Influence of the Novel Urease Inhibitor Hydroquinone on Growing Lamb Nitrogen Utilization

Y. G. Zhang*, A. S. Shan and J. Bao

College of Animal Science and Technology, Northeast, Agricultural University, Harbin 150030, P. R. China

ABSTRACT : Two *in vivo* experiments were conducted to evaluate the effect of novel urease inhibitor hydroquinone (HQ) on ammonia release rate from urea hydrolysis, nitrogen balance, nutrient digestibility and efficiency of microbial protein synthesis. In Exp. 1, twelve crossbred cannulated lambs were randomly assigned within initial body weight block to one of four HQ treatments, which included 0 (control), 30, 60 or 80 mg HQ/kg DM intake. Ammonia concentration and pH of ruminal fluid were immediately measured at 0, 2, 4, 6 and 8 h after feeding. Increasing the dose of HQ tended ($p<0.15$) to linearly decrease NH_3 formation. The ammonia peak concentration (2 h post-feeding) in animals receiving HQ was approximately one-half of that in animals not receiving HQ ($p<0.01$), and a relatively sustained ammonia release could be obtained at the dose of 30 or 60 mg HQ/kg DM. In Exp. 2, sixteen intact crossbred lambs (weight 40±0.8 kg) were used in a 2×2 factorial design experiment. The four rations consisting of soybean meal-based (SBM) or urea-based (Urea) nitrogen source with or without HQ (S1, S0, U1 and U0) were fed in digestion and N balance trials. Apparent digestibility of major nutrients except that of ADF was not affected by either nitrogen source or addition of HQ. Regardless of nitrogen source, supplementation of HQ significantly improved ADF digestibility ($p<0.05$). The various ration had no effects on N metabolism in the presence of HQ. There was significant difference between total purine derivatives (PD), estimated efficiency of microbial N synthesis ($p<0.05$) and urea-N excretion ($p<0.01$) in the urine for the SBM ration and for the Urea ration. However, HQ had little influence on efficiency of microbial N synthesis as proportion of daily intake of total tract digestible OM ($p>0.05$). No interactions between main nitrogen source and HQ were measured throughout the trial. Results of this study suggest that addition of HQ to ration may improve ADF digestion with having no negative effect on N metabolism and microbial protein production. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 992-997)***

Key Words : Hydroquinone, Urea, Nitrogen Utilization, Lamb

INTRODUCTION

A major factor that limits the efficiency of the utilization of urea by ruminants is the rate at which urea is hydrolyzed in the rumen. Urea is converted to ammonia and carbon dioxide and the resulting ammonia is frequently produced more rapidly than the microorganisms can utilize it for amino acid and protein synthesis. One solution to the problem of the rapid rate of ammonia release from urea could be the use of urease inhibitor. Currently, urease inhibitors (e.g. acetohydroxamic acid, AHA; phenylphosphorodiamidate, PPDA; N-(n-butyl) thiophosphoric triamide, NBPT), as a means of retarding the rapid degradation of dietary urea in rumen, have been used with limited success. Research with these urease inhibitors has demonstrated that most of them are unsuitable for long-term use *in vivo* because of the rapid adaptation, whereas some have negative effects on nitrogen metabolism in the animal (Jones and Milligan, 1975; Whitelaw et al., 1991; Ludden et al., 2000a,b). However, many other urease inhibitors are widely used in urea-based commercial fertilizer and livestock manure disposal. Of these, hydroquinone (HQ) has shown be one of the most potent inhibitors of soil urease. There is no report on the use of hydroquinone in animals in the published literature. We have earlier reported inhibitory effects of HQ on ruminal urease in sheep and its kinetics *in vitro* (Zhang et al., 2001). The aim of this study was to determine the influence of HQ on *in vivo* rumen ammonia production, nitrogen balance, nutrient digestibility and efficiency of microbial protein synthesis.

