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# Effect of Rumen Degradable Protein Supplementation on Purine Derivatives Excreted Through Urine and Milk in Lactating Holstein Cows

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**Abstract:** This study was conducted to evaluate the effect of Supplemented Rumen Degradable Protein (SRDP) on excretion of Total Purine Derivatives (TPD; urinary allantoin, urinary uric acid, milk allantoin and milk uric acid) and contribution of purine derivatives excreted through urine or milk in TPD. Multiparous lactating Holstein cows (n = 15) with an average Body Weight (BW) of 643 kg, days in milk; 21 days and milk yield 30 kg days$^{-1}$ fed a basal diet with different levels of SRDP (treatments 1-3 were 0, 50 and 100 g days$^{-1}$ SRDP, respectively). The TPD excretion was 464.81, 468.70 and 480.83 mmol days$^{-1}$ for treatments 1-3, respectively (p = 0.009). Urinary allantoin (p = 0.01) and uric acid (p = 0.04) and milk allantoin (p = 0.02) were affected but milk uric acid was not affected by SRDP. Although, the amounts of excreted purines affected with SRDP, the contribution of Urinary Purine Derivatives (UPD) and Milk Purine Derivatives (MPD) to TPD were approximately constant. Urinary allantoin, urinary uric acid, milk allantoin and milk uric acid accounted approximately 91.63, 4.54, 3.07 and 0.73% of excreted TPD, respectively. In conclusion, regardless the effect of SRDP, the results indicate that urinary allantoin accounted the greatest and milk uric acid accounted the least contribution in TPD excretion in lactating Holstein cows.

**Key words:** Purine derivatives, urine spot sampling, lactating cows, milk, urine, uric acid

## INTRODUCTION

Microbial Nitrogen Flow (MNF) to the duodenum may be regarded as the most important and sensitive indicator to optimize rumen metabolism in dairy cows (Tas and Susenbeth, 2007). Previous study characterizing protein synthesis by the rumen microbial population has required surgical cannulation of the rumen and duodenum (Schager *et al*., 2003) but cannulation is costly, increases animal care concerns and can lower dry matter intake and milk production (Wenham, 1979). Topps and Elliot (1965) 1st proposed the use of purine derivatives excreted through urine as metabolic marker of microbial synthesis in ruminants whereas more recent studies have worked towards establishing methods relating purine derivative excretion to microbial yield (Stangassinger *et al*., 1995). Most of the studies used spot urine sampling technique in animals for estimation of total urine excretion (Valadares *et al*., 1999; Reynal and Broderick, 2005). In previous studies, urinary allantoin (Eriksson *et al*., 2004) or Urinary Purine Derivatives (UPD; urinary allantoin plus urinary uric acid) (Reynal and Broderick, 2005) were used for estimation of microbial protein production. On the other hand, purine derivatives could also secrete in to milk (Gonda and Lindberg, 1997). There has been considerable interest in determining purine derivative concentrations in milk due to the ease of collection, on-farm information of milk production and the opportunity to apply on a commercial scale (Tas and Susenbeth, 2007). Therefore, Milk Purine Derivatives (MPD; milk allantoin plus milk uric acid) also have been used as an indicator for microbial protein production in some of the other studies (Schager *et al*., 2003). The effect of different levels of rumen degradable protein on purine derivatives excretions were investigated in previous works (Moscardini *et al*., 1998; Reynal and Broderick, 2005). However, there is limited data regarding the effect of rumen degradable protein source on both urine and milk purine derivatives. This study was conducted to evaluate the effect of different Supplemented Rumen Degradable Protein (SRDP) levels on excretion of purine derivatives through urine and milk. Furthermore, Researchers investigated the

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proportional contribution of purine derivatives excreted through urine and milk in excreted TPD in lactating Holstein cows.

