Livestock Science 198 (2017) 82–88

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

# Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: A meta-analysis

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ARTICLE INFO

*Keywords:*
Nitrogen
Intake
Excretion
Cattle
Goats
Sheep

ABSTRACT

The quantification of faecal nitrogen (FN) and of urinary urea-N (UUN) and urinary non-urea-N (UNUN) excretion at varying N contents in ruminant rations is an important tool in assessing endogenous N turnover via the rumino-hepatic cycle. Using a statistical analysis based on an extensive database, the aim of this meta-analysis was to evaluate correlations derived previously by deduction. The data were categorised into dairy cattle, growing cattle (bulls and heifers), sheep and goats. Data from 50 publications were considered. The independent variable was the daily N intake (NI, g/day). The dependent variables were the daily quantities (g/day) of FN, urinary N (UN) , UUN, UNUN and N retention. The NI influenced FN to differing extents in goats, dairy cattle, growing cattle and sheep (listed in descending order of influence). Except in sheep, the effect was statistically significant. The influence on UN varied in the order goats, growing cattle, dairy cattle and sheep; the effect was statistically significant only for dairy cattle and growing cattle ($P < 0.001$). The UUN was influenced in the order sheep, goats, dairy cattle and growing cattle ($P < 0.05$). The UNUN could be assessed only in dairy cattle, growing cattle and sheep and was not influenced by NI. The UUN is therefore more strongly dependent on NI than is UNUN and the latter can therefore continue to be seen as obligatory. The FN is indeed influenced by NI but, as a result of higher digestibility of the total ration with increasing crude protein content, an improvement in microbial crude protein synthesis can also be assumed, which is reflected in higher FN levels.

## 1. Introduction

Nitrogen (N) in the form of amino acids is an essential nutrient for ruminants. Besides all the ideas about reducing or avoiding N losses in the feed chain (Tamminga, 1992; Yang et al., 2010), it is important to avoid an inadequate N supply not tailored to the animals' needs, and its influence on performance (Fanchone et al., 2013). An N supply optimised for ruminants is not necessarily accompanied by maximum growing or milking performance, even though this would be desirable (Spek et al., 2013).

Ultimately, all ruminants need absorbable amino acids in the small intestine originating largely from rumen microbial crude protein (MCP) synthesis and, in smaller quantities, from ruminally undegraded dietary CP (UDP). To improve N utilization efficiency, it is vital to expand current data and knowledge. The amino acid content of MCP and UDP, and models for estimating the intermediary, i.e. post-absorptive availability and utilization of these amino acids, especially methionine and lysine, represent an important basis for providing ruminants with a tailored supply of amino acids (Schuba and Südekum, 2013).

To use N-containing feed resources as efficiently as possible, it is important to quantify the minimum supply of N compounds that ruminants require (Pfeffer et al., 2016). The potential of N recycling via the rumino-hepatic cycle can be fully utilised only if the feed N content is as low as possible (Walker et al., 2005), e.g., only 80% of recommended supply with a shortage in rumen-degradable N compounds (Fanchone et al., 2013).

A sufficient volume of reliable data on the N content of faeces (FN) and urine (UN) (including appropriate fractionation into urinary urea-N (UUN) and urinary non-urea-N (UNUN)) is necessary in order to assess N turnover in ruminants and thus to derive recommendations for N supply. From the proportions of UUN in particular, it is possible to draw conclusions regarding the efficiency of MCP synthesis. For example, protein utilization and MCP (indirectly) can be estimated

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http://dx.doi.org/10.1016/j.livsci.2017.01.017
Received 27 August 2016; Received in revised form 28 January 2017; Accepted 30 January 2017
1871-1413/ 

based on the ratio of UUN to allantoin-N (e.g., Kehraus et al., 2006). Moreover, UN and UUN correlate closely with N intake (NI). The UN or UUN fractions can therefore be predicted based on the concentration of CP in the ration (or the urea excreted in milk) (Spek et al., 2013). In contrast to UUN, UNUN and FN are only marginally affected by CP levels in the feed and are therefore unavoidable (Pfeffer et al., 2010). This approach has been confirmed repeatedly in both growing cattle and small ruminants. For example, Cox (2013) showed a 13–88% variation in the fraction of UUN in UN in growing goats fed an increasing supply of CP. In general, therefore, UUN increases with increasing feed N content, whereas UNUN should be seen as virtually independent of N supply.

