



**JOINT FAO/IAEA DIVISION**  
OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE



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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

**REPORT ON**  
**FINAL RESEARCH CO-ORDINATION MEETING**  
**CRP D3.10.21**

**DEVELOPMENT, STANDARDIZATION AND VALIDATION**  
**OF NUCLEAR BASED TECHNOLOGIES FOR**  
**ESTIMATING MICROBIAL PROTEIN SUPPLY IN**  
**RUMINANT LIVESTOCK FOR IMPROVING**  
**PRODUCTIVITY**

## 1. BACKGROUND

Recent advances in ration balancing include manipulation of feed to increase the quantity and quality of protein and energy delivered to the small intestine. Selection of feeds based on high efficiency of microbial protein synthesis in the rumen along with high dry matter digestibility, and development of feeding strategies based on high efficiency as well as high microbial protein synthesis in the rumen will lead to higher supply of protein post-ruminally. The strategy for improving production has therefore been to maximise the efficiency of utilisation of available feed resources in the rumen by providing optimum conditions for microbial growth and then by supplementing provide dietary nutrients to complement and balance the products of rumen digestion to the animal requirement.

Various methods are available for determining microbial protein production, which depend on the external markers such as  $^{15}\text{N}$ ,  $^{32}\text{P}$  and  $^{35}\text{S}$ , and internal markers such as diamino pimelic acid (DAPA), L-alanine and ribonucleic acid (RNA). However, the need to use post-ruminally cannulated animals, and complicated and error-associated procedures to determine digesta flow are major limitations in these methods. The idea of using microbial purine compounds as a specific marker for the rumen microbial biomass was suggested by McDonald in 1954. Purines are heterocyclic rings with nitrogenous bases with varying functional groups. The purine bases, adenine and guanine are found in both DNA and RNA. Microorganisms have high concentrations of purine-containing compounds (RNA and DNA) relative to concentrations in plant and mammalian cells. Furthermore, rumen microbes in general, rapidly degrade purines in diets. They are, therefore, likely to be present in only negligible amounts in digesta leaving the rumen therefore. The purines present in digesta entering the small intestine is, therefore, almost totally of microbial origin. These purine components are then metabolised in ruminants to form purine derivatives (PD) such as xanthine, hypoxanthine, uric acid and allantoin that are excreted, mainly in the urine. All four components are found in urine of sheep, goats, red deer and llamas, but only allantoin and uric acid are present in cattle and buffalo urine.

With estimates of daily urine volume and urinary concentrations of PD, the total daily urinary excretion of PD can be determined and used to predict the rates of absorption of purines from the small intestine using relationships developed for individual ruminant species. Since only urine is needed, an estimation of microbial protein supply can be incorporated into nitrogen balance and digestibility trials without much additional labour inputs. The advantages of this technique are its non-invasive nature and relative ease of use.

As a result of a Consultants meeting held in May 1995 to advise the Joint FAO/IAEA Division on the feasibility of using nuclear and related techniques for the development and validation of techniques for measuring microbial protein supply in ruminant animals, an FAO/IAEA Co-ordinated Research Project (CRP) on 'Development, Standardization and Validation of Nuclear Based Technologies for Measuring Microbial Protein Supply in Ruminant Livestock for Improving Productivity' was initiated in 1996, with a view to validating and adapting this technology for use in developing countries.

## 2. OBJECTIVES OF THE CRP

- To refine and standardise the PD excretion technique for measuring microbial protein supply in ruminant livestock,
- To validate the PD excretion technique for indigenous cattle (*Bos indicus*) and their crosses with exotic breeds, buffaloes, sheep, goats and camel in developing countries,

- To use the PD excretion technique as a robust and inexpensive method for estimating rumen microbial protein supply for developing feeding strategies and as a diagnostic tool for assessing nutritional status of animals in the field and for grazing animals where the quantitative collection of urine output is much more difficult.

The programme was carried out in two phases. During Phase 1 which lasted for 3 years, the technologies developed based on research by the Rowett Research Institute, UK, and other European laboratories, were refined, standardised and validated for indigenous Zebu (*Bos indicus*) cattle and their crosses with exotic breeds (*Bos taurus x Bos indicus*) and buffaloes. During Phase 2, the validated technique were extended for modelling PD in camel, and a spot urine sample approach was validated for different breeds of cattle, buffalo, sheep and camel for use at field level to estimate the supply of rumen microbial protein to the intestine. This spot urine sample approach dispenses with the quantitative recovery of urine.