* Corresponding Author: Y. G. Zhang. Tel: +86-451-5391923, Fax: +86-451-5303336, E-mail: zhangyonggen@sina.com

Received September 13, 2001; Accepted February 15, 2002

MATERIALS AND METHODS

Animals, experimental design and diets

In experiment 1, twelve crossbred cannulated lambs (Texel×Local breed, weight 38±0.9 kg) were used to determine the effective dose range for HQ. Before initiation of the experiment, deworming was done and~ the lambs were adapted to a basal diet (table 1) for approximately one month. The lambs were randomly assigned within block (blocked by initial BW) to one of four HQ treatments, which included 0 (control), 30, 60 or 80 mg HQ/kg DM intake and kept individually in wooden pens equipped with feeder and water troughs. All lambs were fed 600 g of a pelleted complete ration in two equal portions at 08:00 and 18:00 daily for the duration of a 10 day adjustment and 2 day collection.

In experiment 2, sixteen intact crossbred lambs (weight 40±0.8 kg) were used in a 2×2 factorial design experiment to investigate the effects of two nitrogen sources with or

Table 1. Formula and chemical composition of experimental diets[a] in Exp. 1 and Exp. 2

	Exp. 1	Exp. 2			
	Control	S0	S1	U0	U1
Ingredients (% of DM)					
Corn	41.06	38.00	38.00	48.40	48.40
Soybean meal	7.84	11.90	11.90	-	-
Urea	1.0	-	-	1.50	1.50
Cornstover	20.00	20.00	20.00	20.00	20.00
Alfalfa meal	28.00	28.00	28.00	28.00	28.00
Sea salt	0.50	0.50	0.50	0.50	0.50
Limestone	0.60	0.60	0.60	0.60	0.60
Dicalcium phosphate	0.75	0.75	0.75	0.75	0.75
Trace mineral premix[b]	0.10	0.10	0.10	0.10	0.10
Vitamin premix[c]	0.15	0.15	0.15	0.15	0.15
HQ[d] (mg/kg DM)	-	-	30	-	30
Chemical analysis (% of DM)					
OM	85.71	85.84	85.81	85.75	85.73
CP	13.12	13.84	13.67	14.61	14.84
RDP	9.80	5.34	5.27	10.13	10.04
NDF	32.35	33.03	34.01	33.39	32.73
ADF	18.34	18.83	19.29	18.84	18.41
Ca	0.52	0.57	0.53	0.54	0.50
P	0.42	0.43	0.45	0.40	0.41

[a] In exp. 1, other three HQ treatments are inclusion of 30, 60 and 80 mg HQ/kg DM in the diets, respectively. In exp. 2, S0=SBM diet without HQ; S1=SBM diet with 30 mg/kg HQ; U0=Urea diet without HQ; U1=Urea diet with 30 mg/kg HQ.

[b] Trace mineral premix contained (g/100 g): $FeSO_4$ H_2O 23, $CuSO_4$ $5H_2O$ 10, $MnSO_4$ H_2O 14, $ZnSO_4$ H_2O 17, Na_2SeO_4 0.05, KI 0.06 and $CoCl_2$ $6H_2O$ 0.16.

[c] Vitamin premix provided 100,100 IU of vitamin A, 50,000 IU of vitamin D and 1,000 IU of vitamin E per 100 g.

[d] The abbreviation from hydroquinone.

without HQ upon nitrogen balance, major nutrient digestibility and efficiency of microbial protein synthesis. The animals were randomly distributed to four different rations. The four ration supplemented with or without 30 mg HQ/kg DM were basically isonitrogenous and isocaloric varying in the nitrogen source (soybean meal, S and urea, U) and designated as S1, S0, U1 and U0, respectively. Compositions of rations are shown in table 1. The lambs were placed in metabolism crates and managed as described in previous experiment. The nitrogen balance experiment lasted for 15 days with a 10 day adjustment period following by a 5 day collection period.