The UNUN (UNUN=UN-UUN) consists primarily of purine derivatives (mainly allantoin), creatine, hippuric acid and ammonium. In addition, the purine derivative allantoin is a good indicator of the efficiency of microbial protein synthesis, while hippuric acid can be seen as an indicator of the digestibility of plant material. Hippuric acid is an indicator of plant material containing lignocellulose (Kehraus et al., 2006).

Previous studies on the relationship between NI, other dietary factors and N excretion used data of only one ruminant species (cattle; e.g., Reed et al., 2015; Johnson et al., 2016) or category within species (lactating dairy cattle; e.g., Spek et al., 2013; beef cattle, e.g., Waldrip et al., 2013) and some authors also did not differentiate UN into UUN and UNUN (Waldrip et al., 2013; Reed et al., 2015; Johnson et al., 2016). This study used data on small and large ruminants and differentiated UN into UUN and UNUN because the assumption was that, biologically, the response of all ruminant species to increasing NI is not dependent on the species or category (e.g., dairy vs. beef), as surplus N will always result in greater UN, and in particular, UUN excretion.

This meta-analysis therefore aims to pursue and investigate the following hypothesis on the basis of an updated, expanded data set: Regardless of the species or category of ruminant (dairy cattle, growing cattle, sheep or goats), both FN and UNUN are unaffected by a variation in N supply and can therefore be seen as obligatory for derivations of N requirements.

**Table 1**
Statistical description of the database.

| Animal species/category | | Variables (g/day unless otherwise stated) | | | | | |
|---|---|---|---|---|---|---|---|
| | | DMI[a] (kg/day) | NI[d] | FN[e] | UN[f] | UUN[g] | UNUN[h] |
| Dairy Cattle | | | | | | | |
| | n[b] | 136 | 136 | 136 | 136 | 26 | 26 |
| | Mean | 20.3 | 548.4 | 190.3 | 196.7 | 172.6 | 45.8 |
| | Maximum | 30.2 | 827.0 | 386.0 | 342.0 | 240.0 | 77.0 |
| | Minimum | 7.2 | 104.8 | 51.0 | 41.0 | 63.0 | 13.0 |
| | SD[c] | 4.6 | 154.9 | 66.2 | 68.5 | 47.6 | 14.2 |
| Growing Cattle | n | 29 | 29 | 29 | 23 | 18 | 18 |
| | Mean | 6.4 | 130.5 | 40.5 | 50.2 | 30.3 | 18.0 |
| | Maximum | 15.5 | 272.0 | 115.0 | 120.8 | 95.8 | 25.0 |
| | Minimum | 3.9 | 87.6 | 21.1 | 13.6 | 1.5 | 12.1 |
| | SD | 2.9 | 42.3 | 18.5 | 32.1 | 32.8 | 4.3 |
| Sheep | | | | | | | |
| | n | 27 | 27 | 27 | 21 | 27 | 10 |
| | Mean | 1.0 | 21.9 | 6.6 | 11.6 | 8.3 | 3.8 |
| | Maximum | 2.2 | 45.3 | 10.9 | 26.6 | 20.8 | 9.4 |
| | Minimum | 0.5 | 3.5 | 4.1 | 1.8 | 0.8 | 0.9 |
| | SD | 0.4 | 9.5 | 1.7 | 5.4 | 5.6 | 2.6 |
| Goat | | | | | | | |
| | n | 17 | 17 | 17 | 17 | 14 | 11 |
| | Mean | 1.1 | 24.9 | 7.8 | 9.7 | 4.5 | 1.8 |
| | Maximum | 1.8 | 43.9 | 12.0 | 18.1 | 14.6 | 2.5 |
| | Minimum | 0.6 | 7.2 | 2.6 | 1.3 | 0.2 | 1.2 |
| | SD | 0.5 | 12.4 | 3.3 | 5.0 | 4.1 | 0.5 |

[a] DMI=Dry matter intake.
[b] n=Number of rations.
[c] SD=Standard deviation.
[d] NI=Nitrogen intake.
[e] FN=Faecal nitrogen excretion.
[f] UN=Urinary nitrogen excretion.
[g] UUN=Urinary urea-nitrogen excretion.
[h] UNUN=Urinary non-urea-nitrogen excretion.