The first Phase 1 was concluded with a Research Coordination Meeting (RCM) held in Vienna from 24-28 August 1998. The results from the first phase have already been published as an IAEA TECDOC 1093. A Laboratory Manual on the PD methodologies (IAEA TECDOC 945) has also been produced under this project. This manual has been found to be highly valuable to all participants of the CRP and other researchers using the PD excretion technique for estimating rumen microbial protein supply.

The second and final phase of this CRP concluded in May 2002 with the final RCM, which was held in Vietnam. In March 2000, a mid-term review and planning meeting was held in Malaysia.

A total of 15 groups participated in the project. These groups have been listed at the end of this compilation.

### 3. ACHIEVEMENTS OF THE CRP

Modelling purine derivative (PD) excretion and its applications for estimation of rumen microbial protein production in different animal species.

*Camel (Camelus dromedaries) (Morocco)*

- Fasting urinary excretion of PD averaged  $0.230 \text{ mmol/kgW}^{0.75}/\text{d}$ .
- Xanthine oxidase activity was detected in the liver ( $0.038 \text{ } \mu\text{mol /min/g}$ ) and the intestine mucosa ( $0.0047 \text{ } \mu\text{mol /min/g}$ ) but was absent in the plasma.
- Urinary recovery of duodenal purine bases (PB) was about 54 %, although there was some inconsistency in xanthine + hypoxanthine values, which seem to suggest that there is a need for further investigation.
- Microbial samples extracted from the liquid phase of the rumen in camel showed a purine base content and microbial N: total N of  $1.26 \text{ mmol purine base/g N}$  and  $0.088 \text{ MN: Total N}$ , respectively.
- Creatinine excretion in camel was  $0.350 \text{ mmol/kgW}^{0.75}/\text{d}$ . Moreover, the PDC index increased significantly from 83 to 141 with increase in digestible organic matter intake (DOMI) from 1.3 to 3.5 kg/d, confirming that the PDC index could be used as a tool to estimate microbial out flow.

### *Zebu (Bos indicus) and Kedah-Kelantan (Bos indicus) cattle (Thailand)*

- Non-salvageable PD were detected in urine, and allantoin and uric acid constituted the entire PD in urine.
- Fasting urinary excretion of PD was  $0.275 \text{ mmol/kgW}^{0.75}/\text{d}$ .
- Urinary excretion of PD (Y, mmol/d) responded significantly to the duodenal infusion of PB (X, mmol/d). The proposed equation is  $Y = 0.85X + 7.15$  ( $R^2=0.62$ ).
- Creatinine excretion in Kedah-Kelantan (KK) cattle was independent of the level of duodenal infusion of PB. The average daily creatinine excretion in KK-cattle was  $0.89 \text{ mmol/kgW}^{0.75}$  and the PDC index responded significantly and positively to the infusion.
- Profile of urinary PD, endogenous excretion and urinary recovery of duodenally infused PB confirmed the similarity in the response for Zebu cattle and European cattle (*Bos taurus*).

### *Swamp buffalo (Bos bubalus) (Thailand)*

- Urinary PD profile in buffaloes consisted of allantoin and uric acid. Xanthine and hypoxanthine were present in trace levels. Endogenous excretion of PD in buffalo was  $0.2 \text{ mmol/kgW}^{0.75}/\text{d}$ .
- Urinary recovery of duodenal purine in buffalo was consistently lower than those values obtained in other ruminants. The relationship between duodenal input of PB (X, mmol/d) and urinary output (Y, mmol/d) was:  $Y = 0.12X + 12.78$  ( $R^2=0.45$ ).
- Urinary recovery of labelled  $^{14}\text{C}$ -Uric acid administered via the jugular vein was 0.6 and was similar to that in cattle.
- Glomerular filtration rate (GFR) in buffalo ( $12.69 \text{ L/kgW}^{0.75}/\text{d}$ ) was much higher than the value reported in cattle ( $5.55 \text{ L/kgW}^{0.75}/\text{d}$ ).
- Creatinine excretion in buffalo was  $1.23 \text{ mmol/kgW}^{0.75}/\text{d}$  and was slightly higher than in KK cattle ( $0.89 \text{ mmol/kgW}^{0.75}/\text{d}$ ). Moreover, the PDC index increased significantly (from 11.34 to 16.9) in response to duodenal purine base infusion and the response was lower than in cattle.
- The low urinary recovery of duodenal purine bases limits the use of urinary excretion of PD as an index of rumen microbial outflow.