Measurements

In Exp. 1, on day two of the experiment, samples (30 ml) of ruminal fluid were collected from the ventral sac using a suction strainer just before the morning feeding (0 h) and at 2, 4, 6 and 8 h after feeding. The pH of these samples was measured immediately (Portable pH Meter; Orion Research, Inc. USA). Two milliliters of 10% m-phosphoric acid (wt/vol) was added to 10 ml of each sample, and the samples were frozen (-5°C). The samples were thawed and centrifuged at 16,000×g for 15 min at room temperature. Ammonia concentration of the resulting supernatant was determined with an ion analyzer (Expandable Ion Analyzer EA 940, USA). In Exp. 2, nitrogen balance measurements were conducted for five days. Total fecal output was measured and recorded each day, and sub-samples of each lamb (a constant percentage of daily fecal output) were placed in a drying oven (65°C) for a minimum of 72 h. Fecal sub-samples were weighted, composited for each lamb, and ground through a 1 mm screen. Total urinary output was collected each day into plastic collection vessels containing 10 ml 10% of sulphuric acid (vol/vol) to prevent ammonia-N loss by keeping the final pH of the urine below 3, and a daily urine aliquot of each lamb was composited and stored at 4°C until further analyses. Urea ~content of the urine was determined with the diacetylmonoxame method of Marsh et al. (1965) at 520 nm using Spectrophotometer (UV-2401PC-Shimadzu, Japan). Feed offered and feed refusals were weighed everyday, and a representative sample was composited at the end of the experiment and ground through 1 mm screen until further analyses. Representative samples of feeds, orts and feces were subjected to DM and OM determination following the AOAC (1975) procedure. Nitrogen content of the samples were determined by the Kjeldahl method. Neutral detergent fiber (NDF) and acid detergent fiber

(ADF) were analyzed following the procedure of Goering and Van Soest (1970). With the assumption that purine derivative (PD) excretion could be used as estimator of the microbial protein supplied to the animal (Topps et al., 1965; Fujihara et al., 1986; Chen et al., 1990; Verbic et al., 1990). The amount of microbial purine absorbed corresponding to PD excreted was calculated as follows: $Y=0.84X+0.15W^{0.75}e^{-0.25X}$; where Y=purine derivative output in urine (mmol/d). X=The amount of microbial purine absorbed by small intestine (mmol/d), and W=body weight (kg). Microbial purines absorbed and microbial N yield was calculated based on the relationship derived by Chen et al. (1990), namely:

Microbial N (g/d)=0.727X; where X=purine amount absorbed through small intestine (mmol/d). In the present experiment, the term "efficiency of microbial nitrogen supply" (EMNS) is expressed as grams of microbial N per kg of total tract digestible OM. Purine derivatives excreted in the urine of ruminants comprise allantoin, uric acid, xanthine and hypoxanthine. Allantoin was determined by the method of Young and Conway (1942) while xanthine, hypoxanthine and uric acid were analyzed following the method of Fujihara et al. (1987).

Statistical analysis

For statistical analysis, GLM procedures of SAS (1990) were used. Data were subjected to analysis of variance using the randomized complete block model in Exp.1and the two-way factorial model in Exp. 2, respectively. Where appropriate, mean comparisons were made by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Rumen parameters