## 2. Materials and methods

### 2.1. Description of database

The database used in this meta-analysis was constructed from 50 publications (see Appendix A). The breakdown is as follows: 27 publications on dairy cattle, 6 publications on growing cattle, 10 publications on sheep and 7 publications on goats. The crucial selection criterion in each case was that all relevant N fractions in faeces and urine were quantified, rather than calculated or derived. The data set included the species and sample size studied in each publication, plus dry matter intake (DMI), CP, NI, FN, UN, UUN and UNUN. For dairy cattle, the data also included milk urea, milk fat, milk protein and milk yield. All data were expressed both as absolute quantities and as concentrations. However, for reasons of clarity and to improve comparability, this publication only presents the absolute figures (g/day) except for milk fat and protein and milk urea, which were expressed as concentrations. Table 1 summarises the statistical distribution of the variables tested in the data for each animal species or category. For ease of reading, the term 'species' is used throughout, also when referring to a category within the species 'cattle'.

### 2.2. Statistical analyses

Data compiled in the database were analysed using mixed model regression methodology (St-Pierre, 2001; Sauvant et al., 2008). The NI (g/day) was treated as the independent variable and considered as fixed effect. Different studies were considered as random effects. The dependent variables were FN, UN, UUN, UNUN, N retention (NRet), milk yield, milk protein, milk fat and milk urea. Accordingly, the following model was used:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij},$$

where $Y_{ij}$=the dependent variable, $B_0$=overall inter-study intercept (fixed effect), $B_1$=the overall linear regression coefficient Y on X (fixed effect), $B_2$=the overall quadratic regression coefficient Y on X (fixed effect), $X_{ij}$=the value of the continuous predictor variable, $s_i$=the random effect of the ith study, $b_i$=the random effect of study on the regression coefficient of Y on X, and $e_{ij}$=the residual error. The model was applied for each ruminant species, i. e. dairy cattle, growing cattle, sheep and goats. Model statistics used for this study was Akaike's information criterion (AIC), which was applied in model selection to measure the relative goodness of fit of a statistical model. In this study, AIC was used to select whether a model is quadratic or linear (lower AIC indicates better model fit), together with the P-value. When a quadratic model did not significantly explain the relationship between independent and dependent variables, the model was modified into a linear model by eliminating out the $B_2X_{ij}^2$ component. Since data were unbalanced among ruminant species and different variables, the meta-analyses were performed based on the available data.

## 3. Results

The regression analysis results are shown in Table 2, differentiated by species, with NI (g/day) serving as an independent variable in

**Table 2**
Equations for linear regression between dietary nitrogen level (independent variable) and faecal and urinary nitrogen-excretion (response variable; faecal nitrogen, urinary nitrogen, urinary urea nitrogen, urinary non-urea nitrogen, nitrogen retention, milk yield and milk composition of dairy cattle, growing cattle, sheep and goats.