### *Chilean Friesian (Bos taurus) dairy cows (Chile)*

- Creatinine excretion was similar ( $1.175 \pm 0.074$  and  $1.200 \pm 0.104 \text{ mmol/kgW}^{0.75}/\text{d}$ ), when measured using HPLC and colorimetric methods, respectively.
- The inclusion of different levels of concentrate (0, 16, 33 and 50 % in diet) increased the PDC index (Y) ( $P<0.01$ ) showing a significant relationship with DOMI (X, kg/d) ( $Y = 16.85X + 6.23$ ;  $R^2=0.84$ ). When PD excretion was calculated assuming constant excretion of creatinine ( $1.19 \text{ mmol/kg W}^{0.75}/\text{d}$ ), urinary excretion of PD increased with the increasing level of concentrate. The PD : DOMI ratio or MN : DOMI, where MN is microbial nitrogen,

as an index of microbial synthesis efficiency, also increased (from 10.3 to 15.6 g MN/kg DOMI).

- Changes in roughage supply (23 % of Rye grass silage replaced by NaOH-treated straw) did not modify DOMI and the PDC index ( $P=0.13$ ), although among experimental groups there was a significant decrease ( $P<0.05$ ) in milk production (- 1.5 L/d) and decrease in body weight gain (- 0.380 kg/d).

*Local Vietnamese cattle (Bos indicus) and swamp buffalo (Bos bubalus) (Vietnam)*

- Dry matter digestibility in cattle and buffalo was similar (cattle 59 – 63 %; buffalo 53 – 59 %), with no consistent differences due to feeding levels. Protein retention (Y, g/d) increased with increasing level of protein intake (X, g/d) generated with different levels of feeding in both cattle and buffaloes ( $Y=0.63X - 21.2$  buffalo,  $Y=0.66X - 11.0$  cattle;  $Y=0.35X - 9.7$  buffalo,  $Y=0.34X - 8.1$ , cattle), suggesting similar increases in protein supply in buffalo and cattle.
- There were consistent differences in urinary excretion of PD with buffalo at similar intakes, excreting less than half of PD in comparison with cattle. In buffaloes fed mainly forage maize, PD excretion increased from 13.1 - 42.6 mmol/d with the increase in intake from 0.8 - 2.1 kg DOMI. The respective increase for cattle were 26.9 - 82.4 mmol/d for increase in intake of 0.7 - 1.6 kg DOMI. In the second experiment fed mainly rice straw, urinary PD excretion increased from 13.0 - 37.3 mmol/d as a result of increase in DOMI from 0.7 - 1.6 kg in buffaloes and 23.6 - 67.8 mmol/d as a result of increase in DOMI from 0.5 - 1.2 kg DOMI.
- Creatinine excretion ranged between 1.2 - 1.3 mmol/kgW<sup>0.75</sup>/d in buffalo and 1.8-1.9 mmol/kgW<sup>0.75</sup>/d for cattle.
- The PDC index in spot urine samples ranged from 31.6 ( $\pm 1.9$ ) to 23.1 ( $\pm 1.1$ ) for buffalo and 40.6 ( $\pm 0.8$ ) to 35.2 ( $\pm 0.7$ ) for cattle.
- No consistent difference in the PDC index was observed for spot urine samples collected 4 times a day, but buffaloes urinated less frequently than cattle, some times only once daily.

*Bali cattle (Bos sondaicus), Ongole cattle (Bos indicus), Friesian cattle (Bos taurus) and buffaloes (Bos bubalus) (Indonesia)*

- A comparison between four types of large ruminants at one feeding level related to metabolic body weight, Bali cattle, Ongole cattle, Friesian cattle and buffaloes showed clearly that buffaloes were consistently different in urinary PD excretion compared to cattle, with excretion per day from buffaloes being about 25 % of that from cattle. The respective values (mmol/kgW<sup>0.75</sup>/d) were: Bali cattle (0.460), Ongole cattle (0.541), Friesian cattle (0.646), and buffaloes (0.179).

At 4 feeding levels for Ongole cattle  $Y = 11.30X + 8.89$  and for buffaloes  $Y = 2.08X + 3.20$  where Y=PD excretion (mmol/d) and X=DOMI (kg/d).

