creatinine [7] and total-N [1].

2.3. Experiment III. Spot urine sampling at smallholder farms.

30 Yerli Kara cross-breed bulls were selected from 12 different small holder farms. Age, body weight and avarage feed intake were recorded.Selected farms were divided into three Groups I, II and III according to received feeds. Feed samples were collected and analysed for organic matter, dry matter and crude protein. Compound feed containing 65% barley, 25% bran, 6% sunflower seed meal, 3% marmer dust and 1% mineral and vitamin mixture (120 g/kg DM Crude Protein and 950 g/kg DM Organic Matter) was offered total in between 2 to 3kg in two parts one in the morning (07:30 h) and one in the afternoon (17:00 h). Compound feed ingredients were similar given to all animals but Groups I, II and

III animals were receiving 1 to 2kg/d of straw (30 g CP/kg DM ,930 g OM/kg DM), grass hay (70g CP/kg DM, 915g OM/kg DM), straw and grass hay respectively.

Spot urine samples were taken from bulls 2-3 times in two days. Collected urine samples in plastic bottles containing 5% H_2SO_4 were transported to laboratory. Four aliquots of each collected urine were stored – 20^oC until analysis. Urine nitrogen, creatinine [7], allantoin and uric acid [6], concentrations were determined.

Test urine sample were prepared and run at each chemical analysis. The slope and intercept of each standard curve were evaluated for interassay changes.

2.4. Statistical Analysis

Two-factor ANOVA was performed to compare the differences between the measurements. The relationships between PD excretion and feed intake; DOMI and PD:C ratio, were exemined by linear regression analysis. The statistical analysis was aided by Minitab.

3.Results and discussion

3.1 Experiment I. Response of daily PD excretion to feed intake in Yerli Kara-cross breed on state farm.

Nutrient digestibility of diet fed to YK-C breed cattle at four different leves of intake is shown in Table I. The mean values of total PD, allantion, uric acid, creatinine and total urinary nitrogen are presented in Table II. The differences between four treatments were statistically significant ($P<0.01$) with the exception of creatinine for YK-C fed at four different levels.

Table I. Nutrient digestibility of diet fed to yerli kara cross breed bulls at four different levels of intake

	Level of feed intake (%)			
	40	60	80	95
DM digestibility (%)	71.8 (± 1.2)	72.9 (± 3.9)	69.6 (± 10.1)	75.1 (± 5.7)
OM digestibility (%)	71.7 (± 2.45)	72.6 (± 4.8)	68.8 (± 11.3)	74.5 (± 6.1)
DOMI (kg/d)	1.46 (± 0.05)	2.21 (± 0.15)	2.79 (± 0.46)	3.44 (± 0.37)
DDMI (kg/d)	1.56 (± 0.04)	2.37 (± 0.12)	3.03 (± 0.44)	3.70 (± 0.39)

Standard deviations within parenthesis

Table II. Excretion of urinary pd, allantoin, creatinine, uric acid and total nitrogen in yerli kara cross bulls fed at four different levels of intake