Ruminal ammonia and pH traits in the rumen fluid of the lambs receiving 0, 30, 60 and 80 mg/kg DM of HQ.Irrespective of supplemental HQ were found in table 2, the pH of the rumen fluid of all animals dropped slightly following feeding then increased until 6 h after feeding, indicating that rumen pH at different time intervals post-feeding was not affected by supplementation of HQ with all values between 6.06 and 6.78. Administration of HQ resulted in a decrease in the rumen ammonia peak. At the time of peak concentration (2 h post-feeding), the ammonia concentration in animals receiving HQ was approximately one-half of that in animals not receiving HQ ($p<0.01$). The rumen ammonia concentration was lower in lambs fed 80 mg/kg DM than in other lambs at 4 h ($p<0.05$), and by 6 h post feeding no significant differences ($p>0.05$) existed between control and treatments. Rumen ammonia concentrations at 8 h post feeding, the control and 80 mg/kg HQ treatment were lower as compared to the 30 or 60 mg/kg HQ treatments ($p<0.05$). These results indicate that dietary HQ depressed rumen urease and inhibited the hydrolysis of dietary urea. The immediate effect of this inhibition was evident by the decrease in peak concentration of ammonia. In general, the effect of the urease inhibitor was greater at 2 h than at 4, 6 and 8 h post-feeding. Addition of 30 or 60 mg/kg HQ to diet appeared to be beneficial for sustained ammonia release in the rumen. Administrtion of HQ to lambs in conjunction with urea-containing rations depressed the rumen concentration of ammonia supporting the results found in earlier *in vitro* studies (Zhang et al., 2001).

Nutrient digestion

The nutrient digestibility of rations in Exp. 2 is presented in table 3. The digestibility of DM, OM, CP and NDF of Urea ration (U0 and U2) was slightly higher than that of SBM rations (S0 and S1), but differences between the two types of diets did not result in statistical significance ($p>0.05$). Regardless of the type of ration, addition of HQ also had no significant effect on DM, OM, CP and NDF digestibility of the diet ($p>0.05$). The trend to increase NDF digestibility with supplementation of HQ was significant at 10%, but not at the 5% level. Addition of HQ significantly improved ADF digestibility of diets (44.7 vs 36.1, $p<0.05$). No interactions between main nitrogen source and HQ were measured throughout the trial. Research with other urease inhibitors such as AHA has also demonstrated such an effect on nutrient digestibility and fiber degradation (Streeter et al., 1969). Moore et al. (1968) noted that addition of AHA to ruminal fluid stimulated cellulose degradation, and this response was greater with inclusion of urea in the inoculum. In our studies, the addition of HQ increased ADF digestibility, which is consistent with the previous study. As expected, decreasing urea hydrolysis resulted in optimum ruminal fermentation when HQ was supplemented in the diet. In contrast to this result, Ludden et al. (2000b) and Voigt et al. (1980c) noted that some potent urease inhibitors, such as PPDA or NBPT suppressed digestion of cellulose. It is well known that ammonia is an essential nutrient for proper growth of many bacteria. Therefore, inclusion levels of the inhibitor may have been in excess, producing a deficiency in available NH_3-N from thereby decreasing the activity of cellulolytic bacteria. In our studies, the ammonia concentrations resulting from inclusion of HQ at different levels in the diet for Exp.1 were within the recommended range (Satter and Slyter, 1974) for optimum microbial growth, therefore, supporting the ideology that inclusion levels of urease inhibitor must be balanced according to microbial growth.

Nitrogen balance

As shown in table 4, either dietary N source or HQ did

Table 2. Rumen pH and ammonia concentration† in lambs fed hydroquinone

	Post-feeding (h)	Hydroquinone concentration in ration (mg/kg DM)				SEM
		0	30	60	80	
Ammonia (mg/dl)						
	0	11.47	11.04	11.20	7.18	2.04
	2	25.54^{A}	14.64^{B}	15.04^{B}	14.30^{B}	5.45
	4	6.30^{a}	7.76^{a}	8.56^{a}	4.78^{b}	1.67
	6	4.08	4.64	6.00	5.98	0.79
	8	3.90^{b}	6.52^{a}	6.96^{a}	4.96^{b}	1.69
	Mean	10.26	8.92	9.55	7.44	
Rumen pH						
	0	6.69	6.61	6.78	6.75	0.08
	2	6.36	6.20	6.30	6.35	0.10
	4	6.29	6.17	6.27	6.26	0.10
	6	6.24	6.43	6.41	6.06	0.11
	8	6.50	6.63	6.63	6.44	0.09
	Mean	6.42	6.41	6.48	6.37	

† Values are means of 3 lambs.
^{A,B} Means with different superscripts in the same row differ ($p<0.01$).
^{a,b} Means with different superscripts in the same row differ ($p<0.05$).

