

Absorption of Nucleic Acid from the Small Intestines of Swamp Buffaloes and Zebu Cattle

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Abstract

Three male yearling swamp buffaloes and three Kedah Kelantan (KK) zebu cattle of similar age and sex were used. Animals were each fed 4 kg of a total mixed ration diet (TMR) consisting of 40% oil palm frond and 60% concentrate for 2 wk. Cr_2O_3 was used as the indigestibility marker. On day 15, animals were sacrificed and the digesta in the small intestine was collected to study the absorption of purines in the segments, each being 60 cm in length of the small intestine. Deoxyribonucleic acid (DNA) disappearance from the first to the 15th segment did not differ between buffaloes and cattle ($P>0.05$), averaging 94% and 91%, respectively. Ribonucleic acid (RNA) disappearance for buffaloes was lower than that for cattle ($P<0.05$) (82% and 95%, respectively) but did not differ between species ($P>0.05$). Similarly, purine absorption did not differ between buffaloes and cattle ($P>0.05$). Total purine absorbed within 20 segments was 81% and 92% respectively for buffaloes and cattle. Although disappearance of RNA, DNA and the purine bases (PB) in the small intestine did not differ between cattle and swamp buffaloes ($P>0.05$), based on concentration per Cr marker, the quantity of unabsorbed PB in the last 5 segments of the small intestine of buffaloes was higher than that of cattle ($P<0.05$). Although this suggests that absorption of PB in the small intestine of buffaloes was lower than of cattle, it can not explain the large differences in the PD urinary excretion rates between the species.

Key Words: Swamp buffaloes; Kedah Kelantan (KK) zebu cattle; Purine absorption; Nucleic acid

Introduction

Nucleic acids in duodenal digesta are mainly of microbial origin (Perez et al., 1997). However, microbial nucleic acids are extensively degraded by enzymes when they enter the small intestine. Intestinal mucosa are the first site for the degradation of absorbed purines. Xanthine oxidase plays an important role in governing the availability of

exogenous purines for utilization by the animal. The mucosal cell of buffaloes and cattle are rich in xanthine oxidase, which increases the potential for oxidation of absorbed purines before they enter the blood. In contrast, sheep mucosa have only trace amounts of xanthine oxidase (Al-Khalidi and Chaglassian, 1965; Chen et al., 1996). Nucleosides and free bases (except hypoxanthine) are almost

entirely absorbed before reaching the terminal ileum of ruminants (McAllan, 1980). Apparent digestibility values for nucleic acids in the small intestine were reported by Smith and McAllan (1970) to be about 75 to 85%. Various researchers (Smith and McAllan, 1971; Coehlo da Silva et al., 1972; Jackson et al., 1976; McAllan, 1980) have reported that the net digestibility of microbial nucleic acids between the proximal duodenum and distal ileum of sheep and steers are 80 to 90% for ribonucleic acid (RNA) and 75 to 85% for deoxyribonucleic acid (DNA), and that absorbed is excreted via the kidney. Excretion rates of purine derivatives (PD) in the urine reflect, and therefore can be used to predict, flows of microbial purines into the intestinal. Quantitative relationships between total urinary excretion of PD and exogenous purine input have been established for European sheep (Chen et al., 1990b; Balcells et al., 1991) and cattle (Verbic et al., 1990; Vagnoni et al., 1997) to estimate rumen microbial protein synthesis. Liang et al. (1994) reported that the excretion of urinary PD for zebu cattle did not differ from that of European cattle, but that for swamp buffaloes was 43 to 55% lower than cattle (Vercoe, 1976; Liang et al., 1994). The lower urinary PD excretion in buffaloes was postulated to be due, at least partly, their ability to recycle more plasma PD into the rumen (Liang et al., 1994; Chen

et al., 1996). However, this postulation was shown to be invalid based on recovery rate of plasma PD using ^{14}C uric acid as a marker (Pimpa, 2002). In view of this, one possible explanation for the lower urinary PD excretion rate in buffaloes could be due to lower absorption of purine in the small intestine of buffaloes as compared to cattle.

The objective of the study was to elucidate differences in the apparent purine absorption in the small intestine of swamp buffaloes and zebu cattle and to test the hypothesis that the observed lower urinary PD excretion in buffaloes, compared to cattle, was due to lower absorption of purines in the buffaloes.

Materials and Methods

Animals and feeding

Three male swamp buffalo yearlings [232.3 ± 36.26 kg initial body weight (BW)] and three KK zebu cattle (148.3 ± 12.73 kg) of similar age and sex were used. Animals were kept in individual pens and fed 4 kg DM/d of a total mixed ration diet (TMR) consisting of 40% oil palm frond and 60% concentrate (maize grain 24%, soy bean meal 29.5%, cassava chip 29.5% and mineral premix 1.7% on DM basis) mixed and pelleted together (Table 1). Feed was offered in two equal portions, at 07:00 and

Table 1 Dry matter (DM) and major chemical components of dietary ingredients¹

	Dry matter (%)	Crude protein	Neutral Detergent fiber	Acid Detergent fiber	Ash
			-----% DM basis-----		
Oil palm frond (OPF)	95.4	5.49	84.66	56.76	6.75
Concentrate ²	96.4	16.83	41.9	11.1	4.72

¹ Values are means of 4 subsamples of each material assayed.