Purine derivatives	Level of feed intake (%)			
	40	60	80	95
Total PD (mmol/d)	42.2^{a} (± 3.6)	55.0^{b} (± 4.1)	66.0^{c} (± 4.0)	80.8^{d} (± 2.8)
Allantoin (mmol/d)	38.7^{a} (± 3.8)	50.6^{b} (± 4.0)	60.9^{c} (± 3.8)	74.7^{d} (± 2.7)
Creatinine (mmol/d)	61.6 (± 4.2)	59.4 (± 4.9)	61.3 (± 3.6)	59.2 (± 3.9)
Uric acid (mmol/d)	3.49^{a} (± 0.56)	4.44^{b} (± 0.57)	5.11^{c} (± 0.35)	6.12^{d} (± 0.42)
Total–N (g/d)	26.6^{a} (± 2.6)	33.2^{b} (± 2.7)	38.0^{c} (± 2.1)	43.1^{d} (± 4.3)
Total PD/$W^{0.75}$ (mmol/d/kg)	0.78^{a} (±0.10)	1.01^{b} (±0.11)	1.21^{c} (±0.12)	1.49^{d} (±0.17)
Allantoin/ $W^{0.75}$ (mmol/d/kg)	0.71^{a}(±0.10)	0.93^{b} (±0.11)	1.12^{c} (±0.12)	1.38^{d} (±0.16)
Total PD/Creatinine	0.69^{a} (±0.06)	0.93^{b} (±0.08)	1.08^{c} (±0.08)	1.37^{d} (±0.10)
Creatinine/ $W^{0.75}$ (mmol/d/kg)	1.34 (±0.12)	1.10 (±0.14)	1.13 (±0.11)	1.09 (±0.10)
PDC Index	37.6^{a} (±5.5)	51.2^{b} (±9.0)	59.3^{c} (±9.2)	75.0^{d}(±9.5)

Standard deviations within parenthesis
Mean values within a row with different superscripts are significantly different ($P<0.001$)

The relationship between urinary PD excretion and DOMI for cattle is in Fig.1.

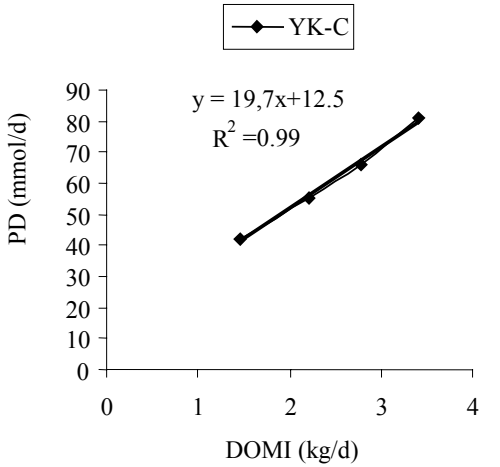

Fig. 1. Urinary PD of YK-C as a function of DOMI

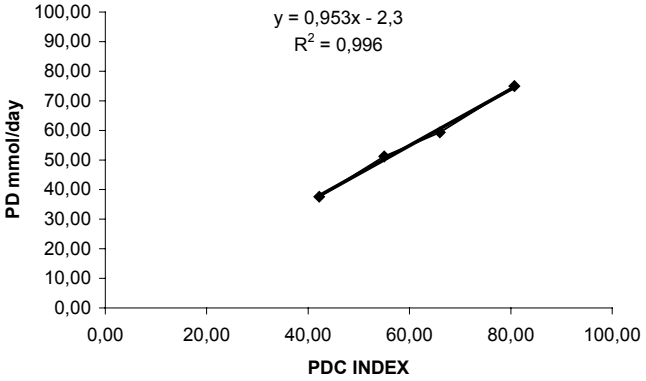

Fig. 2. Urinary PD versus PDC Index of YK-C

There was significant correlation (R^2=0.99) between PD exretion (mmol/d) and DOMI (kg/d) for YK-C cattle. Similar to previous report values for Bos taurus cattle [9, 10]. The profile of PD excretion in the urine of YK-C cattle was similar to other breeds of cattle [2, 8, 11]. The results also showed that PD excretion rate per kg DOMI for YK-C is similar to YK [4, 5] but higher than that for buffalo [11, 12]. PD exceretion (mmol/d) was plotted against [PD]: [Creatinin] x $W^{0.75}$ (PDC Index) to obtain slope and use as constant for the estimation of daily PD exceretion from spot sampling from animals held by small holders. The equation could be expressed as:

$$PD\ (mmol/d) = -2.3 + 0{,}953\ (PDC\ Index) \quad (1)$$

The constant (C)-the slope as mmol/kg $W^{0.75}$ was 0.953.

3.2.Experiment II. Spot urine sampling at state farm.