Table 3. Apparent digestibility of major nutrients of experimental diets

	Diet[1]					Effects[2]		
	S0	S1	U0	U1	SEM	Diet	HQ	Diet×HQ
DM (%)	58.78	57.47	59.35	64.49	1.39	NS	NS	NS
OM (%)	66.37	65.94	68.44	72.63	1.32	NS	NS	NS
CP (%)	75.20	68.72	72.83	75.09	1.38	NS	NS	NS
NDF (%)	43.07	46.02	43.23	51.65	2.01	NS	NS	NS
ADF (%)	37.11	44.55	35.11	44.93	1.92	NS	*	NS

[1] S0=SBM diet without HQ; S1=SBM diet with 30 mg/kg HQ; U0=Urea diet without HQ; U1=Urea diet with 30 mg/kg HQ.
[2] Diet=Main effect of diet; HQ=Main effect of hydroquinone addition; Diet×HQ=Diet by hydroquinone addition interaction.
NS: Not significant ($p>0.05$), HQ: Hydroquinone.
* The main effect of hydroquinone addition is significant ($p<0.05$).

not alter the N balance of animals significantly ($p>0.05$).

This is inconsistent with the study by Ludden et al. (2000b) in which NBPT inclusion to the ration had a negative effect on N metabolism. Voigt et al. (1980b) noted that diets containing urea and PPDA increased ~total amount of urea-N excreted in the urine, indicating that dietary urea was less efficiently utilized by the animal. We did not observe an increase in urinary urea-N with addition of HQ, however, the effect of diet was noticeable. The differences in urea-N excretion in urine between the SBM and the Urea ration were very significant ($p<0.01$, 1.75 vs 4.08). Therefore, the difference in N source ~in terms of rumen degradable rate would explain this result. Animals receiving ration S1 decreased Urea-N excretion by 15% ($p<0.12$) in comparison with S0. As a result, supplementation with HQ tends to decline endogenous urea-N excretion and consequently exert sparing-protein effect. More than half of the urea-N consumed from urea-containing ration was voided in the urine. Urea-N loss through urine accounts for 50.6% of the daily urea-N intake in the HQ fed group, in other words, 49.4% of urea- N made available to the animals, while the corresponding group had 44.3%. Our observations suggest that urea-N utilization could potentially be improved by including HQ to urea-containing rations.

Microbial protein yield

Total purine derivative (PD) excretion was higher on HQ supplemented than unsupplemented groups (12.3 vs 11.35), and the differences approached a significance level of $p<0.10$. The amount of purine derivatives (PD) excreted by lambs fed the Urea ration was significantly higher ($p<0.05$) than that of lambs fed the SBM ration (8.6 vs 15.16). The added RDP from urea promoted higher PD excretion. Urea-containing rations had higher PD excretions with allantoin contributing more than 80% of total PD resulting in a higher estimated microbial N supply as compared to the SBM ration ($p<0.05$). The significant

Table 4. Nitrogen balance, purine derivative excretions, microbial N yield and efficiency of microbial N supply in lambs fed diets differing in nitrogen source with or without hydroquinone

