Nitrogen and phosphorus utilisation and excretion in dairy buffalo intensive breeding

Gianluca Neglia,1 Anna Balestrieri,1 Bianca Gasparri,1 Monica I. Cutrignelli,1 Giovanna Bifulco,1 Angela Salzano,1 Roberta Cimmino,2 Ettore Varricchio,2 Michael J. D’Occhio,3 Giuseppe Campanile1

1Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli Federico II, Italy
2Dipartimento di Scienze e Tecnologie, Università del Sannio, Benevento, Italy
3Faculty of Agriculture and Environment, The University of Sydney, Camden, Australia

Abstract

The aim of this study was to quantify nitrogen (N) and phosphorus (P) utilisation and excretion in intensive dairy buffaloes. Italian Mediterranean buffaloes were divided into 4 groups (n=6/group) as follows: Groups M50, M125 and M225 at 50, 125 and 225 days of lactation, respectively, and Group NL which was non-lactating. Lactating buffaloes had greater (P<0.05) dry matter and organic matter intake than NL buffaloes. Buffaloes in Group M50 had a lower (P<0.05) crude protein (CP) apparent digestibility than buffaloes in Groups M125 and M225. Group NL had a higher (P<0.01) real CP digestibility than Group M50. Buffaloes in Groups M50 and NL had a negative N balance (-79 and -12 g, respectively) whilst buffaloes in Groups M125 and M225 had a positive N balance (26 and 16 g, respectively). Buffaloes in Group M50 had more (P<0.05) N in urine (204 g) than Group NL (87 g). Buffaloes in Group NL had less (P<0.01) P in both faeces (12.9 g) and urine (0.8 g) compared with the three groups of lactating buffaloes combined (25 g in faeces and 12 g in urine) and they also had greater (P<0.05) P digestibility (68%) than lactating buffaloes combined (43%). The present study has shown that buffaloes have a relatively high efficiency of N and P utilisation.

Introduction

The efficiency of nutrient retention and utilisation by buffaloes has an important environmental relevance (Tammenga, 1996). This applies in particular when buffaloes are used in intensive production systems in developed countries (Campanile et al., 2010), but it is also relevant in developing countries where the vast majority of buffaloes are located (Zicarelli, 1994). In both intensive and extensive ruminant production systems it is desirable to reduce the environmental release of nitrogen (N) and phosphorus (P) in urine and faeces. Excess N can directly cause leaching and soil nutrient imbalance (Marini and Van Amburgh, 2005) and it can also be converted to nitrous oxide which has a greenhouse gas potential that is around 300 times that of carbon dioxide (Eckard et al., 2010). Despite apparent differences in nutrient retention and excretion, buffaloes are usually categorised together with cattle when accounting for environmental impact even though intensive buffalo dairies likely have substantially lower nutrient outflows than intensive dairy cattle systems (Campanile et al., 2010). This has important implications for policy and regulation (Golub et al., 2013) and also in the design and operation of feeding and waste management and nutrient re-cycling systems in intensive livestock production systems (Bouwman et al., 2013). In Italy, in fact, buffalo breeding has reached a great level of innovation, similar to that performed in dairy cattle. Animals are maintained in paddocks, automated milking is performed twice and sometimes three times a day, diets are characterised by a 50:50 forage:concentrate ratio and overall management aims to milk production. The sustainable intensification of livestock requires environmental considerations and the environmental component of food production industries will be part of the future contribution of agricultural and food systems to ecosystem services (Eisler et al., 2014). The aim of the present study was to quantify N and P retention and utilisation in buffaloes at a greater precision than is currently available. This information is considered essential for increased accuracy in nutrient metabolism of buffaloes, highlighting differences with cattle, and other economically important livestock.