- With duodenally cannulated buffaloes and cattle it was shown that differences in urinary PD excretion was not due to differences in flow of nucleic acid (11.4 and 13.2 mg RNA/g DOMI for buffalo and cattle respectively).
- Creatinine excretion ( $\text{mmol/kgW}^{0.75}/\text{d}$ ) was: Bali 0.862, Ongole 0.720, Freisian 0.813 and buffalo 0.628.
- Spot urine samples taken every 3 hours during five 24-hour periods with 4 animals showed no consistent differences in accuracy in predicting total urinary PD excretion except that the early morning sample gave poor accuracy.

*Indigenous Sri Lankan cross-bred cattle (Sri Lanka)*

- Urinary excretion of PD (Y, mmol/d) was closely related to DOMI (X, kg/d) as:  $[Y = 22.9X + 10.7 (R^2 = 0.7)]$
- Creatinine excretion averaged  $0.96 \text{ mmol/kgW}^{0.75}/\text{d}$ .
- In spot samples taken 4 times per day during 4 days, no significant difference in the PDC index was observed, indicating little variation with time.

*Yerli Kara crossbred cattle (Turkey)*

- Urinary excretion of PD (Y, mmol/d) was closely related to DOMI (X; kg/d) as:  $(Y = 19.7X + 12.5 [R^2 = 0.9])$
- Creatinine excretion averaged  $1.09 \text{ mmol/kgW}^{0.75}/\text{d}$
- In spot urine samples taken from the field study (3 times during 2 days), no significant difference in the PDC index was observed, indicating no significant variation with time.
- The DOMI (Y, g/d) was positively related to PDC index (X) as:  $Y = 48.7X + 344 (R^2 = 0.9)$
- The relationship between the PDC index in spot urine sample and PD excretion (mmol/d) was:  $\text{PD (mmol/d)} = -2.3 + 0.953 \text{ PDC}$ , and could be used to develop a banding system to predict protein nutrition status of animals in the field.

*Chinese Yellow Cattle (China)*

- In two feeding trials in which 8 Chinese Yellow Cattle fed with 4 levels of feed intake, total excretion of PD (Y, mmol/d) was found to be highly correlated with DOMI (X, kg/d):

$$Y = 21.35X + 18.86 (R^2 = 0.97, n = 8, p < 0.01).$$

The intercept 18.86 mmol/d was equivalent to  $0.19 \text{ mmol/kgW}^{0.75}/\text{d}$ . PD in the urine consisted of 84% allantoin and 16% uric acid.

Daily excretion of creatinine in Chinese Yellow Cattle was  $1.15 \pm 0.08$  mmol/kgW<sup>0.75</sup> (mean of 8 animals). Creatinine excretion was rather consistent and not affected by feed intake.

- In another experiment conducted in 5 farms with a total 18 Chinese Yellow Cattle, with the objective of examining whether the PDC index in spot urine samples responded to an improvement in feeding regime [from the two typical low-energy rations used by farmers to their supplementation with concentrate (3 diets)] resulted in a significant increase in the PDC index, implying a significant increase in microbial protein synthesis in the rumen. The work also showed that by taking the average of 4 spot samples, diurnal variability was small (CV=4%) in the PDC index.
- The response curve of PD excretion to DOMI was similar to those observed with *Bos taurus*, it appears that the equation relating PD excretion to microbial protein production for *B. taurus* may be used for Chinese Yellow Cattle. The results also showed that where total urine collection is not feasible, spot urine sample can be used. A banding system relating PD to creatinine ratio in spot urine sample and the microbial protein supply status was proposed for use under field condition.

#### *Sabi sheep and small East African goats (Zimbabwe)*

- Preliminary work was conducted to study the PD excretion in four indigenous Sabi sheep and four small East African goats given 4 different levels of star grass (*Cynodon nlemfuensis*) hay. PD excretion (Y, mmol/d), which consisted of allantoin, uric acid, xanthine plus hypoxanthine, increased with DOMI (X, kg/d). With goats, the response curve was  $Y = 5.86X - 0.33$  ( $R^2 = 0.99$ ), while for sheep, the response was low and variable:  $Y = 2.97X + 0.15$  ( $R^2 = 0.72$ ). It was clear that qualitatively, PD excretion increased with feed intake, but at a much lower rate compared to European sheep. However, the present work needs to be repeated and extended in order to reveal the quantitative aspects of the response.