	Diet[1]					Effects[2]		
	S0	S1	U0	U1	SEM	Diet	HQ	Diet×HQ
N intake (g/d)	21.80	24.92	26.85	25.46	1.35	NS	NS	NS
Fecal N (g/d)	5.45	7.77	7.14	6.85	0.37	NS	NS	NS
Urinary N (g/d)	5.45	6.23	6.71	6.36	0.16	NS	NS	NS
Retained N (g/d)	10.90	10.92	13.00	12.25	0.65	NS	NS	NS
N retention (%)	50.00	43.82	48.41	48.11	2.29	NS	NS	NS
Urea N (g/d)	1.89	1.61	3.92	4.22	0.43	**	NS	NS
Total PD (mmol/d)	7.99	8.81	14.71	15.77	1.46	*	NS	NS
Allantoin (mmol/d)	6.31	6.99	12.86	12.93	1.28	*	NS	NS
Uric acid (mmol/d)	1.38	1.62	1.35	2.03	0.15	NS	NS	NS
Xanthine+hypoxanthine (mmol/d)	0.29	0.19	0.49	0.81	0.10	*	NS	NS
Microbial N (g/d)	6.68	7.71	14.79	16.00	1.73	*	NS	NS
Total tract digestible OM (kg/d)	0.55	0.63	0.66	0.67	0.06	NS	NS	NS
EMNS[3] (g/kg DOM)	12.36	12.24	22.41	23.88	0.91	*	NS	NS

[1] S0=SBM diet without HQ; S1=SBM diet with 30 mg/kg HQ; U0=Urea diet without HQ; U1=Urea diet with 30 mg/kg HQ.
[2] Diet=Main effect of diet; HQ=Main effect of hydroquinone addition; Diet×HQ=Diet by hydroquinone addition interaction.
[3] Efficiency of microbial N synthesis (EMNS) calculated as g microbial N/kg of total tract digestible OM (DOM).
NS: Not significant ($p>0.05$), HQ: Hydroquinone, PD: Purine derivatives.
** The main effect of diet is very significant ($p<0.01$).
* The main effect of diet is significant ($p<0.05$).

increase in microbial N yield from the urea ration was due to the abundant supply of rumen degradable nitrogen. Khandaker et al. (1997) found highly significant correlations between RDP intake and total PD excretion in cattle ($p<0.01$, $r=0.69$). Our findings are in agreement with Khandaker et al. (1997) results. The EMNS was significantly lower for the SBM ration than the Urea ration ($p<0.05$). This difference is probably related to a relatively low degradability of soybean meal protein in contrast to urea. Moreover, variations in the EMNS are mainly related to the supply of microbial nucleic acid (Verbic et al., 1990). However, HQ had little influence on efficiency of microbial N synthesis ($p>0.05$).

CONCLUSIONS

In conclusion, the present experiments have shown that feeding HQ at levels that effectively depress ammonia production from urea does not result in changes either in the digestibility of major nutrients (except for ADF) and in N metabolism. Irrespective of nitrogen source in the diet, HQ had little effect on efficiency of microbial protein production depending largely on the availability of carbohydrates and rumen degradable nitrogen. A relatively sustained release of ammonia caused by administration of HQ appeared to be conducive to a significant increase in ADF digestibility. Direct addition of HQ to the complete ration revealed a tendency to decline endogenous and exogenous Urea-N loss through urine, which implies favorable application of HQ as a urease inhibitor in practical ruminant rations. Further investigation of the effects of long-term administration of HQ *in vivo* on nitrogen utilization of ruminants is warranted.

REFERENCES

AOAC. 1975. Official Methods of Analysis (12th Ed.). Association of Official Analysis Chemists, Washington, DC.

Chen, X. B., F. D. Deb, Hovell, E. R. Orskov and D. S. Brown 1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. Br. J. Nutr. 63:131-142.

Fujihara, T., E. R. Orskov, P. J. Reeds and D. J. Kyle. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. J. Agric. Sci. (Camb). 107:12.

Goering, H. K. and P. J. VanSoest. 1970. Forage fiber analysis. In: Agriculture Handbook No. 379. Agriculture Research Services. Washington, DC. US.

Jones, G. A. and J. D. Milligan. 1975. Influence on some rumen and blood parameter of feeding acetohydroxamic acid in a urea-containing ration for lambs. Can. J. Anim. Sci. 55:39-47.