Materials and methods

Animals and management

The study utilised 24 Italian Mediterranean buffaloes (Bubalus bubalis) at an intensive commercial buffalo dairy farm in Southern Italy [6.2±1.3 years old; 613.7±14.1 kg live weight (LW)]. Six buffaloes were not lactating and the other 18 buffaloes were at different stages of lactation. The buffaloes were housed individually in tie-stalls for the duration of the study according to the guidelines of Guide for the Care and Use of Agricultural Animals in Research and Teaching. The non-lactating and lactating buffaloes received different rations that were specifically formulated for their respective dietary requirements (Table 1). The diet of lactating buffaloes was formulated to meet the weight requirements for buffaloes of approximately 600 kg and to produce 16 kg/day of standard buffalo milk [energy corrected milk (ECM)=740 kcal] according to the formula (Campanile et al., 2003):

\[
\text{[(fat (g·kg⁻¹)-40+protein (g·kg⁻¹)-31)×0.01155]+1}]×\text{milk yield}
\]

Non-lactating buffaloes received a rationed diet that was formulated to meet the maintenance requirements of pregnant buffaloes, while the lactating buffaloes received ad libitum a diet calculated for a 10% refusal (Table 1) (Campanile, 1997). The respective diets were provided as a total mixed ration once daily at 08:00 h.
**Experimental design**

The buffaloes were allocated (n=6/group) to a non-lactating (NL) group and three groups of lactating buffaloes that were at different stages of lactation at the start of the study as follows: Group M50, 50±8 days milking; Group M125, 125±55 days milking; Group M225, 225±26 days milking. Live weight and age were similar for the four groups and milking number and milk yield were similar for the three groups of lactating buffaloes. The latter buffaloes were milked twice daily at 06:00 and 16:00 h.

The experimental period comprised 14 days of adaptation to the respective diets for non-lactating and lactating buffaloes followed by 14 days of data collection. During the second period of 14 days, feed intake was determined daily from the amount of unconsumed feed (remains) left at 07:00 h after buffaloes had been fed at 08:00 h the previous day. Milk yield was recorded daily for 14 days. During the final 3 days of the study, samples were daily obtained of the ration and remains, mixed in equal proportion, and stored at -20°C until subsequent analyses. Milk samples were also obtained in the morning and afternoon during the final three days. The morning and afternoon samples were mixed in proportion to the respective milk yields and stored at -20°C until analysis. Faecal and urine samples were additionally collected over the final three days. Faeces were collected on steel trays and daily output was determined before faeces were mixed thoroughly and a sample (10% of daily output) was stored at -20°C for future chemical analyses. Total urine output was collected using indwelling Bardex Foley bladder catheters (26 Fr, 75-cc ribbed balloon, lubricious-coated; C.R. Bard Inc., Murray Hill, NJ, USA). Catheters were inserted at 08:00 h on Day 26 (Day 12 of the second 14-day period) and connected to urine collection tubing until Day 28. Urine was collected into 20-L Carboy polyethylene containers to which 150 mL of concentrated HCl had been added to achieve a urine pH<3. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile N. Daily urine output was measured, mixed thoroughly, and 5% of the daily volume was stored at -20°C until analysed for total N. Crude protein (CP) (and nitrogen) real digestibility was calculated according to McDonald et al. (1995).

**Analyses**

The analyses of feed and remains for individual buffaloes were carried out according to AOAC (2004). Energy values (Milk Forage Units-MFU=1700 kcal NEL) were calculated according to the INRA equations (Jarrige, 1988). Milk samples were analysed for protein and fat using a near infrared analyser (Foss System 4000; Foss Electric, Hillerød, Denmark) according to AOAC (2004). Faecal samples were separated into two aliquots. One aliquot (approximately 5 g) was used to determine CP by the macro-Kjeldahl procedure and total N content using a Kjetc 2400 autoanalyser after ether extraction according to the methods described by AOAC (AOAC, 2004). The second faecal aliquot was transferred to aluminium pans and held at 60°C in a forced-air oven for 48 h (AOAC, 2004) for dry matter (DM) determination. Dried faeces were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Ipswich, UK) and analysed for ash content by combustion in a muffle furnace at 550°C for at least 8 h. The organic matter (OM) was determined as the difference between DM and ash.