² Consisted of maize grain 24%, soy bean meal 29.5%, cassava chip 29.5% and mineral premix 1.7% on DM basis.

16:00 h for the 3 wk study (1 wk of adaptation and 2 wk of data collection). During data collection, chromium oxide (Cr_2O_3) was used as an indigestible marker and was mixed with the concentrate at 1 g/kg DM intake (DMI, McAllan, 1980). Clean drinking water was freely available at all times. The BW of the animals were recorded on the last 3 d before the animals were sacrificed on the last day of the experiment.

Collection of digesta

On the last day (day 15) of the 2 wk data collection, the 3 buffaloes and 3 cattle were sacrificed 4 h after the morning feeding. The body cavities were opened and intestine exposed. The small intestine was immediately tied with a string at approximately 60 cm intervals, starting from the pylorus, to prevent mixing of the digesta. The end small intestine were tied off and severed at the abomasal and ileo-caecal junctions. Digesta from each section of small intestine was removed intact, weighed and homogenized (Heidolph, Model DIAX 600, Germany) at 6000 x g for 5 min and divided equally into 3 portions. One portion was laid on a flat surfaced plastic tray, weighed and stored at -20°C before freeze drying for later analysis for PB. The second portion was weighed and used for determination of DM and marker analysis. The last portion was kept for DNA and RNA analysis. The digesta content from each segment of each animal was labeled for identification of each segment within individual animal.

Analytical methods

Determination of DM and Cr_2O_3 marker in digesta

The second portion of the homogenized digesta from each segment from each animal (approximately 50 to 60 g) was divided into two duplicate sub-portions and immediately dried to constant weight at 103°C for determination of DM, and subsequently for ash content (0.5 g DM of sample, combustion at 500°C for 16 h). The ash samples were solubilized (Siddons et al., 1985) and analysed for Cr_2O_3 content

by atomic absorption spectrometry (Varian® SpectAA-400, Australia) procedure, using a nitrous oxide acetylene flame.

RNA and DNA analysis

The RNA and DNA contents of homogenized digesta were determined by the procedures for RNA and DNA of the Molecular Research Center (MRC), Inc., using TRI Reagent® LS for liquid samples (cat. No. TS 120). The final preparations of total RNA and DNA were detected (Spectronic® Genesys™ 8, England) by U.V. absorption at λ 260 and 280 nm (MRC, 2000).

Purine base analysis

Adenine and guanine were determined by reversed phase HPLC, using two spherisorb C-18 ODS-5 (4.6 x 250 mm) columns, according to Balcells et al. (1992a) with the modification of Martin Orue et al. (1995).

Data analysis

The concentration of RNA, DNA and PB (guanine plus adenine) from the digesta of each 60 cm segment of small intestine of each animal were calculated from marker Cr_2O_3 concentration. The recovery (R) of RNA, DNA and PB in each 60 cm segment ($R_{\text{in each segment}}$; in % of the first segment) was calculated as;

$$R_{i \text{ th segment}} = \left[\frac{[\text{PB}_{i \text{ th segment}} / \text{Cr}_2\text{O}_3]_{i \text{ th segment}}}{[\text{PB}_{1 \text{ st segment}} / \text{Cr}_2\text{O}_3]_{1 \text{ st segment}}} \right] \times 100,$$

where $i = 2 \dots n$ segment

The disappearance (Dis, %) of PB from the intestinal tract between each 60 cm segment ($\text{Dis}_{\text{segment 1} - \text{segment 2}}$) was calculated as; $\text{Dis}_{\text{segment 1} - \text{segment 2}} = 100 - R_{2 \text{ nd segment}}$. Similarly, the disappearance (%) of RNA and DNA were calculated. The disappearance of RNA, DNA among 15 intestinal segments and PB (%) among 20 intestinal segments of each animal was compared between species. The correlation coefficient and the non-linear equation were evaluated from NLIN Procedure of SAS (1988). The difference in slope of

non-linear equations of RNA, DNA and PB from the two species were analyzed by the analysis of variance (ANOVA) procedures of the Statistic System Institute (SAS, 1988).

