Biuret Research Review

Introduction

Biuret is a slow-release non-protein nitrogen (NPN) product produced by ADM and has been used in beef cattle and sheep feeds since the 1960s. Biuret was approved for use in lactating dairy cattle in 2003. Biuret is formed by condensing two molecules of urea into a single molecule of biuret, which retains three of the original nitrogen atoms from the two urea molecules.



Feed-grade biuret contains a minimum of 55% biuret, a maximum of 15% urea, and a maximum of 30% triuret and cyanuric acids. Guaranteed analysis shows a minimum of 38.5% nitrogen and a crude protein (from NPN) between 240 and 255%. Biuret is free-flowing and its chemical properties make it less hydroscopic (attracts less water) compared to urea. Biuret is also less soluble than urea. The combination of structural and physical characteristics along with low levels of biuretolytic activity from biuretase enzyme (converts biuret to ammonia, carbon dioxide, and urea) in the rumen of animals not adapted to biuret slows the rumen digestion of biuret. Numerous studies have clearly indicated that biuret, even when fed at high levels is a safe source of nitrogen for ruminants grazing forages or for cattle fed in confinement. It is stable to temperature and pressure and can be used in pellets, cubes and pressed molasses tub products.

ADM suggests biuret can be used in dairy rations exactly the same as encapsulated urea products, such as Nitroshure or Optigen. However, there are clear differences that need to be considered. Recommendations from ADM include using biuret in combination with urea to reduce costs. Per ADM literature a 50:50 ratio may be reasonable for diets with more fermentable energy sources, with the amount of biuret rising as the level of NPN is increased and SIP is raised beyond 33% CP. A feeding rate of 0.1 to 0.2 lb/head/day is suggested. One should keep in mind that feed grade biuret does contain up to 15% urea.

Summary of Literature

A ruminal nitrogen release study comparing biuret to urea and soybean meal was conducted by Bartle et al. (1998). Six Holstein steers were used in a replicated 3X3 Latin square with 7-day periods. Steers were intraruminally dosed with .25 g/kg BW (.20 g/kg, 2nd and 3rd periods) of urea and isonitrogenous quantities of biuret and SBM. The authors suggested that these results (graph below) indicate that ruminal nitrogen is released from biuret similarly to SBM and suggest that the nitrogen release rate from biuret did not exceed the ability of the rumen microflora to capture the ammonia. However, the authors did not specify the dietary protein level and if the animals were adapted (and if so for how long) to biuret both of which could impact the release curve of biuret.



Figure 1. Rumen nitrogen release from biuret, urea and soybean meal

Adaptation to Biuret

A review by Fonnesbeck et al. (1975) provides an excellent summarization of literature addressing adaptation of ruminants to biuret. Biuret naïve cows have essentially no rumen biuretolytic activity. Schroder (1970. J. Agric. Sci. Camb.) conducted studies to look at biuretolytic activity and ruminal dynamics in sheep that were or were not adapted (4 weeks) to biuret (Table 1).

Sheep	Experiment no.	Average daily feed consumption (g)	Biuretolytic activity (mg %/24 h)	μ_a (%)	μ_p (%)	$(\%)^{\mu_m}$
$\mathbf{K}22$	1	931	Nil	63.4	36.6	0
K 22	2	1140	Nil	30.0	70.0	0
K 32	3	703	Nil	44.4	55.6	0
K 32	4	767	Nil	30.8	$69 \cdot 2$	0
K34	7	1110	Nil	28.0	72.0	0
K 33	5	1004	118	63.3	9.8	26.9
K33	6	967	164	29.1	33.8	37.1
K41	10	1472	214	43 ·0	15.8	41.2
K46	8	1029	140	45.1	15.9	$39 \cdot 1$
K51*	9	1846	330	48.7	11.3	40.0
		* 30 g adm	inistered only to F	51.		
$\mu_a \%$ = abs	orbed from th	e rumen μ ٩	% = bypassed ru	men ۳ _m %	6 = degrade	ed in rumen

Table 1. Comparison of rumen biuretolytic activity and dynamics in adapted vs. un-adapted sheep

In non-adapted animals, biuret is not degraded in the rumen, but it does appear to be absorbed to some extent directly form the rumen or subsequently from the small intestine (SI). Tiwari et al. (1973a) observed that in vitro about 20% of biuret added to rumen fluid (from biuret adapted sheep) was bound in a complexed form associated with the soluble fraction. The complex did not assay as biuret. If this occurred in the work by Schroder (1970) it could account for some of the biuret assumed to have been absorbed from the rumen. Tiwari et al. (1973a) did show that the complexed biuret was released at low pH. Schroder and Gilchrist (1969) reported a rapid decrease in biuret concentrations within 6.5 hr of feeding biuret while ammonia nitrogen was little affected.

Once biuret is introduced into the rumen biuretolytic activity begins to develop within a few days. However, it can take several weeks for rumen adaptation to achieve maximum biuretolytic activity. How long it takes to fully adapt to biuret appears to be dependent on the dietary crude protein level of the diet. Schroder and Gilchrist (1969) noted that sheep fed on average of 4.4%, 5.9% or 10.3% CP required 15, 29 and 71 d respectively (Figure 2), to achieve maximum biuretolytic activity.



Figure 2. Effect of dietary crude protein on time to achieve maximum biuretolytic activity in sheep

De-adaptation and Re-adaptation

Another interesting aspect of feeding biuret is the impact of discontinuing biuret use on rumen biuretolytic activity. As discussed above adaptation to biuret can take quite a while to achieve. However, Schroder and Gilchrist (1969) observed that once biuret was removed from the diet biuretolytic activity quickly decreased and was essentially gone within just a few days at most (Figure 3). In addition, when biuret was re-introduced the return to maximum biuretolytic activity again could take several weeks to achieve. This has major implications on how biuret use needs to be managed particularly in dairies.