## MATERIALS AND METHODS

**Cows, management and diets:** The fifteen multiparous lactating Holstein cows in early lactation with an average Body Weight (BW) of 643 kg (±17), days in milk 21 days (±5) and milk yield 30 kg day$^{-1}$ (±0.8 kg) were assigned in a completely randomized design (5 cows treatment$^{-1}$). Based on the previous studies which have been used caseinate as a degradable protein in rumen (Vanhatalo *et al*., 2003), this supplement was used in this study (0, 50 and 100 g day$^{-1}$ supplemented caseinate as treatments 1-3, respectively). Basal diet was formulated with CPM-Dairy (CPM Dairy; University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY and William H. Miner Agric. Res. Inst., Chazy, NY) that contained 1.72 Mcal kg$^{-1}$ Net Energy for lactation (NEl) and 17.2% crude protein and was consisting of 440 g kg$^{-1}$ forage (DM basis), comprised of 210 g kg$^{-1}$ alfalfa hay and 230 g kg$^{-1}$ corn silage (DM basis). The rest 560 g kg$^{-1}$ was consisted of mixed concentrate. The study lasted 49 days and consisted of a 14 days adjustment period and a 35 days collection period. The cows were kept in individual stalls and were fed twice daily at 8:00 and 16:00 h and milked 3 times daily at 2:00, 10:00 and 18:00 h. The cows had free access to water and salt block and also they had enough space to walk. Orts were collected and weights recorded once daily at 7:00 h and the feeding rate were adjusted daily to yield orts of about 5-10% intake.

**Experimental procedures and chemical analysis:** The DM was determined in weekly composites of corn silage and alfalfa by drying at 60°C for 48 h (AOAC, 1990). Intake of DM was computed based on the 60°C DM determinations for total mixed ration and orts. After drying, ingredients were ground through a 1 mm screen (Wiley mill) and composites were prepared by mixing equal DM. Composite samples were analyzed for total nitrogen, DM at 105°C, ash and organic matter (AOAC, 1990). Body weights and body condition score of the cows (Wildman *et al*., 1982) were recorded at the beginning and at the end of the study. Milk yield was recorded at daily three consecutive milkings throughout the sample collection period. Milk was sampled 3 times week$^{-1}$ at 3 daily consecutive milkings and samples were analyzed for fat, protein, lactose and solids non fat (Milkoscan; Foss Electric, Hillerod, Denmark). Milk sampled from 3 daily consecutive milking on day 10, 25 and 35th and the samples were deproteinized (Broderick and Clayton, 1997). The deproteinized samples then stored at -20°C for analysis of purine derivatives. After thawing, the samples were recentrifuged (15,000×g 4°C, 15 min) and analyzed for allantoin and uric acid (Chen and Gomes, 1992). The urine samples for determination of urinary purine derivatives were taken in the same days of milk sample collection. Two urine samples per day approximately at 9:00 and 14:00 h were collected from all cows when cows urinated spontaneously. The 10 mL aliquots were diluted immediately with 90 mL 0.036 N sulfuric acid and stored at -20°C for later analysis. After thawing concentration of creatinine, allantoin and uric acid were measured in urine samples (Chen and Gomes, 1992).

**Calculations and statistical analysis:** Spot urine sampling technique was used for estimating daily urine volume (Valadares *et al*., 1999). Creatinine excretion was used for estimation of daily urine excretion and purine derivatives/creatinine ratio was used to estimate the daily excretions of purine derivatives through urine (Chizzotti *et al*., 2008). Estimated urine volumes were used to compute daily excretion of urinary allantoin and urinary uric acid (mmol day$^{-1}$). Total allantoin and uric acid secreted into milk (mmol day$^{-1}$) was calculated by the multiplication of milk allantoin and milk uric acid concentrations (mmol L$^{-1}$) by daily milk volume. The UPD, MPD and TPD excretions calculated as follow:

$$\text{UPD (mmol day}^{-1}\text{)} = \text{Urinary allantoin (mmol day}^{-1}\text{)} + \text{Urinary uric acid (mmol day}^{-1}\text{)}$$

$$\text{MPD (mmol day}^{-1}\text{)} = \text{Milk allantoin (mmol day}^{-1}\text{)} + \text{Milk uric acid (mmol day}^{-1}\text{)}$$

$$\text{TPD (mmol day}^{-1}\text{)} = \text{Urinary allantoin (mmol day}^{-1}\text{)} + \text{Urinary uric acid (mmol day}^{-1}\text{)} + \text{Milk allantoin (mmol day}^{-1}\text{)} + \text{Milk uric acid (mmol day}^{-1}\text{)}$$