Results

Concentrations of RNA and DNA

The concentrations of RNA in the first segment of the intestine of buffaloes and KK zebu cattle were 1.36 and 1.75 mg/mg Cr_2O_3 , respectively (Table 2). The concentrations continuously decreased to 0.25 mg/mg Cr_2O_3 for swamp buffalo and to 0.08 mg/mg Cr_2O_3 for KK zebu cattle, in the 15th segments. The disappearance of RNA (as % of the first segment) was 22.1% in the second segment and cumulatively reached 81.6% at the 15th segment for swamp buffaloes, while the equivalent values for KK zebu cattle were 36.6 to 95.4%. The non-linear equation between the RNA disappeared (Y , % of the first segment) and the respective small intestine segment (calculated from mid point of each segment X , cm) for buffaloes was $Y = 93.004 (1 - e^{0.00208X})$; $r^2 = 0.97$, while for KK zebu cattle was $Y = 95.358 (1 - e^{0.00335X})$; $r^2 = 0.89$, (Figure 1). The slopes of the two equations did not differ between species ($P > 0.05$).

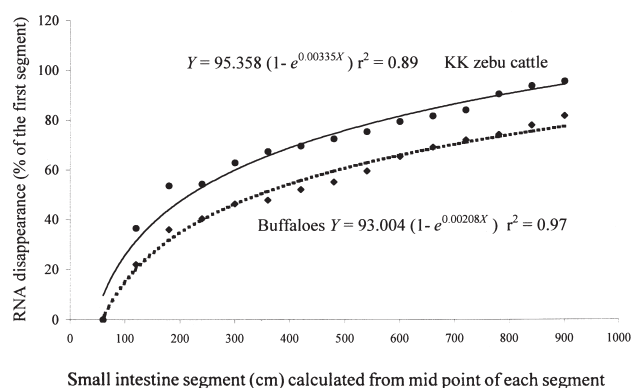


Figure 1 The RNA disappearance in small intestine of buffaloes and KK zebu cattle.

Similarly, concentrations of DNA in the digesta decreased continuously in the 15 segments, from 0.98 to 0.06 mg/mg Cr_2O_3 for buffaloes and 1.23 to 0.11 mg/mg Cr_2O_3 for KK zebu cattle (Table 2). Based on

these values, the disappearance of DNA for buffaloes did not differ from KK zebu cattle (Figure 2). The disappearance of DNA by the last segment are 93.9% and 91.1% for swamp buffaloes and KK zebu cattle, respectively. The non-linear equations for the relationships between DNA disappeared (Y , % of the first segment) and intestine segments (X , cm) were $Y = 103.93 (1 - e^{0.0028X})$; $r^2 = 0.93$ for buffaloes and $Y = 101.78 (1 - e^{0.0024X})$; $r^2 = 0.95$ for KK zebu cattle. The slopes of the two equations did not differ between species ($P > 0.05$).

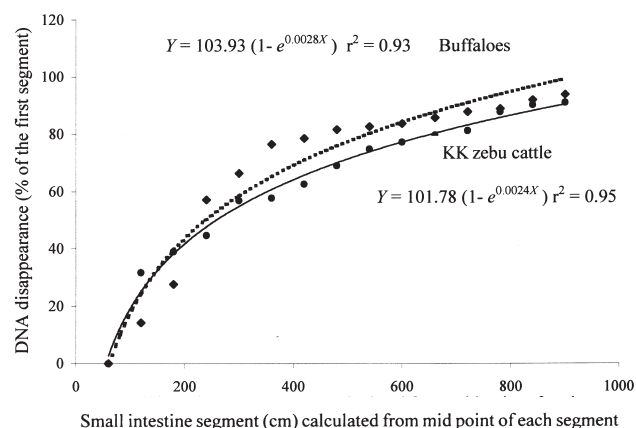


Figure 2 The DNA disappearance in small intestine of buffaloes and KK zebu cattle.

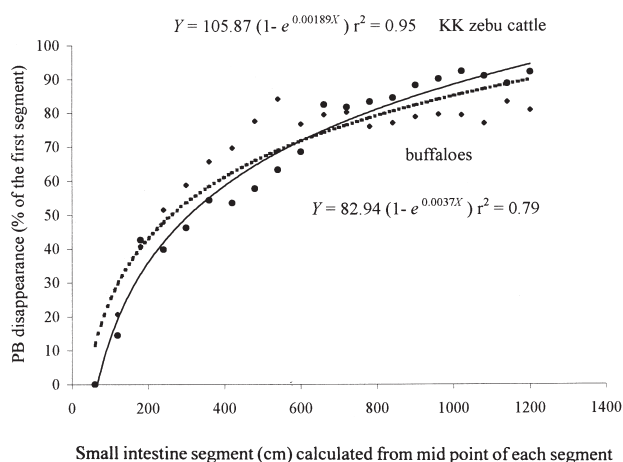
Concentration of purine bases in digesta

The concentration of guanine for swamp buffaloes decreased continuously from 240.3 in the first segment to 40.1 $\mu\text{mol/mg Cr}_2\text{O}_3$ in the 20th segment and that of adenine also continuously decreased from 145.0 to 33.6 $\mu\text{mol/mg Cr}_2\text{O}_3$ (Table 3). The total PB for swamp buffaloes was 385.3 $\mu\text{mol/mg Cr}_2\text{O}_3$ in the first segment and decreased to 73.7 $\mu\text{mol/mg Cr}_2\text{O}_3$ in the 20th segment. The disappearance of PB was 20.7% in the second segment and increased to 80.9% in the 20th segment (Table 4). The non-linear equation between PB absorbed (Y , %) and intestine segments (X , cm) was $Y = 82.938 (1 - e^{0.0037X})$; $r^2 = 0.79$ for swamp buffaloes (Figure 3).