| Independent Variable=NI[a] | | | | | Parameter estimates | | | | | | Model statistics |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Dependent Variable | Unit | Model[b] | n[c] | Intercept | SE[e]intercept | P int | Slope | SE slope | P slope | AIC[d] |
| Dairy Cattle | *FN*[f] | g/d | Q | 136 | 117.3 | 53.5 | 0.038 | 3.24 | 0.47 | < 0.001 | |
| | | | | | | | | −0.0043 | 0.001 | < 0.001 | 1509 |
| Growing Cattle | | | L | 29 | 17.51 | 22.01 | 0.457 | 2.6 | 0.54 | 0.005 | 271.9 |
| Sheep | | | L | 27 | 10.47 | 6.45 | 0.156 | 1.66 | 1.07 | 0.182 | 146.9 |
| Goat | | | L | 17 | −2 | 2 | 0.391 | 3.7 | 0.26 | < 0.001 | 89.5 |
| Cattle Dairy | *UN*[g] | g/d | L | 136 | 322.3 | 25.4 | < 0.001 | 1.18 | 0.1 | < 0.001 | 1447.3 |
| Growing Cattle | | | Q | 23 | 41.25 | 8.13 | 0.004 | 1.89 | 0.2 | < 0.001 | |
| | | | | | | | | −0.005 | 0.001 | 0.025 | 170.3 |
| Sheep | | | L | 21 | 11.86 | 2.3 | 0.002 | 0.88 | 0.27 | 0.017 | 132.8 |
| Goat | | | Q | 17 | 5.07 | 0.79 | 0.008 | 2.57 | 0.37 | 0.006 | |
| | | | | | | | | −0.05 | 0.01 | 0.023 | 78.4 |
| Cattle Dairy | *UUN*[h] | g/d | L | 26 | 412.8 | 33.6 | < 0.001 | 1.35 | 0.2 | 0.001 | 240.5 |
| Growing Cattle | | | L | 18 | 76.16 | 8.7 | 0.0123 | 1.29 | 0.07 | 0.003 | 132 |
| Sheep | | | Q | 27 | 3.52 | 2.51 | 0.203 | 3.53 | 0.69 | 0.001 | |
| | | | | | | | | −0.11 | 0.03 | 0.011 | 132.7 |
| Goat | | | L | 14 | 14.5 | 4.7 | 0.091 | 1.52 | 0.32 | 0.041 | 60.5 |
| Cattle Dairy | *UNUN*[i] | g/d | L | 24 | 700.5 | 48.4 | < 0.001 | −1.01 | 1.09 | 0.395 | 261.3 |
| Growing Cattle | | | L | 18 | −85.69 | 20.82 | 0.054 | 11.02 | 1.13 | 0.01 | 151.8 |
| Sheep | | | L | 10 | 14.37 | 5.33 | 0.074 | 2.21 | 1.91 | 0.333 | 65.7 |
| Cattle Dairy | *NRet*[j] | g/d | L | 61 | 568 | 29.5 | < 0.001 | 0.46 | 0.47 | 0.342 | 734.1 |
| Growing Cattle | | | L | 19 | 48.82 | 14.08 | 0.026 | 2.3 | 0.3 | 0.005 | 135.3 |
| Sheep | | | L | 16 | 15.66 | 2.73 | 0.011 | 1.4 | 0.66 | 0.124 | 93.2 |
| Goat | | | L | 11 | 13.38 | 9.86 | 0.404 | 2.49 | 1.13 | 0.271 | 62.6 |
| Cattle Dairy | *Milk yield* | kg/d | Q | 119 | 519.4 | 60.5 | < 0.001 | −6.65 | 4.7 | 0.173 | |
| | | | | | | | | 0.26 | 0.09 | 0.008 | 1308.5 |
| Cattle Dairy | *Milk protein* | % | L | 119 | 470.8 | 160.7 | 0.008 | 36.53 | 50.22 | 0.476 | 1323.5 |
| Cattle Dairy | *Milk fat* | % | Q | 119 | 620.4 | 29.5 | < 0.001 | −8.88 | 3.97 | 0.037 | |
| | | | | | | | | 0.07 | 0.03 | 0.038 | 1346.8 |
| Cattle Dairy | *Milk urea* | mg/dL | L | 66 | 477.6 | 39.1 | < 0.001 | 1.17 | 0.27 | 0.001 | 680.5 |

[a] NI=Nitrogen intake (g/day).
[b] Q=quadratic; L=linear.
[c] n=Number of rations used.
[d] AIC=Akaike's information criterion.
[e] SE=Standard error.
[f] FN=Faecal nitrogen excretion (g/day).
[g] UN=Urinary nitrogen excretion (g/day).
[h] UUN=Urinary urea-nitrogen excretion (g/day).
[i] UNUN=Urinary non-urea-nitrogen excretion (g/day).
[j] NRet=Nitrogen retention (g/day).

relation to the various dependent variables (FN, UN, UUN, UNUN, NRet, all in g/day).

FN correlated positively with increasing NI in all species, in the order goats (quadratic model), dairy cattle, growing cattle and sheep. Only in sheep this effect was not statistically significant.

The UN also correlated positively with increasing NI in all species. For UN, the clearest effect was again shown in goats, in a quadratic model with a slope value of 2.57, followed by growing cattle (quadratic model), dairy cattle and sheep. This effect was significant for all species ($P < 0.05$).

UUN also showed a positive correlation with increasing NI for all four species. For UUN, the clearest effect of a variation in NI was shown for sheep, with a slope value of 3.53 in the quadratic model, followed by goats, dairy cattle and growing cattle. All effects were significant ($P < 0.05$).

For UNUN, no values could be ascertained for goats due to the limited data set. Sheep and growing cattle showed a positive correlation with increasing NI, and dairy cattle a negative correlation. With a slope value of 11.02 in growing cattle, a strong influence was identified following NI variation ($P < 0.01$). Contrary to growing cattle, sheep data had a much smaller slope value of only 2.21 and, just like in dairy cattle with a slope value of −1.01, no significant relationship between NI and UNUN was determined.

In the case of NRet, all four species showed positive correlations with increasing NI. Once again, it was in goats that NRet was most dependent on NI variations (slope value=2.49), followed by growing cattle, sheep and dairy cattle. However, this effect was statistically significant only in growing cattle ($P < 0.05$).