Urine samples underwent a similar procedure for total N determination as described for faeces. Dry matter in urine was obtained by drying a 3 mL sample in an oven at 60°C for 12 h and ash and OM were determined as described above (AOAC, 2004). Faeces (1.2 g) and urine (1.2 mL) samples were also utilised for P determination by combustion in a muffle furnace at 550°C for 5 h with ZnO and processing by a colorimetric method with ascorbic acid and Na2MoO4*2H2O. Phosphorus determination was carried out by ultraviolet-visible spectroscopy.

**Statistical analysis**

No data were collected and analysed during the first 14 days of adaptation to the diet. Feed intake, remains and milk production data from each buffalo over the last 14 d of data collection were analysed using SPSS version 17.0 (2009). The data obtained during the last three days of the study (feed intake, remains, digestibility and N, P and water balances) were individually analysed by using ANOVA for repeated measures. Treatment group was used as the experimental unit and the effect of the time and the interaction treatment×time was also analysed. Significant differences between groups were evaluated by Turkey test and statistical differences are reported as P<0.05 or P<0.01. Trends for significance are reported as P<0.10. Correlation analyses (SPSS, 2009) were also performed for the range of parameters measured.

### Results

**Dry matter and organic matter intake, excretion and digestibility**

Results for feed intake, dry matter intake (DMI), organic matter intake (OMI) and milk production are shown in Table 2. The DMI for all lactating buffaloes was around 2.16% of LW and ranged from 1.98% for buffaloes in Group M50 to 2.34% in Group M125. Lactating buffaloes had a greater (P<0.05) DMI than non-lactating buffaloes. The DMI per kg of ECM averaged 0.25 kg/kg ECM for all lactating buffaloes and tended (P<0.10) to be lower for buffaloes in Group M50 compared with buffaloes in Group M125 and Group M225, which did not differ. Both DM and OM digestibility were similar among the four groups of buffaloes (Table 3). No significant interaction time×treatment was recorded.

| Table 1. Feed and chemical composition of the diets for lactating and non-lactating buffaloes. |
|---------------------------------------------------------------|
| Diet A | Diet B |
| Feed, kg | | |
| Reye grass silage | 15.0 | - |
| Corn silage | - | 10.0 |
| Reye grass hay | 3.5 | - |
| Alfalfa hay | 2 | - |
| Concentrate³ | 8.2 | - |
| Straw | - | 5.5 |
| Soybean meal | - | 0.5 |
| Corn meal | - | 1 |
| Vitamins³ | - | 0.04 |
| Mineral supplement⁴ | - | 0.36 |
| Chemical composition | | |
| DM, % | 69.2 | 50.9 |
| OM, % DM | 90.4 | 92.8 |
| EE, % DM | 5.6 | 2.1 |
| CP, % DM | 15.4 | 8.5 |
| NDF, % DM | 42.4 | 52.5 |
| ADF, % DM | 22.6 | 28.6 |
| ADL, % DM | 5.2 | 2.7 |
| Ash, % DM | 9.6 | 7.2 |
| Total N, % DM | 2.5 | 1.4 |
| P, % DM | 0.4 | 0.5 |
| Ca, % DM | 0.8 | 0.5 |

Diet A, diet of lactating buffaloes; Diet B, diet of non-lactating buffaloes; DM, dry matter; OM, organic matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; N, nitrogen; P, phosphorus; Ca, calcium. °Concentrate chemical characteristics: CP 20%; EE, 8%; crude fibre, 4%; ash, 7%; NDF, 18%; ADF, 7%. "Vitamins and mineral supplement (for 1 kg of concentrate): Ca, 11 mg; P, 7.9 mg; vitamin A, 40,000 IU; vitamin D3, 800 IU; vitamin E, 60 mg; vitamin B3, 480 mg; vitamin B2, 200 mg; iron carbonate, 800 mg; calcium iodide, 2400 mg; cobalt carbonate, 80 mg; zinc oxide, 12000 mg; sodium selenite, 12 mg.