Table 2 RNA and DNA concentrations (mg/mg Cr₂O₃) and cumulative disappearance from the first segment (%) in digesta of swamp buffalo and KK zebu cattle at different segments of the small intestines (values are means of 3 animals + SE)

Gut segment ¹	RNA				DNA			
	Buffalo		Cattle		Buffalo		Cattle	
	Concentration mg/mg Cr ₂ O ₃	Cumulative disappearance %	Concentration mg/mg Cr ₂ O ₃	Cumulative disappearance %	Concentration mg/mg Cr ₂ O ₃	Cumulative disappearance %	Concentration mg/mg Cr ₂ O ₃	Cumulative disappearance %
1	1.36±0.587		1.75±0.425		0.98±0.341		1.23±0.453	
2	1.06±0.461	22.1	1.11±0.066	36.6	0.84±0.255	14.3	0.84±0.431	31.7
3	0.87±0.315	36.0	0.81±0.293	53.7	0.71±0.175	27.6	0.75±0.466	39.0
4	0.81±0.277	40.4	0.80±0.292	54.3	0.42±0.127	57.1	0.68±0.414	44.7
5	0.73±0.297	46.3	0.65±0.191	62.9	0.33±0.105	66.3	0.53±0.279	56.9
6	0.71±0.300	47.8	0.57±0.198	67.4	0.23±0.041	76.5	0.52±0.266	57.7
7	0.65±0.322	52.2	0.53±0.202	69.7	0.21±0.045	78.6	0.46±0.298	62.6
8	0.61±0.287	55.1	0.48±0.187	72.6	0.18±0.053	81.6	0.38±0.225	69.1
9	0.55±0.230	59.6	0.43±0.156	75.4	0.17±0.050	82.7	0.31±0.208	74.8
10	0.47±0.215	65.4	0.36±0.108	79.4	0.16±0.046	83.7	0.28±0.215	77.2
11	0.42±0.159	69.1	0.32±0.135	81.7	0.14±0.034	85.7	0.25±0.192	79.7
12	0.38±0.129	72.1	0.28±0.153	84.0	0.12±0.030	87.8	0.23±0.192	81.3
13	0.35±0.109	74.3	0.17±0.158	90.3	0.11±0.035	88.8	0.15±0.164	87.8
14	0.30±0.091	77.9	0.11±0.157	93.7	0.08±0.029	91.8	0.12±0.166	90.2
15	0.25±0.056	81.6	0.08±0.117	95.4	0.06±0.021	93.9	0.11±0.156	91.1

¹Each segment was 60 cm, calculated from mid point of each segment.

**Figure 3** The purine bases disappearance in small intestine from buffaloes and KK zebu cattle.

The concentration of guanine for KK zebu cattle decreased continuously from 226.9 to 17.3 $\mu\text{mol/mg}$ Cr₂O₃ in the 20 segments, and adenine decreased from 173.9 to 14.5 $\mu\text{mol/mg}$ Cr₂O₃ (Table 3). These values give a total PB concentration of 400.7 $\mu\text{mol/mg}$ Cr₂O₃ in the first segment and 31.8 in the last segment. The disappearance of PB was 14.5% in the second segment and increased to 92.1% in 20th segment (Table 4). The non-linear equation between PB disappearance (Y , % of the first segment) and intestinal segment (X , cm) was $Y = 105.87 (1 - e^{0.00189X})$; $r^2 = 0.95$ for KK cattle (Figure 3).

The non-linear equations of total PB disappearance for buffaloes and KK zebu cattle did not differ ($P > 0.05$; Table 5).