Data were analyzed using Proc mixed in SAS (2000) (Version 8.1; SAS institute Inc., Cary, NC). The following model was used for variables which were repeated measurements over time (milk and urinary purines, urine volume and urinary creatinine):

$$Y_{ijk} = \mu + T_i + Z_k + ZT_{ik} + \varepsilon_{ijk}$$

Where:

$Y_{ijk}$ = The dependent variable
$\mu$ = The overall mean
$T_i$ = The effect of treatment i
$Z_k$ = The effect of time k

$ZT_{ik}$ = The interaction between time k and treatment i
$\varepsilon_{ijk}$ = The residual error

Differences between least squares means were considered significant at $p<0.05$ and differences were considered to indicate a trend toward significance at $0.05<p<0.10$ using PDIFF in the LSMEANS statement.

## RESULTS AND DISCUSSION

**DMI and lactation performance:** The data for Dry Matter Intake (DMI) and lactation performance of the cows are shown in Table 1. Neither DMI (p = 0.42) nor milk yield (p = 0.57) was affected by SRDP. The milk fat yield tended to be rise by SRDP (p = 0.09) and consequently, the greatest 3.5% FCM production was for treatment 3 (p = 0.02). Higher degradable protein in rumen could positively affect fiber digestibility (Yang, 2002) that have potential to increase milk fat yield (Cyriac *et al*., 2008). Treatment 3 has contained the highest rumen degradable protein, therefore more nitrogen content in the rumen probably caused the greater digestibility of fiber and consequently increased 3.5% FCM. Body weights and body condition score of the cows were not affected by treatments.

**Urinary creatinine excretion and urine volume:** Urinary creatinine excretion was 88.4, 91.2 and 91.8 mg dL for treatments 1-3, respectively and there was no effect of SRDP on creatinine excretion (p = 0.37). Urine volumes which estimated based on the creatinine excretion were 24.4, 24.2 and 23.8 L $day^{-1}$ for treatments 1-3, respectively and it was not affected by SRDP (p = 0.61). Reynal and Broderick (2005) found no significant effect of different levels of RDP on urine excretion estimated by spot sampling technique. The estimated urine volume in spot sampling technique is calculated based on creatinine excretion and the creatinine excretion is a function of body weight (Valadares *et al*., 1999). Because body weight changes of the cows were not affected with SRDP, neither urinary creatinine nor estimated urine volume was affected among treatments.

Table 1: Dry matter intake and lactation performance of lactating Holstein cows supplemented with rumen degradable protein

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Items | 1 | 2 | 3 | p-values | SE |
| DMI (kg $day^{-1}$) | 22.91 | 23.32 | 23.65 | 0.42 | 0.62 |
| Milk yield (kg $day^{-1}$) | 38.70 | 39.54 | 39.32 | 0.57 | 0.43 |
| 3.5% FCM[2](kg $day^{-1}$) | $36.37^{b}$ | $37.82^{ab}$ | $38.53^{a}$ | 0.02 | 0.26 |
| Milk fat (kg $day^{-1}$) | 1.21 | 1.24 | 1.28 | 0.09 | 0.03 |
| Milk protein (kg $day^{-1}$) | 1.19 | 1.23 | 1.23 | 0.46 | 0.05 |
| Milk total solids (kg $day^{-1}$) | 4.55 | 4.67 | 4.68 | 0.71 | 0.10 |
| BW change (kg $day^{-1}$) | 0.08 | 0.12 | 0.14 | 0.23 | 0.03 |
| Body condition score | 2.90 | 2.80 | 2.90 | 0.88 | 0.03 |