Changes of total-N (g/L), allantion, uric acid, creatinine, total-PD (mmol/L) and PDC Index in spot urine samples in YK-C at four different levels of intake are shown in Table III

Each value was the mean of 8 spot urine samples. There were significant differences between treatments at the same spot sampling times of each parameter ($P<0.01$) with the exception of allantoin, total-N and creatinine between 60% and 95% of feeding levels. The mean values of total-N, PD and uric acid were not different between spot urine collection times at 40% feeding level but significant for allantion, creatinine and PDC Index ($P<0.05$) The mean values of total-N,

allantion, uric acid PD and PDC Index were not significant but significant (P<0.05) for creatinine at 60% feeding level between the spot urine collection times.

Table III. Changes of uric acid, allantoin, total-pd (mmol/l), creatinine, total-n (g/l), and pdc index in spot urine samples in yk-c cattle at four different levels of intake

Variable	Sampling Time	Feeding Level			
		%40	%60	%80	%95
1	2	3	4	5	6
Uric Acid	1 m	$0.97^{a} \pm 0.10$	$1.41^{a} \pm 0.08$	$1.52^{a} \pm 0.06$	$1.89^{a} \pm 0.06$
(mmol/L,	2 m	$1.28^{a} \pm 0.41$	$1.44^{a} \pm 0.05$	$1.61^{b} \pm 0.05$	$1.81^{a\,b} \pm 0.05$
X.±SD)	3 a	$1.05^{a} \pm 0.07$	$1.45^{a} \pm 0.06$	$1.37^{c} \pm 0.05$	$1.75^{b} \pm 0.07$
Allantoin	1 m	$10.28^{a} \pm 0.42$	$12.54^{a} \pm 0.49$	$15.13^{a} \pm 0.65$	$19.36^{a} \pm 0.38$
(mmol/L,	2 m	$10.12^{a} \pm 0.32$	$12.51^{a} \pm 0.46$	$14.77^{a} \pm 0.79$	$18.71^{a\,b} \pm 0.65$
X±SD)	3 a	$9.71^{b} \pm 0.42$	$12.62^{a} \pm 0.40$	$14.92^{a} \pm 0.59$	$18.05^{b} \pm 0.69$
PD	1 m	$11.25^{a} \pm 0.52$	$13.95^{a} \pm 0.56$	$16.65^{a} \pm 0.70$	$21.25^{a} \pm 0.43$
(mmol/L,	2 m	$11.40^{a} \pm 0.63$	$13.94^{a} \pm 0.50$	$16.37^{a} \pm 0.82$	$20.50^{bc} \pm 0.70$
X±SD)	3 a	$10.76^{a} \pm 0.46$	$14.07^{a} \pm 0.45$	$16.29^{a} \pm 0.62$	$19.80^{c} \pm 0.75$
Creatinin	1 m	$18.07^{a} \pm 0.16$	$19.43^{a} \pm 0.22$	$18.61^{a} \pm 0.34$	$19.27^{a} \pm 0.33$
(mmol/L,	2 m	$18.38^{b} \pm 0.17$	$19.15^{b} \pm 0.19$	$19.62^{b} \pm 0.39$	$18.38^{b} \pm 0.40$
X±SD)	3 a	$18.47^{c} \pm 0.31$	$20.36^{c} \pm 0.40$	$19.21^{c} \pm 0.32$	$19.25^{a} \pm 0.37$
Total-N	1 m	$12.45^{a} \pm 0.33$	$12.74^{a} \pm 0.43$	$11.96^{a} \pm 0.35$	$12.03^{a} \pm 0.22$
(gN/L,	2 m	$12.22^{a} \pm 0.27$	$12.00^{a} \pm 0.40$	$12.30^{a} \pm 0.24$	$12.74^{b} \pm 0.43$
X±SD)	3 a	$12.39^{a} \pm 0.25$	$11.99^{a} \pm 0.58$	$12.17^{a} \pm 0.21$	$13.23^{c} \pm 0.27$
PDC Index	1 m	$34.49^{a} \pm 1.47$	$39.78^{a} \pm 1.22$	$49.55^{a} \pm 1.44$	$61.11^{a} \pm 0.78$
(X±SD)	2 m	$34.37^{a} \pm 1.80$	$40.35^{a} \pm 1.45$	$46.22^{b} \pm 1.66$	$61.83^{a} \pm 0.95$
	3 a	$32.26^{b} \pm 0.91$	$38.29^{a} \pm 0.78$	$46.98^{b} \pm 1.49$	$56.96^{b} \pm 1.28$