Table 3 Concentrations ($\mu\text{mol}/\text{mg Cr}_2\text{O}_3$) of purine bases in digesta from different segments of swamp buffalo and KK zebu cattle small intestines (values are means of 3 animals \pm SE)

Gut segment ¹	Buffaloes			Cattle		
	Guanine	Adenine	Total PB	Guanine	Adenine	Total PB
1	240.3 \pm 34.31	145.0 \pm 24.93	385.3 \pm 58.83	226.9 \pm 29.55	173.9 \pm 22.58	400.7 \pm 45.19
2	196.4 \pm 39.75	109.2 \pm 29.26	305.6 \pm 69.02	186.2 \pm 16.05	156.5 \pm 28.92	342.7 \pm 44.72
3	163.5 \pm 42.15	64.7 \pm 19.13	228.2 \pm 60.89	137.5 \pm 9.77	92.1 \pm 12.83	229.6 \pm 11.54
4	115.5 \pm 22.71	71.2 \pm 15.29	186.7 \pm 30.53	151.9 \pm 13.27	89.5 \pm 17.86	241.4 \pm 21.92
5	95.6 \pm 8.86	63.2 \pm 13.24	158.9 \pm 15.99	138.3 \pm 10.11	77.1 \pm 23.75	215.4 \pm 33.85
6	88.7 \pm 18.61	43.3 \pm 10.71	132.0 \pm 28.84	138.0 \pm 31.11	44.8 \pm 16.64	182.7 \pm 37.76
7	58.8 \pm 7.59	58.0 \pm 22.93	116.7 \pm 28.98	121.2 \pm 19.67	65.2 \pm 19.15	186.3 \pm 37.91
8	56.9 \pm 15.05	29.6 \pm 8.17	86.5 \pm 21.43	125.7 \pm 23.36	43.4 \pm 11.97	169.1 \pm 27.89
9	34.5 \pm 4.37	26.4 \pm 6.77	60.9 \pm 11.00	99.5 \pm 28.78	47.5 \pm 4.27	147.0 \pm 31.75
10	59.4 \pm 8.84	29.9 \pm 8.41	89.3 \pm 17.18	87.7 \pm 22.13	38.5 \pm 12.33	126.2 \pm 25.20
11	56.5 \pm 13.35	22.4 \pm 2.07	78.8 \pm 11.29	49.0 \pm 11.05	21.1 \pm 0.80	70.2 \pm 10.27
12	44.2 \pm 9.81	31.6 \pm 7.48	75.7 \pm 11.78	44.0 \pm 14.63	29.3 \pm 6.78	73.3 \pm 21.40
13	48.6 \pm 7.34	43.7 \pm 12.92	92.3 \pm 11.76	38.4 \pm 7.49	28.6 \pm 6.70	67.0 \pm 13.69
14	49.6 \pm 16.49	38.5 \pm 14.52	88.1 \pm 23.39	32.3 \pm 7.34	29.8 \pm 7.12	62.2 \pm 14.42
15	38.1 \pm 10.90	43.2 \pm 12.28	81.3 \pm 15.96	22.4 \pm 6.71	24.8 \pm 9.69	47.3 \pm 16.19
16	42.5 \pm 8.88	36.0 \pm 5.84	78.5 \pm 11.62	24.6 \pm 9.97	15.0 \pm 10.45	39.6 \pm 20.42
17	42.3 \pm 11.28	37.6 \pm 4.26	79.4 \pm 17.95	16.0 \pm 4.80	15.0 \pm 12.23	31.1 \pm 17.03
18	51.1 \pm 4.26	37.6 \pm 4.26	88.7 \pm 0.91	16.7 \pm 9.51	19.8 \pm 12.65	36.5 \pm 22.16
19	31.5 \pm 6.84	33.0 \pm 1.70	64.5 \pm 7.52	18.5 \pm 8.08	26.6 \pm 11.97	45.1 \pm 19.34
20	40.1 \pm 10.03	33.6 \pm 3.28	73.7 \pm 12.83	17.3 \pm 6.21	14.5 \pm 7.09	31.8 \pm 12.78

¹Each segment was 60 cm, calculated from mid point of each segment.

Although the quantity of total digesta from the 20 segments of the small intestine of the two species did not significantly differ, the digesta in the last 4 segments of intestine of buffaloes was much higher than for cattle (Table 6), resulting in a higher quantity of unabsorbed PB in the last 5 segments in buffaloes than for cattle ($P < 0.05$).

Discussion

Results of this study are consistent with those of Smith and McAllan (1971) and Jackson et al. (1976), in that nucleic acids were extensively degraded in the small intestine. However, the disappearance of RNA and DNA within the whole tract did not differ between species, and values were close to the ranges previously reported for calves, sheep and steers

(Smith and McAllan, 1971; Coelho da Silva et al., 1972; Jackson et al., 1976; McAllan, 1980).

Disappearance of PB in the small intestine for buffaloes was within the range of 75 to 85% of that leaving the abomasum, as reported for bovines by Smith et al. (1969), while that for KK zebu cattle was higher. Although, Condon et al. (1970) and McAllan (1980) reported that adenine, guanine and uracil were completely absorbed from the small intestines of lambs and steers, PB was still detected in the last few segments of the small intestine in both species, especially buffaloes, in our study.