#### *Buffalo (Italy)*

- PD excretion (mmol/kgW<sup>0.75</sup>/d) from buffaloes given 4 kg or 8 kg fescue hay was 0.23 and 0.31. The respective values for creatinine were 0.61 and 0.70 mmol/kgW<sup>0.75</sup>/d, and of the PDC index 49.4, 55.9. The value of PD excretion, mmol/kg of DOMI was 9.2. These values were similar to those observed in Malaysian for buffaloes.
- HPLC methods for determination of PD and creatinine in urine have been standardised, modified, and improved. The values obtained have been compared with the spectrophotometric methods reported in IAEA TECDOC-945. The values for the PD using the spectrophotometric methods were lower than those obtained by the HPLC method; therefore, it is necessary to specify the analytical method used and to utilize the regression equation to compare data. The values for creatine were comparable by the two methods.
- FT-NIR predicted the main chemical components, i.e. allantoin, uric acid, creatinine, total nitrogen, and sum of nitrogen allantoin and uric acid with a satisfactory accuracy: the determination coefficient ( $R^2$ ) of validation ranged from 0.888 for uric acid to 0.982 for total nitrogen, while the coefficients of determination of allantoin, creatinine, and sum of nitrogen allantoin and uric acid were 0.925, 0.894 and 0.905, respectively. Hypoxanthine and

xanthine in urine samples were not detectable by NIRS, probably because of their low concentrations, and hence they were not considered for instrumental calibration.

#### *Merino Sheep Cross (Australia)*

- After intravenous injection of  $^{14}\text{C}$ -allantoin in Merino cross sheep, recovery of radioactivity in urine was >94% and in gut <6%. Low amount of  $^{14}\text{C}$ -bicarbonate in the blood confirmed that only a small fraction of the allantoin (<2%) leaving the bloodstream was degraded in the sheep, probably by gut microorganisms.
- Total recovery of intravenous injected allantoin in urine took 3 days. Allantoin has a long half time in blood and therefore is probably sequestered into other fluid compartments. The recovery of  $^{14}\text{C}$  label in urine was not affected by level of feed intake. There is no need to alter the existing model equations for sheep.

#### *Bali and Ongole Cattle (Indonesia)*

- Using  $^{14}\text{C}$ -allantoin, the recovery of allantoin in the urine varied from 80 to 85% in Bali cattle and from 72 to 75% in Ongole cattle. While there was a positive relationship between glomerular filtration rate and allantoin recovery, this could not explain all of the variation.

#### *Suffolk cross sheep (UK)*

- The allantoin recovery in the urine of Suffolk cross sheep, as  $^{14}\text{C}$ -allantoin, was on average 83% but there was an individual variation from 66 to 95% with the remainder excreted via the gut.

#### *Holstein-Friesian (Spain)*

- Labelling microbial or exogenous duodenal purine bases may constitute a useful methodology to estimate the ratio of endogenous PD: exogenous PD in urine and further the endogenous contribution to the urinary excretion.
- Endogenous contribution ( $\text{mmol}/\text{kgW}^{0.75}/\text{d}$ ) was  $0.31 \pm 0.031$  in dry cows,  $0.236 \pm 0.006.0$  in growing steers and  $0.512 \pm 0.036$  in lactating Holstein-Friesian.

#### *Validation of the PDC index as an index of microbial production in small-holders buffalo farm in northeast of Thailand (Thailand).*

- Further validation of the PDC index under local conditions confirmed that PD excretion (Y;  $\text{mmol}/\text{d}$ ) in buffaloes seems to be poorly correlated with infusion of PB (X,  $\text{mmol}/\text{d}$ ) ( $Y = 0.098X + 15.17$ ;  $R^2=0.31$ ) while a higher correlation was recorded in KK cattle:  $Y = 0.83X + 26.93$  ( $R^2=0.87$ )
- The relationship between the daily excretion of PD (X,  $\text{mmol}/\text{d}$ ) and the PDC index (Y) in spot urine samples was not significant for buffaloes, where  $Y = -0.144X + 14.88$  ( $R^2=0.03$ ) but was significant for cattle  $Y = 0.36X + 19.1$  ( $R^2=0.59$ ). The results indicate the potential use of the PDC index for the estimation of microbial supply for zebu cattle but not for buffalo.

- In the farm investigations conducted with 2 samplings per day, the PDC index was not significantly different considering the time (morning and afternoon) and season (dry vs. rainy).