Khandaker, Z. H. and A. M. M. Tareque. 1997. Effect of rumen degradable protein in straw based ration on purine derivatives excretion and microbial nitrogen supply in cattle. Asian-Aus. J. Anim.Sci. 10: 364-370.

Ludden, P. A., D. L. Harmon, B. T. Larson and D. E. Axe. 2000a. Influence of the novel urease inhibitor N-(n-butyl) thiophosphoric triamide on ruminant nitrogen metabolism:I*n vitro* urea kinetics and substrate digestion. J. Anim. Sci. 78: 181-187.

Ludden, P. A., D. L. Harmon, B. T. Larson and D. E. Axe. 2000b.

Influence of the novel urease inhibitor N-(n-butyl) thiophosphoric triamide on ruminant nitrogen metabolism: Ⅱ. Ruminal nitrogen metabolism, diet digestibility and nitrogen balance in lambs. J. Anim. Sci. 78:188-197.

Marsh, W. H., B. Fingerhut and H. Miller. 1965. Automated and manual direct methods for determination of blood urea. Clinical Chem. 11:624-627.

Moore, M. J., W. R. Woods and T. J. Kopfenstein. 1968. Nitrogen metabolism as influenced by acetohydroxamic acid. J. Anim. Sci. 27:1172-1173.

SAS. 1990. SAS/STAT User`s Guide. Volume 2, Version 6, Fourth Edition. SAS Institute Inc., SAS Campus Drive, Cary, NC27513.

Satter, L. D. and L. L. Slyter. 1974. Effect of feeding urea supplemented and urea-treated wheat straw on rumen fermentation pattern and fiber digestibility in crossbred cattle. Indian J. Anim. Nutr. 7(2):107-112.

Streeter, C. L., R. R. Oltjen, L. L. Slyter and W. N. Fishbein. 1969. Urea utilization in wethers receiving the urease inhibitor acetohydroxamic acid. J. Anim. Sci. 29:88-93.

Topps, J. H. and R. C. Elliott. 1965. Relationship between concentrations of ruminal nucleic acids and excretion of purine derivatives by sheep. Nature 205:498-499.

Voigt, J., K. Krawielitzki and B. Piatkowski. 1980b. Studies on the effect of phosphoric phenly ester diamide as inhibitor of the rumen urease of dairy cows:2. The metabolism of ^{15}N-urea.Arch.Tierernaehr. 30:825-834.

Voigt, J., K. Krawielitzki and B. Piatkowski. 1980c. Studies on the effect of phosphoric phenly ester diamide as inhibitor of the rumen urease of dairy cows: 3. Digestibility of the nutrients and bacterial protein synthesis. Arch. Tierernaehr. 30:835-840.

Voigt, J., K. Krawielitzki and B. Piatkowski. 1980a. Studies on the effect of phosphoric phenly ester diamide as inhibitor of the rumen urease of dairy cows: 1. Influence of urea hydrolysis ammonia release and fermentation in the rumen. Arch. Tierernaehr. 30:811-823.

Verbic, J., X. B. Chen, N. A. Macleod and E. R. Ørskov. 1990. Excretion of purine derivatives by ruminants: effect of microbial nucleic acid infusion on purine derivative excretion by steers. J. Agric. Sci. Camb. 114:243-248.

Whitelaw, F. G., J. S. Milne and S. A. Wright. 1991. Urease inhibition in the sheep rumen and its effect on urea and nitrogen metabolism. Br. J. Nutr. 66:209-225.

Young, E. G. and C. F. Conway. 1942. On the estimation of allantoin by Rimini-Schryner reaction. J. Biol. Chem. 142: 839-853.

Zhang, Y. G., A. S. Shan and J. Bao. 2001. Effect of hydroquinone on ruminal urease in the sheep and its inhibition kinetics *in vitro*. Asian-Aust. J. Anim. Sci. 14:1216-1220.