Although the disappearance of PB within the 20 segments of this study did not differ between the species, that the quantity of PB in the last 5 segments for buffaloes was higher than for cattle seems to

Table 4 Cumulative disappearance from the first segment (%) of purine bases in digesta from different intestinal segments of swamp buffalo and KK zebu cattle (values are mean of 3 animals \pm SE)

Segment ¹	Buffaloes			Cattle		
	Guanine	Adenine	Total PB	Guanine	Adenine	Total PB
2	18.3 \pm 12.53	24.7 \pm 11.92	20.7 \pm 12.25	17.9 \pm 7.46	10.0 \pm 4.93	14.5 \pm 4.43
3	32.0 \pm 12.75	55.4 \pm 11.54	40.8 \pm 11.54	39.4 \pm 14.19	47.0 \pm 10.24	42.7 \pm 7.88
4	51.9 \pm 6.86	50.9 \pm 20.09	51.5 \pm 10.44	33.0 \pm 8.33	48.5 \pm 12.66	39.8 \pm 2.02
5	60.2 \pm 5.88	56.4 \pm 19.78	58.8 \pm 10.80	39.0 \pm 4.44	55.7 \pm 11.30	46.2 \pm 2.89
6	63.1 \pm 8.66	70.1 \pm 10.51	65.7 \pm 9.20	39.2 \pm 8.32	74.2 \pm 11.45	54.4 \pm 5.44
7	75.5 \pm 7.87	60.0 \pm 20.63	69.7 \pm 12.15	46.6 \pm 2.18	62.5 \pm 12.46	53.5 \pm 6.21
8	76.3 \pm 11.58	79.6 \pm 8.45	77.6 \pm 10.31	44.6 \pm 5.52	75.1 \pm 8.59	57.8 \pm 3.11
9	85.6 \pm 3.09	81.8 \pm 6.99	84.2 \pm 4.51	56.1 \pm 8.80	72.7 \pm 3.81	63.3 \pm 4.25
10	75.3 \pm 8.17	79.4 \pm 10.05	76.8 \pm 8.86	61.3 \pm 6.02	77.9 \pm 8.84	68.5 \pm 4.08
11	76.5 \pm 2.90	84.6 \pm 4.94	79.5 \pm 1.08	78.4 \pm 4.95	87.9 \pm 1.79	82.5 \pm 2.31
12	81.6 \pm 1.78	78.2 \pm 5.63	80.3 \pm 1.24	80.6 \pm 4.58	83.1 \pm 5.01	81.7 \pm 4.79
13	79.8 \pm 1.50	69.9 \pm 9.52	76.0 \pm 2.93	83.1 \pm 2.14	83.5 \pm 5.25	83.3 \pm 3.44
14	79.3 \pm 3.22	73.5 \pm 9.06	77.1 \pm 4.06	85.7 \pm 1.62	82.8 \pm 4.50	84.5 \pm 2.57
15	84.1 \pm 2.68	70.2 \pm 7.66	78.9 \pm 1.84	90.1 \pm 1.91	85.7 \pm 5.87	88.2 \pm 3.44
16	82.3 \pm 1.41	75.2 \pm 4.67	79.6 \pm 1.03	89.1 \pm 2.54	91.4 \pm 4.27	90.1 \pm 3.35
17	82.4 \pm 3.10	74.1 \pm 2.16	79.4 \pm 2.71	92.9 \pm 0.81	91.3 \pm 5.28	92.3 \pm 2.90
18	78.7 \pm 3.77	74.1 \pm 6.81	77.0 \pm 4.54	92.6 \pm 3.09	88.6 \pm 4.98	90.9 \pm 3.97
19	86.9 \pm 4.75	77.2 \pm 6.04	83.3 \pm 5.05	91.8 \pm 2.88	84.7 \pm 6.22	88.7 \pm 4.21
20	83.3 \pm 4.74	76.8 \pm 6.73	80.9 \pm 5.19	92.4 \pm 2.11	91.6 \pm 2.93	92.1 \pm 2.47

¹Each segment was 60 cm, calculated from mid point of each segment.

Table 5 The disappearance rates of RNA, DNA and PB (Y, % of the first segment) from different intestinal segments (X, cm) of swamp buffalo and KK zebu cattle

	Swamp buffaloes	KK zebu Cattle
RNA	$Y = 93.004 (1 - e^{0.00208X})$, $r^2 = 0.97$, $P < 0.001$	$Y = 95.358 (1 - e^{0.00335X})$, $r^2 = 0.89$, $P < 0.001$
DNA	$Y = 103.93 (1 - e^{0.0028X})$, $r^2 = 0.93$, $P < 0.001$	$Y = 101.78 (1 - e^{0.0024X})$, $r^2 = 0.95$, $P < 0.001$
Total PB	$Y = 82.938 (1 - e^{0.0037X})$, $r^2 = 0.79$, $P < 0.001$	$Y = 105.87 (1 - e^{0.00189X})$, $r^2 = 0.95$, $P < 0.001$

indicate that the quantity of PB absorbed in small intestine of buffaloes was lower than that of cattle. However, the procedure adopted in the present study could not quantify the actual amount of total PB absorbed in different segments of small intestine and this possibility requires further investigation.