Table 2 also includes the results for milk yield, milk protein, milk fat and milk urea in dairy cattle. In a linear model, a positive effect with increasing NI was found for milk urea (mg/dL) with a slope value of 1.17 ($P < 0.001$).

## 4. Discussion

### *4.1. Relationship between nitrogen intake and faecal nitrogen excretion*

It can generally be assumed that FN is influenced only marginally, even with increasing NI (Röhrmoser et al., 1984; Kreuzer and Kirchgeßner, 1985; Fanchone et al., 2013). The FN usually remains unchanged or even falls slightly with increasing NI (Röhrmoser and Kirchgeßner, 1982). This study now shows a close correlation with NI across almost all species (not significant for sheep only), which needs to be discussed.

Faecal N can be divided into undigested feed N compounds (mainly fibre-bound N) and metabolic N compounds (Mason, 1969; Schwarm et al., 2009). Metabolic N consists of an endogenous fraction and, as main component, of microbial N. The proportion of microbial N excreted in faeces (i.e, undigested MCP from either ruminal or large intestinal synthesis) is particularly influenced by feed quality and, specifically, by the digestibility of the feed. If the digestibility of the feed (especially roughage) increases, the CP content of the feedstuff usually increases as well (*e.g.*, because forage is fertilised more intensively and harvested earlier). In addition, higher feed digestibility means that more fermentable energy is available to the ruminal microbes. Faecal excretion of MCP increases as a result, because MCP is not completely digested postruminally (Lukas et al., 2005). It is unlikely that varying amounts of undigested microbial CP from large intestinal synthesis would impact on this general relationship, as Richard et al. (2017), using as large dataset on mammals, found that “current empirical data does not support a concept of differences in metabolic losses between digestion types”. Digestion types also encompassed herbivores with foregut or hindgut fermentation chambers in the study of Richard et al. (2017).

Rodehutscord et al. (2000) nicely demonstrated this relationship for faecal phosphorus (P) and N losses in goats. Although equal quantities of P and N (g/day) were ingested in the three feeding groups, faecal P excretion and FN increased in the ration variant with higher digestibility. In this meta-analysis too, this connection initially appears to be the cause of the relationships found. However, in the authors' view, this correlation between NI and FN cannot be categorically assumed because, on closer inspection of the individual data on which this meta-analysis is based, 93% of the data were in the range below 11 g FN/kg DMI (see also Pfeffer et al. (2016)). Therefore, FN values over 11 g/kg DMI were rare and, almost without exception, found in the species ‘dairy cattle’.

In addition, the proportion of FN in rations containing tannins is usually higher even where the digestibility of the feed remains constant (Robbins et al., 1987; Tiemann et al., 2008; Alkindi et al., 2013). Tiemann et al. (2008) showed a dose-effect relationship with targeted application of tannin-rich fodder plants. The formation of tannin-protein complexes, which are virtually inaccessible for rumen microbes (Carulla et al., 2005), promotes a shift in N excretion from UN to FN (Robbins et al., 1987; Waghorn and McNabb, 2003; Carulla et al., 2005; Tiemann et al., 2008). However, the effect on the results presented here is almost negligible, as only one of the publications in this meta-analysis involved targeted application of tannins (Alkindi et al., 2013). A tannin content of $>5$ g/kg DM, relevant for N binding (Barry and McNabb, 1999), is found in only a few common species of legume and foliage. Discussion is needed on whether this effect can be disregarded generally, that is to say for all domesticated ruminants. On the one hand, the introduction of tannins by feeding foliage is of relevance in the feeding of livestock particularly for low-producing ruminants in the tropics and subtropics. This kind of data was not included in the data set of this study. On the other hand, tannin levels in common legume species such as red clover (*Trifolium pratense*), lucerne (*Medicago sativa*) (Barry and McNabb, 1999), soybean and soybean commodities (*Glycine max*), faba bean *(Vicia faba)* and pea *(Pisum sativum)* (Reddy et al., 1985) are low. The feeding of legume species with a high ($>45$ g/kg DM) native content of condensed tannins such as birdsfoot trefoil *(Lotus pedunculatus)* (Min et al., 2003; Terril et al., 1992), bigt trefoil *(Lotus corniculatus)* (Min et al., 2003) and sulla *(Hedysarum coronarium)* (Terril et al., 1992) is of minor practical relevance and, in small quantities as part of a mixed ration, would be so diluted that no effect could be expected.