1m : 1st. morning collection
2m : 2nd. morning collection
3a : 3th collection, afternoon collection
Within columns, for each variable, means with a common superscript do not differ significantly (P>0.05).

There were significant differences (P<0.05) for creatinine, uric acid and PDC Index between spot sampling times but not for PD, total-N and allantion at 80% feeding level.

The differences were significant (P<0.05) for creatinine, uric acid, allantion, PD and PDC Index between morning and afternoon spot urine collection times at 95% feeding level.

The coefficient of variation (CV) for the uric acid, allantion, PD, creatinine, total-N, PDC Index in spot urine samples of four treatments were less than 5%.

3.2.Experiment III. Spot urine sampling at small holder farms .

Mean values of uric acid, allantion, PD and creatinine in mmol/L; metabolic weights at Groups I, II and III; estimated daily PD excretion by using C Constant obtained from Experiment I and [PD]:[C]x$W^{0.75}$ (PDC Index) of small holders YK-C cattle, are shown in Table IV. There were not significant difference for each parameter between the spot urine sampling times of Groups I, II and III.

Table IV. Mean values of uric acid, allontoin, pd, creatinine, total nitrogen, pdc index in spot urine samples; estimated daily pd and metabolic weight of yerli kara cross bulls held by small holder farms

	Group I (n=10)		Group II (n=10)		Group III (n=10)	
	m	a	m	a	m	a
Uric acid (mmol/L)	1.3±0.2	1.3±0.2	1.4±0.2	1.4±0.2	1.5±0.2	1.4±0.1
Allantoin (mmol/L)	11.7±1.0	11.9±1.0	10.7±1.5	11.2±1.3	11.0±0.9	11.1±1.0
PD mmol/L	13.0±1.0	13.2±1.0	12.2±1.5	12.6±1.4	12.5±1.0	12.6±1.2
Creatinine (mmol/L)	17.1±1.1	17.1±1.1	17.9±1.0	17.9±0.9	18.3±1.7	18.2±1.4
Total–N (g/L)	11.6±1.1	11.5±0.8	11.5±0.9	11.6±0.8	11.9±0.7	11.8±0.7
PD Index	49.7±13.5	50.2±13.5	44.7±15.0	46.5±15.1	48.6±14.5	49.3±15.3
PD Estimated mmol/d	47.3±12.9	47.8±12.8	42.6±14.3	44.3±14.4	46.3±13.8	47.0±14.6
$W^{0.75}$(kg)	64.1±13.2		65.0±16.4		70.6±17.8	

m : morning collection
a : afternoon collection
n : number of animals
PDC Index :[Total Purin Derivatives-mmol/L]:[Creatinine-mmol/L]x $W^{0.75}$(Metabolic Weight)
p>0.05

The banding system was prepared by using PDC Index obtained from spot sampling under the controlled Experiment II, PD excretion from Experiment I, Microbial-N was estimated using the model developed for YK cattle [4, 5] and the equation given for European breed of cattle [6]. The corresponding values for the daily PD excretion and microbial-N supply in YK-C at four different ranges of PDC Index is shown in Table V.

As suggested by Chen et al [3], this banding system can be used for YK and YK-C cattle for the spot urine sample measurement.