Chen et al. (1996) reported that mucosal cells of buffaloes and cattle were rich in xanthine oxidase (EC 1.2.3.2), which increased the potential for oxidation of absorbed purines before they entered the blood system. Similarly, Balcells et al. (1992b) reported that PD, but not the nucleosides guanosine and adenosine,

Table 6 The quantity of fresh digesta (g) and PB (μmol) in each segments of small intestine of swamp buffalo and KK zebu cattle (values are means of 3 animals \pm SE)

Gut segment ¹	Buffaloes		Cattle	
	Digesta	Total PB	Digesta	Total PB
1	47.5 \pm 14.78	344 \pm 191.5	84.9 \pm 52.54	447 \pm 285.1
2	51.2 \pm 8.03	297 \pm 110.1	32.7 \pm 9.21	264 \pm 41.9
3	38.3 \pm 12.99	141 \pm 36.6	56.9 \pm 30.11	280 \pm 148.3
4	75.4 \pm 54.65	227 \pm 170.2	42.7 \pm 18.56	124 \pm 33.1
5	127.9 \pm 55.79	637 \pm 399.3	46.3 \pm 28.87	166 \pm 73.2
6	110.6 \pm 56.35	315 \pm 148.4	56.9 \pm 26.65	116 \pm 8.4
7	72.5 \pm 34.71	231 \pm 157.2	70.4 \pm 24.86	157 \pm 88.9
8	83.2 \pm 37.85	323 \pm 244.3	111.7 \pm 29.73	366 \pm 167.9
9	140.3 \pm 62.21	278 \pm 158.8	72.7 \pm 29.65	296 \pm 231.5
10	136.9 \pm 48.76	201 \pm 84.2	103.6 \pm 40.45	466 \pm 280.7
11	106.4 \pm 24.36	346 \pm 216.5	133.1 \pm 32.74	276 \pm 62.9
12	78.3 \pm 9.83	201 \pm 79.4	159.2 \pm 35.36	556 \pm 402.7
13	62.2 \pm 21.98	91 \pm 0.8	100.7 \pm 38.32	192 \pm 129.8
14	38.7 \pm 14.59	71 \pm 29.3	129.3 \pm 30.07	222 \pm 149.6
15	75.4 \pm 20.37	147 \pm 48.9	170.2 \pm 58.62	277 \pm 130.2
16	88.7 \pm 16.91	413 \pm 247.9	97.2 \pm 49.34	195 \pm 102.2
17	96.7 \pm 14.68	445 \pm 131.3	61.4 \pm 0.75	63 \pm 38.0
18	86.7 \pm 15.89	338 \pm 162.5	89.8 \pm 70.75	76 \pm 68.5
19	105.5 \pm 16.10	451 \pm 323.4	75.3 \pm 35.46	53 \pm 21.2
20	148.1 \pm 34.81	573 \pm 279.5	86.4 \pm 7.42	79 \pm 48.8
Total	1772.6 \pm 343.13	6069 \pm 2614.7	1728.8 \pm 305.30	4559 \pm 1126.2

¹Each segment was 60 cm, calculated from mid point of each segment.

and the purines guanine and adenine were detected in the plasma samples of sheep and steers. Balcells et al. (1992b) suggested that the absorbed nucleosides and bases were metabolized during absorption through the intestinal mucosa and, therefore, the purine absorption rate could be measured from the plasma concentration. In addition, Chen et al. (1990a) reported that the concentrations of PD did not differ between portal and jugular blood. Therefore the concentration of plasma PD, as sampled from the jugular vein, could be used to determine the absorption rate of purines. Several studies (Liang et al., 1994; Pimpa, 2002) have shown that plasma

PD for buffaloes was much lower than that for cattle, implying that the absorption of PB in buffaloes is lower than that in cattle. Further studies are required to validate this possibility.