[1]Treatments 1-3 were 0, 50 and 100 g $day^{-1}$ dietary supplementation of caseinate, respectively; [2]FCM = Fat Corrected Milk; [a-c]Least squares means within the same row without a common superscript differ ($p<0.05$)

**Urinary purine derivatives:** The data for purine derivates excreted through urine are shown in Table 2. The SRDP increased both allantion (p = 0.01) and uric acid (p = 0.04) excretions through urine. Reynal and Broderick (2005) found a significantly increase in UPD excretion from 352-435 mmol $day^{-1}$ by increasing the RDP content of the diet from 10.6-13.2%. They suggested that increasing the amount of RDP offered to animal caused to maximize microbial growth and the amount of N that is captured into rumen microbial cells was elevated. Maximizing the capture of degradable N improves the supply of microbial amino acids to the small intestine and consequently, the urinary excretion of purines which are originated from microbial source will increase (Reynal and Broderick, 2005; Tas and Susenbeth, 2007).

**Milk purine derivatives:** Milk allantoin was affected by SRDP in this study (p = 0.02) but milk uric acid was not affected with RDP supplementation (p = 0.31) (Table 2). Valadares *et al*. (1999) found a significant effect of concentrate level on milk allantoin. Timmermans *et al*. (2000) found that excretion of allantoin and uric acid in milk averaged 8.43 and 1.06 mmol $day^{-1}$, respectively. Valadares *et al*. (1999) concluded that milk allantoin accounted for 4-6% of the purine derivative excretion. Timmermans *et al*. (2000) found that milk uric acid accounted about 12% of purine derivatives excreted via milk but Gonda and Lindberg (1997) reported that approximately 28% of the uric acid was secreted in milk. In the present study, milk uric acid accounted about 19.2% of purine derivatives excreted via milk. Giesecke *et al*. (1994) reported that allantoin excretion through the mammary gland accounted for a mean of 1.6% of total (renal plus mammary) excretion. Researchers found that

Table 2: Purine derivatives excretion through urine and milk in lactating Holstein cows supplemented with rumen degradable protein

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Items (mmol $day^{-1}$) | 1 | 2 | 3 | p-values | SE |
| Urinary allantoin | $464.81^{b}$ | $469.70^{ab}$ | $480.83^{a}$ | 0.010 | 4.12 |
| Urinary uric acid | $22.41^{b}$ | $24.83^{a}$ | $23.02^{ab}$ | 0.040 | 0.65 |
| UPD[2] | $487.22^{c}$ | $494.53^{b}$ | $503.85^{a}$ | 0.009 | 5.22 |
| Milk allantoin | $14.83^{b}$ | $16.05^{a}$ | $16.67^{a}$ | 0.020 | 0.45 |
| Milk uric acid | 3.59 | 4.12 | 3.64 | 0.310 | 0.10 |
| MPD[3] | $18.42^{b}$ | $20.17^{a}$ | $20.31^{a}$ | 0.030 | 1.07 |
| TPD[4] | $506.64^{b}$ | $514.70^{ab}$ | $524.16^{a}$ | 0.004 | 6.23 |

[1]Treatments 1-3 were 0, 50 and 100 g $day^{-1}$ caseinate dietary supplemented, respectively; [2]UPD = Urinary Purine Derivatives (urinary allantoin+urinary uric acid); [3]MPD = Milk Purine Derivatives (milk allantoin + milk uric acid); [4]TPD = Total Purine Derivatives (urinary allantoin + urinary uric acid + milk allantoin+milk uric acid); [a-c]Least squares means within the same row without a common superscript differ ($p<0.05$)

allantoin excretion through milk was about 3.25% of total allantoin excreted via milk plus urine (average of total allantoin excretion was 487.63 mmol $day^{-1}$).