The corresponding microbial-N values to PDC Index of groups I. II and III (Table V) were fallen into the same Band II and is 15-25 g/d. This is the indication of insufficient feeding under small holder farms condition since the value matches with 60%, feeding value, (Experiment I). Experimentaly estimated DOMI was 2.21±0.15 kg/d

In order to use of PDC Index to estimate DOMI (g/d) for YK-C cattle, DOMI values obtained from Experiment I were plotted against PDC Index which is obtained from Experiment II. Plott is shown in Fig. 3.

$$DOMI = 344 + 48.7 \times PDC\ Index \quad (2)$$

By using the equation (2) obtaind from Fig.8 DOMI (g/d) of Groups I, II, and III were estimated as 2.8±0.6, 2.6±0.7 and 2.7±0.7 respectively. CV% of Groups I, II and III were 22, 27 and 26% respectively.

Table V. The corresponding values for the daily pd excretion and microbial - n supply in yerli kara cros breed cattle at four different ranges of pdc index

Band	PDC Index [PD mmol/L]:[C mmol/L]×kg W $^{0.75}$	PD excretion (mmol/d)	Estimated Microbial N (g/d)
I	<30	<35	<15
II	30-45	35-60	15-25
III	45-60	60-70	25-40
IV	>60	>70	>40

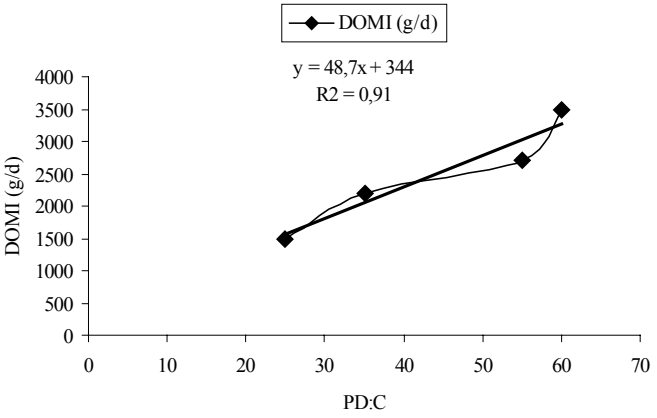

Fig. 3: The digestible organic matter intake (DOMI) of the YK-C plotted against PDC Index, which is expressed as (mmol/L PD divided by mmol/L Creatinine)×kg $W^{0.75}$

Changes in intercepts and slopes of standard curves of allantoin, uric acid and creatanine with days of analysis are shown in Fig. 4. and Fig. 5. CV% of slopes of standard curves of allantoin, uric acid and creatanine were less than 5%.

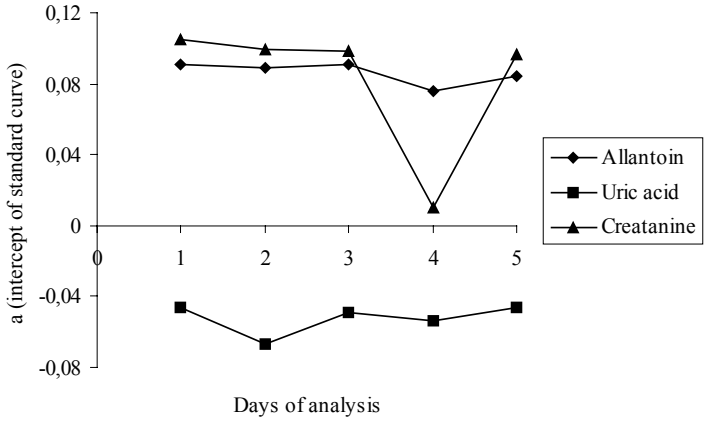

Fig. 4: Changes in intercepts of standard curves of allantoin, uric acid and creatanine with days of analysis