Neglia et al.
Buffaloes in Group NL defecate less (P<0.05) than Groups M50 and M225 and showed a lower (P<0.01) daily faecal weight (Appendix). There was a relatively high correlation (r=0.549; P<0.05) between DMI and DM of faeces. For all groups combined, buffaloes excreted around 0.42 kg DM of faeces/kg of DMI and there were no significant differences between the groups. Buffaloes in Group NL urinated less (P<0.01) than buffaloes in the three lactating groups (Appendix) and the former buffaloes showed less (P<0.05) daily urine output (Appendix). There was a relatively high correlation between total urine and excreted N (r=0.959; P<0.01). No significant interaction time×treatment was recorded.

Crude protein, nitrogen and phosphorus digestibility and balance

Buffaloes in Group M50 had a lower (P<0.05) CP apparent digestibility than buffaloes in Groups M125 and M225 (Table 3). Buffaloes in Group NL had a CP apparent digestibility similar to that of buffaloes in Groups M225, M125, and M50. The latter showed a CP apparent digestibility significantly lower compared to other lactating groups. No differences were found among NL, M125 and M225 groups in CP real digestibility. However, there was a difference (P<0.01) between Group NL and Group M50 buffaloes in CP real digestibility (Table 3). The N intake of lactating buffaloes was greater (P<0.01) than the N intake of non-lactating buffaloes (Table 4). Negative N balance was not different among the four Groups of buffaloes. The efficiency of

Table 2. Effect of stage of lactation on feed intake, dry and organic matter intake, and milk production in buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Results are means±standard error.

|                      | NL     | M50    | M125   | M225   | Lactating groups combined |
|----------------------|--------|--------|--------|--------|---------------------------|
| Live weight, kg      | 700±11 | 597±20 | 596±3  | 585±15 | 615±14                    |
| Feed intake, kg/day  | 17.4±0.8 | 14.3±1.7 | 20.4±3.1 | 17.2±1.2 | 17.3±1.2 |
| DMI, kg/day          | 8.9±0.4 | 11.9±1.0 | 13.9±1.5 | 12.9±0.7 | 12.9±0.7 |
| OMI, kg/day          | 8.2±0.4 | 10.8±0.9 | 12.6±1.3 | 11.7±0.6 | 10.9±0.6 |
| DMI/live weight, %   | 1.5±0.1 | 2.3±0.3 | 2.2±0.1 | 2.1±0.1 | 2.3±0.3 |
| DMI for maintenance, kg | 11.0±1.3 | 11.0±0.1 | 10.5±0.2 | 10.1±0.2 | 10.0±0.2 |
| DMI for production, kg DMI/kg ECM | 0.1±1.4 | 0.4±1.1 | 0.3±1.1 | 0.2±1.1 | 0.3±1.1 |
| Feed intake, kg/day  | 17.4±0.8 | 14.3±1.7 | 20.4±3.1 | 17.2±1.2 | 17.3±1.2 |
| Total N excretion, g/day | 132±20 | 392±43 | 306±46 | 298±34 | 291±30 |
| N intake, g/day      | 119±5   | 312±30 | 332±71 | 314±29 | 275±29 |
| N balance, g/day     | -12±25c | -79±32c | 26±22d | 16±45d | -15±20 |
| N utilisation for milk production, % | -20±1.3 | 16±1.4 | 14±2.5 | 17±1.3 | 17±1.3 |

Table 3. Effect of stage of lactation on dry and organic matter, and crude protein digestibility in buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Data for real crude protein digestibility are also reported. Results are means±standard error.

|                      | NL     | M50    | M125   | M225   | Lactating groups combined |
|----------------------|--------|--------|--------|--------|---------------------------|
| DM                   | 60.9±4.5 | 51.4±5.0 | 65.6±5.6 | 56.8±5.1 | 58.0±2.8 |
| OM                   | 83.6±4.2 | 54.7±4.4 | 68.8±5.0 | 60.3±4.9 | 61.2±2.6 |
| CP                   | 62.6±2.6 | 71.7±4.0 | 73.5±2.5 | 74.2±2.0 | 67.2±2.1 |
| Real CP              | 99.0±2.0 | 94.0±5.0 | 94.0±2.0 | 94.0±2.0 | 91.0±2.0 |