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References

- Al-Khalidi, U.A.S. and Chaglassian, T.H. (1965) The species distribution of xanthine oxidase. *Biochemical Journal* 97: 318-320.
- Balcells, J., Guada, J. A., Castrillo, C. and Gasa, J. (1991) Urinary excretion of allantoin and allantoin precursors by sheep after different rate of purine infusion into the duodenum. *Journal of Agricultural Science (Cambridge)* 116: 309-317.
- Balcells, J., Guada, J. A., Peiro, J. M. and Parker, D. S. (1992a) Simultaneous determination of allantoin and oxypurines in biological fluids by high-performance liquid chromatography. *Journal of Chromatography* 575: 153-157.
- Balcells, J., Parker, D. S. and Seal, C. J. (1992b) Purine metabolite concentrations in portal and peripheral blood of steers, sheep and rats. *Comparative Biochemistry and Physiology* 101B, 4: 633-636.
- Chen, X.B., Hovell, F.D.DeB. and Ørskov, E.R. (1990a) Excretion of purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut. *British Journal of Nutrition* 63: 197-205.
- Chen, X.B., Hovell, F.D.DeB., Ørskov, E.R. and Brown, D.S. (1990b) Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition* 63: 131-142.
- Chen, X.B., Samaraweera, L., Kyle, D.J. and Ørskov, E.R. (1996) Urinary excretion of purine derivatives and tissue xanthine oxidase (EC 1.2.3.2) activity in buffaloes (*Bubalis bubalis*) with special reference to difference between buffaloes and *Bos taurus* cattle. *British Journal of Nutrition* 75: 397-407.
- Coelho da Silva, J. F., Seeley, R. C., Beever, D. E., Prescott, J.H.D. and Armstrong, D.J. (1972) The effect in sheep of physical form and stage of growth on the site of digestion of a dried grass.
2. Sites of nitrogen digestion. *British Journal of Nutrition* 28: 357.
- Condon, R.J., Hall, G. and Hatfield, E.E. (1970) Metabolism of abomasally infused ¹⁴C-labelled RNA adenine, uracil and glycine. *Journal of Animal Science* 31: 1037-1038 (abstr).
- Christian, R.R. and Coup, M.R. (1954) Measurement of feed intake by grazing cattle and sheep. VI. The determination of chromic oxide in faeces. *New Zealand Journal of Science and Technology*. Sec. A. 36: 328-330.
- Jackson, T.C., Schelling, G.T., Mitchell, G.E. and Tucker, R.E. (1976) Nucleic acid bases in sheep ingesta. *Journal of Animal Science* 43: 325-326.
- Liang, J.B., Matsumoto, M. and Young, B.A. (1994) Purine derivative excretion and ruminal microbial yield in Malaysian cattle and swamp buffalo. *Animal Feed Science and Technology* 47: 189-199.
- Martin Orue, S. M., Balcells, J., Guada, J. A. and Castrillo, C. (1995) Endogenous purine and pyrimidine derivative excretion in pregnant sows. *British Journal of Nutrition* 73: 375- 385.
- McAllan, A.B. (1980) The degradation of nucleic acid in, and the removal of breakdown products from the small intestines of steers. *British Journal of Nutrition* 44: 99-112.
- Molecular research center (MRC), Inc. (2000) Manufacturer's protocol 1995, TRI Reagent® LS "DNA, RNA and protein from liquid samples, single step method. MRC. Inc. Cincinnati, Ohio, USA.
- Perez, J.F., Balcells, J., Guada, J.A. and Castrillo, C. (1997) Contribution of dietary nitrogen and purine bases to duodenal digesta: comparison of duodenal and polyester-bag measurements. *British Society of Animal Science* 65: 237-245.
- Pimpa, O. (2002) Urinary purine derivatives excretion as a method for estimation of rumen microbial protein production in swamp buffaloes

- and Zebu cattle. PhD Thesis, Universiti Putra Malaysia.
- Statistical Analysis Systems Institute. (1988) SAS® User's Guide statistic, Version 6.03, Cary, NC.
- Siddons, R.C., Paradine, J., Beever, D.E. and Cornell, P.R. (1985) Ytterbium acetate as a particulate-phase digesta-flow marker. *British Journal of Nutrition* 54: 509-519.
- Smith, R.H. and McAllan, A.B. (1970) Nucleic acid metabolism in the ruminant. 2. Formation of microbial nucleic acids in the rumen in relation to the digestion of food nitrogen and the fate of dietary nucleic acids. *British Journal of Nutrition* 24: 545-556.
- Smith, R.H. and McAllan, A.B. (1971) Nucleic acid metabolism in the ruminant 3. Amounts of nucleic acids and total ammonia nitrogen in digesta from the rumen, duodenum and ileum of calves. *British Journal of Nutrition* 25: 181-190.
- Smith, R.H. McAllan, A.B. and Hill, W.B. (1969) Nucleic acids in bovine nutrition. 3. Fate of nucleic acid presented to the small intestine. *Proceeding of the Nutrition Society* 28, 28A.
- Vagnoni, D.B., Broderick, G.A., Clayton, M.K. and Hatfield, R.D. (1997) Excretion of purine derivatives by Holstein cows abomasally infused with incremental amounts of purines. *Journal of Dairy Science* 80: 1695-1702.
- Verbic, J., Chen, X.B., MacLeod, N.A. and Ørskov, E.R. (1990) Excretion of purine derivatives by ruminants: effect of microbial nucleic acid infusion on purine derivative excretion by steers. *Journal of Agricultural Science (Cambridge)* 114: 243-248.
- Vercoe, J.E. (1976) Urinary allantoin excretion and digestible dry-matter intake in cattle and buffalo. *Journal of Agricultural Science (Cambridge)* 86: 613-615.