#### 4. CONCLUSIONS

- The PD excretion technique has been proved to be a powerful technique for predicting the rumen microbial protein supply in ruminant livestock. It is being increasingly used in a variety of research programmes. This method has the advantage of being non-invasive, simple to use, and inexpensive.
- The DOMI (Y, kg/d) is significantly positively related to daily PD excretion, and more specifically to the PDC index (X) in different species of cattle. *B indicus* (e.g. Yerli Kara, KK, Ongole), *B. taurus* (Friesian), *B. sondaicus* (Bali), seem to be very similar and the data can be combined to give a single prediction equation as follows:  $Y = 0.0597X + 0.6776$  (n=44,  $R^2=0.97$ ).
- Buffaloes have much lower (and more variable) urinary excretion of PD per unit DOMI than cattle. The urinary PD excretion in buffalo is not related to lower supply of microbial cells from the rumen, nor to absorption of purines from the small intestine, but to differences in tissue metabolism for which mechanisms are not fully understood. For these reasons, at present, the use of the PD prediction method in buffaloes is not recommended.
- For the first time, data showing increases in urinary PD excretion being closely related to DOMI is reported for camel. and can be used for prediction of rumen microbial outflow in this species. The slope for this species (10-12 mmol/kg DOMI) is lower than that in cattle. Xanthine oxidase activity was not detected in blood plasma from camels and activity of this enzyme in all tissues examined in camel was lower than in other ruminants. Creatinine excretion in camel was about one-third of that in other large ruminants examined. The recovery of PB infused into the duodenum was about 64 % of that of other ruminants.
- Tracer studies indicated that the rate of excretion of allantoin in sheep is affected by its movement through multiple fluid compartments in the body and its recovery in the urine (mean 84%) may be more variable (range 65 - 95%) than previously thought.
- Protocols for determination of purine derivatives using a spectrophotometer and HPLC, and for quality control of these assays, including a software assisting the quantification of purine derivatives and creatinine have been developed.
- The protocols and the TECDOC-945 developed for use in this CRP have proved to be a successful way of helping participants to collect, process and analyse biological samples. Standardized methods and procedures outlined in the laboratory manual (TECDOC-945) have enabled the participants to carry out their experiments confidently and produce results that are scientifically valid. Some of the HPLC and spectrophotometric methods have now been updated. NIRS has also been examined and has the potential in laboratories that have the instrument.
- In addition, the document outlining protocols and guidelines ensured that analytical determinations are uniformly standardized in the laboratories of all participants and that the data generated are reliable. This has also introduced a quality assurance ethos into the programme.
- Microbial protein production using spot urine sample – validation studies:

- The PDC index developed in this programme in general appears to respond positively, as expected, to changes in DOMI and the creatinine excretion has proved to be a robust means of predicting quantitative recovery of PD. In general, the time of collection of samples does not appear to affect the PDC index.
- The index has potential as a research tool to optimise feed resources.
- Determination of the index from urine samples collected from groups of animals on farm may allow these groups to be categorised into 'bands' corresponding to their nutritional status. This may assist advisors to diagnose nutritional problems.

## 5. RECOMMENDATIONS

- The papers should be published as a book through a reputed publisher.
- The tools developed in this project could effectively be used in the IAEA TC projects for promoting animal production in developing countries.
- The work to develop more rapid tests for PD for diagnostic use, particularly in field, e.g. dipstick (paper-litmus type) tests, biosensor, easy-to-use kits, etc. should be undertaken.
- Further research to elucidate mechanisms that cause buffaloes and camels to be different from cattle, sheep and goats with respect to PD excretion should be supported. Aspects that need attention are the quantitation of renal vs. non-renal losses of allantoin in buffaloes, and analysis of the absorption and metabolism of the PD in intestinal mucosa and liver of buffaloes.
- Work is also needed to exploit full potential of NIRS for measuring PD excretion..
- The PDC index and the use of 'spot urine test' have been found to be a powerful tool for both research and diagnostic purposes. Training workshops should be organised to assist competence in use of the PD and related techniques.
- The results of the project should be collated and presented at an appropriate international conference as achievements of the joint FAO/IAEA purine project.
- Success of this CRP was enhanced by attention to, and integration of, quality control protocols throughout this program, and the use of such an approach is recommended for all future research programs.

Note:

The PDC index is the PD:C ratio (corrected for metabolic weight)

$$= PD : C = \frac{[PD]}{[Creatinine]} \times W^{0.75}$$

where W is the body weight (kg), [PD] and [Creatinine] are PD and creatinine concentrations respectively in mmol/L.

The PD:C ratio used here is expressed as:

$$(mmol L^{-1} PD / mmol L^{-1} Creatinine) \times kg W^{0.75}.$$

The term of metabolic body weight is included here because the creatinine excretion is a function of protein mass of the animal.