Table 4. Effect of stage of lactation on nitrogen balance in buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Results are means±standard error.

|                      | NL     | M50    | M125   | M225   | Lactating groups combined |
|----------------------|--------|--------|--------|--------|---------------------------|
| N intake, g/day      | 119±5   | 312±30 | 332±71 | 314±29 | 275±29 |
| Faecal N, g/day      | 44±4    | 123±16 | 92±21b | 82±9ab | 88±10 |
| Urinary N, g/day     | 87±22b  | 204±28c | 157±16eb | 171±26e | 160±16 |
| Milk N, g/day        | -       | 63±8   | 54±11  | 43±7   | 53±4  |
| Total N excretion, g/day | 132±20 | 392±43 | 306±46 | 298±34 | 291±30 |
| N balance, g/day     | -12±25c | -79±32c | 26±22d | 16±45d | -15±20 |
| N utilisation for milk production, % | -20±1.3 | 16±1.4 | 14±2.5 | 17±1.3 | 17±1.3 |
dietary N utilisation for milk protein production tended to be greater (P<0.10) for buffaloes in Group M50 than the other lactating buffaloes. The amount of N secreted in milk and excreted in faeces and urine as a percentage of N intake ranged from 94.30% for buffaloes in Group M125 to 126.5% for buffaloes in Group M50.

There were no differences in P intake between buffaloes in Group NL and the three groups of lactating buffaloes. Buffaloes in Group M225 had a higher (P<0.05) P intake than buffaloes in Group M50 (Table 5). The amount of P in both faeces and urine was lower (P<0.01) for buffaloes in Group NL than the three groups of lactating buffaloes. Consequently, P digestibility in Group NL was greater than in Group M50 (P<0.01) and Group M125 (P<0.05), and similar to Group M225 (Table 5). A negative P balance was observed in Group M50 together with a lower P digestibility (Table 5).

No differences were recorded in water balance for buffaloes in all groups (Table 6). Drinking water intake (DWI) represented around 93% of total water intake (TWI) and this was similar across treatment groups. Buffaloes in Group M50 had a greater (P<0.05) DWI relative to TWI (96.3±1.2%) compared with buffaloes in Group NL (90.4±2.0%), Group M125 (88.9±2.2%) and Group M225 (92.8±0.6%). Overall, lactating buffaloes ingested a greater (P<0.05) amount of total water than non-lactating buffaloes and the former buffaloes excreted a greater (P<0.05) amount of water in faeces. The amount of water excreted in urine (UWE) also tended to be greater in lactating buffaloes although this was only significant (P<0.05) for Group M50 compared with Group NL.

No significant interaction time-treatment was recorded for all parameters.

### Discussion

The management of animal waste is one of the main challenges in intensive livestock production and there is an increased social awareness of the impact of livestock system on the environment (Martinez et al., 2009). The aim of the present study was to precisely quantify the utilisation and excretion of N and P for buffaloes in an intensive dairy system. This information is important in order to make informed and accurate predictions of the environmental impact of buffalo production and to optimise the design and operation of waste management systems in intensive buffalo dairies. The information will also guide the development of policy and regulation for intensive buffalo dairy systems based specifically on the nutrient physiology of the species.