**Contribution of urine and milk purine derivatives in TPD:** The data for proportional contribution of urine and milk purine excretions in TPD are shown in Table 3. Different combination of purines was considered as TPD in previous researches. Urinary allantoin (Eriksson *et al*., 2004), urinary allantoin plus urinary uric acid (Johnson *et al*., 1998; Reynal and Broderick, 2005; Wanga *et al*., 2009), milk allantoin (Schager *et al*., 2003) and milk allantoin plus milk uric acid (Timmermans *et al*., 2000) have been used as purine derivatives. In the present study, total purine derivatives excreted through urine plus secreted through milk was used to determine TPD excretion. Supplementation of RDP affected TPD excretion ($p = 0.004$). Considering the average of all treatments, approximately 96.18% of excreted TPD among treatments was derived from UPD (which 91.63% of that was for urine allantoin and 4.54% was for urine uric acid) and MPD accounted approximately 3.81% of TPD excretion (which 3.07% of that was for milk allantoin and 0.73% was for milk uric acid). Although, RDP supplementation increased TPD excretion among treatments but the proportional contribution of UPD and MPD in excreted TPD were not affected (Table 3). Urinary allantoin was the most effective component in TPD excretion and accounted about 91.63% of TPD excretion among treatments and the milk uric acid was the least effective component which accounted only about 0.73% of TPD excretion among treatments. Valadares *et al*. (1999) has been considered urinary allantoin, urinary uric acid and milk allantoin as TDP excretion. They concluded that urinary allantloin excretion cows. Vagnoni *et al*., (1997) fed alfalfa hay or alfalfa silage accompanied by different levels of energy (supplied by high moisture corn) to mid-lactating dairy accounted approximately 90% of TPD excretion in dairy cows. They found that urinary allantoin and urinary uric acid excretions affected by treatments but there was no significant effect on purine secretions through milk. The results of the present study show that despite the significant effect of SRDP on UPD and MPD excretions, researchers found no significant effect of RDP supplementation on proportions of UPD and MPD or individual purines in excreted TPD. The results suggest that proportional contribution of both UPD and MPD in TPD is approximately is constant and is not a function of dietary changes.

Table 3: Proportional contribution of urine and milk purines in excreted total purine derivative in lactating Holstein cows supplemented with rumen degradable protein

| | Treatments[1] | | | | |
|---|---|---|---|---|---|
| Items | 1 | 2 | 3 | p-value | SE |
| **TPD[2] (%)** | | | | | |
| Urinary allantoin | 91.92 | 91.25 | 91.73 | 0.82 | 1.09 |
| Urinary uric acid | 4.43 | 4.82 | 4.39 | 0.68 | 0.88 |
| UPD[3] | 96.35 | 96.08 | 96.12 | 0.73 | 1.13 |
| Milk allantoin | 2.93 | 3.11 | 3.18 | 0.38 | 0.26 |
| Milk uric acid | 0.70 | 0.80 | 0.69 | 0.36 | 0.04 |
| MPD[4] | 3.64 | 3.91 | 3.87 | 0.35 | 0.94 |

[1]Treatments 1-3 were 0, 50 and 100 g $day^{-1}$ caseinate dietary supplemented, respectively; [2]TPD = Total Purine Derivatives (urinary allantoin+urinary uric acid+milk allantoin+milk uric acid); [3]UPD = Urinary Purine Derivatives (urinary allantoin+urinary uric acid); [4]MPD = Milk Purine Derivatives (milk allantoin+milk uric acid); [a-c]Least squares means within the same row without a common superscript differ ($p<0.05$)

## CONCLUSION

Supplementation of RDP in lactating Holstein cows increased urinary allantoin, urinary uric acid and milk allantoin but not milk uric acid. Although, individual purine derivatives affected by SRDP (except than milk uric acid), contribution of urine and milk purine derivatives to TPD excretion was approximately constant. In conclusion, urinary allantoin accounted the greatest contribution and milk uric acid accounted the least contribution in TPD excretion in lactating Holstein cows.

## NOMENCLATURE

TPD = Total Purine Derivatives
UPD = Urinary Purine Derivatives
MPD = Milk Purine Derivatives
SRDP = Supplemented Rumen Degradable Protein

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