However, precisely because effects can be clear in individual cases, future studies should always consider the aspect of N binding or a possible N shift from UN to FN depending on tannins or other N-binding substances.

### 4.2. Relationship between nitrogen intake and urinary nitrogen

These results underline previous results (e.g. Kreuzer and Kirchgeßner, 1985; Fanchone et al., 2013) showing that UN correlates strongly with NI. These data also emphasise that UN alone is not suitable for estimating which proportion of urinary N is obligatory and which is not obligatory and is therefore – at least partially – avoidable.

### 4.3. Relationship between nitrogen intake and urinary urea nitrogen

Based on these results, UUN can continue to be seen as an excellent indicator of the efficiency of N turnover in ruminants. High UUN values indicate elevated and therefore excessive feed N content. Because absolute values across species of different body size and, therefore, DMI are not a meaningful measure, one can use feed N content for the purpose of comparison. Based on the derivations presented by Pfeffer et al. (2016), ruminant rations should contain the following N concentrations (per kg DM) to account for obligatory losses: 11 g for compensating losses as faecal N, 4 g for compensating losses as urinary non-urea-N and 1 g for compensating inevitable losses as urinary urea-N, totalling 16 g N/kg DM. Consequently, all feed N concentrations largely exceeding 16 g/kg DMI can be regarded as being excessive. Based on the same study, a ratio of UUN to UNUN $>1.5$ is indicative of excessive feed N content. The correlations established by this meta-analysis confirm earlier studies (e.g. Kiran and Mutsvangwa, 2010), but also substantiate the previous assumptions by Pfeffer et al. (2016) founded on basic considerations and derived using a deductive and descriptive approach.

### 4.4. Relationship between nitrogen intake and urinary non-urea nitrogen

The precise statement postulated by Pfeffer et al. (2016), that UNUN should be regarded as virtually independent of NI and therefore as obligatory, cannot be confirmed in dairy cattle with a negative correlation (slope value=−1.01) in a linear test. In addition, only growing cattle (slope value=11.02) show a positive dependency with increasing NI ($P<0.05$) in a linear test. The reasons for this finding, different from the other species, are not clear. We will therefore continue to assume that UNUN should be regarded as obligatory (Pfeffer et al., 2010; Cox, 2013).

### 4.5. Relationship between nitrogen intake and nitrogen retention

Nitrogen retention can be calculated as the difference between NI minus the total of FN and UN. In linear tests it showed consistently positive correlations with NI. However, only those for growing cattle were statistically significant (p=0.005). The statistically derived results show the difficulty in interpreting NRet. Kreuzer and Kirchgeßner (1985) presented a significant influence on NRet with variations in NI in dairy cattle. From the results of three feeding variants (meeting requirements, 25% above and 25% below requirements), they inferred that the variation in NRet is determined mainly by the influence of NI on UN (see also Pfeffer et al. (2010). Cox (2013), however, was able to demonstrate this in only one of three balance periods in growing goats (two feeding variants: NI=8.0 or 18.3 g/day). Here too, the effect was explained by an increase in UN. However, two further balance periods (each with three feeding variants: NI=7.5, 11.6, 18.0 or 7.2, 14.1, 18.4 g/day) showed no significant differences. This result is analogous to the conclusions of Kiran and Mutsvangwa (2010).

Some of the correlations shown also permit conclusions about the complexity of N turnover in ruminants. Positive effects on NRet are often associated with a concurrent improvement in ruminal energy supply. With a resulting increase in microbial protein synthesis, UUN will fall on the one hand and FN increase on the other (4.1.), which might even lead to an unchanged NRet. In the light of these considerations, therefore, the results for NRet cannot be assessed without also considering quantities of easily fermentable carbohydrates or rumen-available energy sources.

## 5. Conclusions

Even on the basis of an updated, expanded data set, this meta-analysis found for all species studied that UN and UUN in particular are clearly dependent on NI. In contrast, FN and UNUN should be seen as obligatory and are therefore influenced only marginally by increasing NI. The results for FN also show clearly that, in most of the trials, two linked variables influence microbial protein synthesis, namely dietary CP and feed digestibility. The hypothesis that FN should be seen as obligatory can be proven in principle. However, in order to obtain even better derivations of quantitative requirements, it might be necessary to separate a variation in N supply from a variation in digestibility of feed and total ration. This was not possible on the basis of the available data.

## Conflicts of interest statement

No conflict of interest exists.

## Appendix A. List of references used to construct the database

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