Although a similar amount of N was ingested by lactating buffaloes in the present study,

| Table 5. Effect of stage of lactation on phosphorus balance in buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Results are mean±standard error. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | NL             | M50             | M125            | M225            | Lactating groups combined |
| P intake, g/day | 40.8±1.9b       | 38.2±5.7b       | 46.2±7.3b       | 58.9±5.4b       | 46.4±3.5                |
| Faecal P, g/day | 12.9±1.8b       | 26.6±1.8a       | 23.5±1.3b       | 24.2±1.8b       | 22.3±1.7                <0.001 |
| Urinary P, g/day| 0.8±0.2b        | 19.4±6.6a       | 9.9±1.1b        | 6.3±2.9b        | 9.6±2.8                0.007 |
| Milk P, %       | -              | 1.4±0.2         | 1.1±0.2         | 0.8±0.1         | 1.1±0.1                0.087 |
| Total P excretion, g/day | 13.7±1.9b | 46.0±6.1a       | 33.4±2.4b       | 30.4±4.3b       | 31.8±1.6                <0.001 |
| P balance, g/day| 27.1±3.0a       | -2.9±4.7c       | 11.7±5.2a       | 27.7±8.0a       | 13.6±5.3                <0.001 |
| P digestibility, % | 68.2±4.8b | 25.8±10.7        | 47.7±5.0a       | 58.1±4.4b       | 48.8±5.7                0.02 |

NL, non-lactating buffalo; M50, 50±8 days milking; M125, 125±55 days milking; M225, 225±26 days milking; P, phosphorus. °Data reported only for lactating buffaloes. a,bValues in the same row without common superscripts differ significantly at P<0.05; A,Bvalues in the same row without common superscripts differ significantly at P<0.01.

| Table 6. Water balance results for non-lactating buffaloes and buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Results are mean±standard error. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | NL             | M50             | M125            | M225            | Lactating groups combined |
| FWI            | 2.7±1.1        | 2.4±0.9         | 6.5±1.8         | 4.3±0.6         | 3.9±0.7                0.11 |
| DWI            | 24.5±8.0b      | 64.5±10.5b      | 51.3±6.3b       | 55.0±4.4b       | 50.4±5.5                0.03 |
| TWI            | 27.1±9.1b      | 66.9±10.9b      | 57.8±7.9b       | 59.3±4.9b       | 54.3±5.7                0.03 |
| UWE            | 7.2±2.2b       | 19.2±3.1a       | 14.3±3.8b       | 15.6±2.7b       | 14.6±1.6                0.04 |
| FWE            | 11.6±1.3a      | 30.8±3.4a       | 20.6±2.8a       | 23.1±1.7a       | 22.3±2.3                <0.001 |
| NWE°          | -              | 8.2±0.2         | 5.9±1.0         | 5.5±1.2         | 5.1±0.8                0.14 |
| TWE            | 19.3±1.7a      | 58.1±5.9a       | 40.7±5.5a       | 44.0±4.8a       | 42.0±4.4                <0.001 |
| Water balance  | 7.9±7.5        | 8.8±5.1         | 17.1±2.5        | 15.2±1.9        | 12.2±2.3                0.42 |

NL, non-lactating buffalo; M50, 50±8 days milking; M125, 125±55 days milking; M225, 225±26 days milking; FWI, feed water intake; DWI, drinking water intake; TWI, total water intake; UWE, urine water excretion; FWE, faecal water excretion; NWE, milk water excretion; TWE, total water excretion. °Data reported only for lactating buffaloes. a,bValues in the same row without common superscripts differ significantly at P<0.05; A,Bvalues in the same row without common superscripts differ significantly at P<0.01.
the stage of lactation influenced nitrogen digestibility and balance. A previous study on Italian Mediterranean buffaloes reported that urea levels in milk and blood were affected by the stage of lactation and CP intake (Campanile et al., 2003). Buffaloes fed a diet with low CP content showed fluctuations in blood urea levels that were reflective of changes in DMI during milking, whereas buffaloes fed a diet with high CP content had relatively constant blood urea levels throughout milking (Campanile, 1997). These data were interpreted to suggest that buffaloes that receive relatively low levels of CP recycle N via the kidneys. The apparent N digestibility for non-lactating and lactating buffaloes combined was around 67%. Similar values have been reported in previous studies performed in buffalo species (Shekhar et al., 2010; Saxena et al., 2010). Apparent N digestibility was greater in buffaloes at mid- and late-lactation than those in early-lactation. Cattle fed diets with elevated CP have greater apparent N digestibility due partly to the intake of more digestible feed and partly to the dilution of metabolic faecal N (Broderick, 2003).