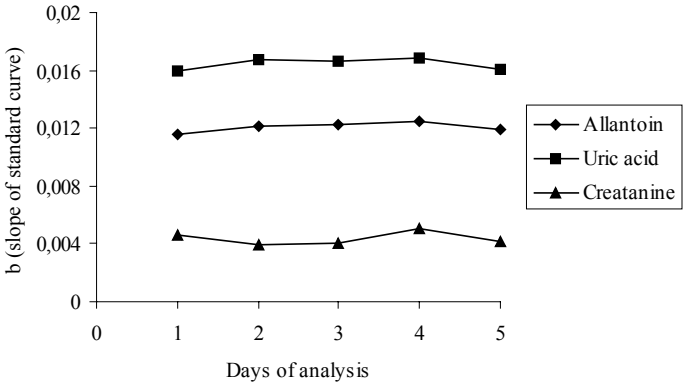

Fig. 5: Changes in slopes of standard curves of allantoin, uric acid and creatanine with days of analysis

In conclusion, the PDC Index in spot urine samples could be used under farm condition as an indicator of microbial protein supply in YK-C cattle, and also DOMI can be estimated from PDC Index in spot urine samples under field conditions.

4. Acknowledgements

Authors are grateful to International Atomic Energy Agency, Turkish Atomic Energy Authority and Republic of Turkey, Ministry of Agriculture and Rural Affair for providing financial and material support.

References

1. AOAC, Official Methods of Analysis, 15th Edn. Association of Official Analytical Chemists, Arlington. VA. (1990)
2. Chen, X.B., Orskov, E.R., Hovell, F.D. DeB., Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep. Br.J. Nutr. 63(1990)121-129.
3. Chen, X.B., Mejia, A.T. Kyle, D.J. Orskov, E.R., Evaluation of the use of purine derivative:creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants:studies in sheep. J. Agric. Sci., 125(1995)137-143.

4. Cetinkaya, N., Yaman, S., Gucus, A.I. Ozcan, H., Uluturk, S., Urinary excreation of purine derivatives in Yerli Kara cattle. T. J. Nuc. Sci. 27(2000)13-32.
5. Cetinkaya, N., Yaman, S., Gucus, A.I. Ozcan, H., Uluturk, S., Measuring microbial protein supply from purine excreation in Yerli Kara cattle. IAEA. TECDOC-1093. Vienna, (1999)69-79.
6. International Atomic Energy Agency, Estimation of rumen microbial protein production from purine derivatives in urine, IAEA-TECDOC-945, IAEA, Vienna (1997)22-24.
7. Hawk, P.B., Oser, B.L., Summerson, W.H., Prac. Physiol. Chem. 14th Ed., McGraw Hill Publishing Company Ltd., London (1976).
8. Verbis, J., Chen, X.B., Macleod, N.A., Orskov, E.R., Excreation of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivatives excretion by steers. J. Agric. Sci., Camb. 14(1990)243-248.
9. Daniels, Z.M., Chen, X.B., Kyle, D.J., Sinciatr, K., Orskov, E.R., Purine derivatives in urine and plasma of lactating cows given differed levels of food intake, Anim. Prod. 58 (1994) 463 A.
10. Giesecke, D., Balsliemke, J., Suderum, K.H., Stangassinger, M., Plasma level, clerance and renal excretion of endogenous and ruminal purines in the bovine, J. Anim. Physiology Anim. Nutr. 70 (1993) 180-189.
11. Chen, X.B., Samaraweera, L., Kyle, D.J., Orskov, E.R., Abeygunawardene, H., Urinary excretion of purine derivatives and tissue xanthine oxidase activity in buffaloes with special reference to differences between buffaloes and Bos Taurus cattle. Brit. J. Nutr. 75(1996)397-407.
12. Liang, J.B., Matsumoto, M., Young, B.A., Purine derivative excretion and ruminal microbial yield in Malaysian cattle and swamp buffalo, Anim. Feed Sci. Technol. 47 (1994) 189-199.