In this study non-lactating buffaloes excreted less N in urine than the average of the three groups of lactating buffaloes (approximately 90 and 180 g/day, respectively). The former buffaloes showed relatively low CP digestibility (63%) and relatively high real N digestibility (99%) and this was interpreted to suggest that non-lactating buffaloes had greater N recycling by the kidneys (Campanile et al., 2003). All ruminants are able to recycle endogenous urea-N to the gastrointestinal tract (Lapierre and Lobley, 2001). In the rumen, urea-N can be utilised for microbial protein synthesis which compensates for the losses of N that occur as a result of amino acid deamination and the transfer of ammonia to blood (Lapierre and Lobley, 2001), amino acid catabolism (Lobley et al., 2003), and microbial sequestration of N in purines and pyrimidines (Kristensen et al., 2010). The efficiency of dietary N utilisation in ruminants decreases dramatically as requirements are met and excess N is excreted, mainly in urine (Marini and Van Amburgh, 2005). Ruminants appear to have adjusted to N deficiency by decreasing N clearance by the kidneys, increasing ruminal return and decreasing blood levels of urea (Fanchone et al., 2013). Buffaloes evolved in the tropics (Zicarelli, 1994) and urea concentrations in blood and milk are elevated in buffaloes that are fed a low protein diet for an extended period, and urea levels decline quickly when buffaloes are returned to a diet of normal protein content (Campanile et al., 1998). It was proposed that an increase in circulating insulin reduced or blocked amino acid catabolism and hence reduced urea levels in the blood of buffaloes on a normal diet (Campanile et al., 1998).

Accurate information on N utilisation and excretion is also fundamental for the proper design of effluent management systems that aim to minimise ammonia loss into the atmosphere (Martinez et al., 2009; Li et al., 2013). In this regard, N utilisation is particularly relevant as a large proportion of ammonia is typically volatised before animal waste reaches storage facilities for processing (Xiccato et al., 2005). The results of this study on N excretion (faeces + urine) allows the calculation of annual N secretion for buffaloes in an intensive dairy system. It can be assumed that the respective duration of the lactation and non-lactation periods in buffaloes are 270 days and 95 days, respectively. Also, milking can be divided into early lactation (90 days), mid-lactation (120 days) and late-lactation (60 days). By combining this information with the results for N excretion in the three periods of lactation into a formula (faeces N+urine N) number of days in each period one can calculate that in 365 days buffaloes would excrete 87.1 kg N per year. Assuming a volatilisation loss of 28% (Xiccato et al., 2005). 62.6 kg N are present in the manure management system. The value of 62.6 kg annual N excretion for buffaloes is substantially less than the value of around 85 kg reported for dairy cows (Flis and Wattiaux, 2005; Xiccato et al., 2005).

The lower protein-to-energy ratio in buffalo milk compared with bovine milk indicates that buffaloes are able to efficiently utilise forage that has relatively low protein content (Zicarelli, 1994; Paul et al., 2008). This feature of buffaloes has allowed them to adapt to environments where forage is characterised by a low protein-to-energy ratio (Zicarelli, 1994). Overall, buffaloes are able to cope better than cattle to diets that are deficient in protein and buffaloes also more efficiently utilise diets that have a relatively large protein-to-energy ratio. With regard the latter, it is possible that buffaloes have a rumen biota with a greater capacity to utilise ammonia than the biota in cattle and buffaloes may also have a greater ability to synthesise urea in the liver. The outcome from either or both would be lower levels of ammonia in circulation in buffaloes compared with cattle. Buffaloes were also reported to have the capacity to better cope with a diet deficient in carbohydrate compared with cattle (Campanile et al., 2003). However, there are limits to the capacity of buffaloes to cope with reduced energy intake: Bhadawari buffaloes fed a diet containing 80% of recommended energy showed decreased N utilisation and a reduced growth rate (Singh et al., 2009).

The combined apparent P digestibility in the present study was around 49% and ranged from 25% in buffaloes that were at Day 50 of lactation at the start of the study to 68% in non-lactating buffaloes. Buffaloes that were at Day 50 of lactation at the start of the study had a lower P digestibility compared with buffaloes at later stages of lactation and also non-lactating buffaloes. This may have been due to the transitioning of buffaloes at Day 50 of lactation to a new diet in which the source of P was mainly phytates which have relatively low digestibility (Hill et al., 2008). Non-lactating buffaloes showed the highest P digestibility and, since the amount of ingested P was similar for the four groups of buffaloes, it is likely that non-lactating buffaloes were able to meet their demand for P by reducing kidney clearance and the amount excreted. In fact, urinary and faecal excretion of P were lower in NL compared to lactating groups.

Dry matter intake in the present study was similar to previous reports in buffaloes (Campanile et al., 2003, 2008) and dairy cows (National Research Council, 2001). In both buffaloes (Campanile, 1997) and cattle (Weiss et al., 2009)DMI influences faecal and urine excretion and DM content of faeces. Buffaloes at Day 50 of lactation at the start of the study had a lower DMI per kg ECM compared with buffaloes at Days 125 and 225 of lactation. This reflected the reduced appetite and intake relative to energy requirements of Day 50 buffaloes and the negative energy balance at this stage of lactation. Drinking water intake was appreciably lower for non-lactating buffaloes than lactating buffaloes. The factors that determine DMI in non-lactating dairy cows include DMI, the percentage of DM in the diet, and dietary CP content (National Research Council, 2001). Also, DMI is related to the need to dilute and excrete N that exceeds the requirements. In the present study, non-lactating buffaloes had a N digestibility of 99% suggesting that they required less DMI to excrete N. This is the first study that reports water balance in non-lactating and lactating buffaloes.

Conclusions

In summary, the present study has conclusively shown that buffaloes have a greater efficiency of N and P utilisation compared with cattle. Conversely, buffaloes excrete less N and
clear differences in N and P utilisation conditions of management and physiology but status. Nevertheless, under the same general conditions of management and physiology buffaloes have a reduced environmental impact than cattle in terms of excreted nutrients in comparable intensive dairy systems.

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## APPENDIX

Appendix. Effect of stage of lactation on the number of defecations in 24 h, daily faecal weight, faecal dry matter, number of urinations in 24 h, urine volume and dry matter in non-lactating buffaloes and buffaloes at days 50, 125 and 225 of lactation at the start of the study and in non-lactating buffaloes. Results are means±standard error.

|                         | Group | NL   | M50  | M125 | M225 | P    |
|-------------------------|-------|------|------|------|------|------|
| Defecation, n/24 h      |       | 8.0±1.1^b | 17.8±2.3^a | 10.0±1.0^b | 16.3±0.6^a | 0.021 |
| Faecal weight, kg       |       | 15.0±1.0^b | 36.5±3.4^a | 25.3±3.2^a | 28.8±2.0^a | <0.001 |
| Faecal DM, kg           |       | 3.5±0.4^b | 5.7±0.5^c | 4.7±0.7^ab | 5.7±0.8^c | 0.051 |
| Urination, n/24 h       |       | 5.7±1.3^b | 39.5±7.0^a | 36.0±9.3^a | 29.5±2.5^a | 0.003 |
| Urine volume, L         |       | 8.0±2.2^b | 19.6±3.1^a | 15.0±1.7^ab | 16.2±2.8^ab | 0.026 |
| Urine DM, %             |       | 4.6±0.7 | 3.7±0.8 | 4.8±0.2 | 4.4±0.5 | 0.410 |

NL, non-lactating buffalo; M50, 50±8 days milking; M125, 125±55 days milking; M225, 225±26 days milking; DM, dry matter. ^a,bValues in the same row without common superscripts differ significantly at *P<0.05. *^abValues in the same row without common superscripts differ significantly at *P<0.01.*