Figure 3. Discontinuation and re-introduction of biuret on rumen biuretolytic activity



A = Last dose of biuret. B= First dose of biuret. 4.9% CP diet.

Metabolism and Degradation

Undegraded biuret that is absorbed either through the rumen wall or from the SI does not appear to be stored in tissues, recycled or metabolized to other compounds. Schroder (1970) injected biuret intravenously into sheep and measured urinary excretion. He noted that biuret was quantitatively

excreted in the urine (Figure 4). Hence, unlike absorbed urea that can be, to some extent, recycled, absorbed biuret is lost and of no nutritional value. As adaptation increases urinary excretion does appear to decrease (Farlin et al., 1968. Figure 5). However even after as much as 51 days of adaptation approximately 30% of biuret fed was still excreted in urine. Tiwari et al. (1973b) estimated that 35% of biuret fed to sheep adapted for 15 weeks still passed from the rumen, which would potentially end up in urine. Small amounts of biuret unabsorbed from the SI may pass to the large intestine and be found in feces.

Figure 4. Urinary excretion of biuret either administered by IV injection or duodenal infusion



Performance

A majority of the research investigating biuret, particularly in the areas of adaptation and metabolism, was performed 50-60 years ago. Much of that work was done with sheep and cattle on low protein poor quality forage diets. Searches conducted of the Journal of Dairy Science and Journal of Animal Science for work done in lactating dairy cows (published since January 2000) that included biuret in the key words or title came up with only a single three-paper series. That series was conducted with wethers and late gestation Angus X Hereford cows.

Waite et al. (1968) looked at the effects on milk yield in cows fed concentrates containing either plantbased proteins or NPN (43% of total concentrate N) from urea or biuret. Cows were 60 to 80 DIM at the start of the trial. A common diet (not containing NPN) was fed for four weeks. Cows were then switched to the experimental diets. The averaged CP level fed during the experimental period was 12.6% which is still considerably below levels fed in more modern dairy cows. Milk production is shown in Figure 6. Cows switched to the NPN based concentrates had lower milk production compared to the control cows. Biuret fed cows produced less milk than urea fed cows, initially. After about 5 weeks the biuret cows caught up to the urea fed cows (suggesting increasing adaptation during this period) but were still producing less milk than the control cows. By 11 weeks biuret cows and control cows were producing at the same level and both had higher milk production than urea fed cows, respectively.

Figure 5. Nitrogen balance and urinary excretion of biuret during adaptation



Figure 5. Waite et al. (1968). Effects on milk yield of feeding a control (plant protein based) concentrate compared to concentrates containing urea or biuret (43% of concentrate total nitrogen)



Triuret and Cyanuric Acid

Both triuret (Clark et al., 1965) and cyanuric acid (Altona and Mackenzie, 1964), which can make up as much as 30% of feed grade biuret, are not toxic to ruminants even when fed at high levels. In a nitrogen balance study using non-adapted sheep Clark et al. (1965) reported that urea, biuret, triuret and cyanuric acid all had similar nitrogen retention over 4 periods (8 weeks). In contrast Miyazaki et al. (1974) looked at nitrogen balance in sheep fed soy isolate, urea or triuret and noted that triuret had significantly lower nitrogen retention than urea which was significantly lower than soy isolate. Altona and Mackenzie (1964) looked at cyanuric acid as a source of NPN in sheep and reported that " when fed in combination with maize meal as a supplement to low quality roughage it can be utilized by sheep as a source of non-protein nitrogen."

Implications

Biuret, based on numerous toxicity studies in sheep and beef cattle, is a safe form of NPN. There is a significant base of research, again with beef cattle and sheep, that biuret can be used successfully to improve performance particularly when feeding or grazing low-protein poor quality forages. There is a lack of research however, particularly in the last 20-30 years, looking at how biuret may, or may not perform in higher producing cows on higher protein diets. Research clearly shows that cows need an adaptation period in order to maximize use of biuret as an NPN source. In beef and sheep, the length of time to maximum adaptation depends on dietary protein level. At higher protein levels (10-11% CP) in the beef and sheep studies maximum adaptation could take as much as 70 or more days. Modern dry cow rations can greatly exceed 10-11% CP. This raises questions about how, at even higher CP levels (or other dietary factors), adaptation be might affect. This may be a major concern when considering biuret use in dairy rations, particularly early lactation diets. If biuret is to be used in early lactation diets it would be important to feed biuret during some portion of the dry period, if not during the entire dry period, to allow for adequate rumen adaptation before the onset of lactation. If the needed adaptation period exceeds the length of the dry period continuous feeding may be necessary. Failure to achieve an optimum degree of adaptation before the onset of lactation may limit RDP at a critical stage of lactation where getting adequate nutrients into the cow is already a challenge.

Another important consideration is how much NPN will biuret really deliver to the cow. As noted above as much as 30% or more of biuret may bypass the rumen and subsequently be excreted in urine. This was reported in sheep on low protein, low quality forages. Dairy cows have a much faster rate of passage than sheep or beef cattle and are fed much higher protein diets. How might these factors impact the amount of biuret bypassing the rumen? As already noted, nitrogen from rumen bypassed biuret, when absorbed in the SI, will be quantitatively excreted in urine, and hence cannot contribute to recycled nitrogen. So how is the protein (RDP) value of biuret potentially impacted? What is the correct protein value to put on biuret?

Summary

Biuret is a slow-release non-protein nitrogen product that is safe to feed to dairy cows. It has been described as having an in vitro release profile, similar to that of SBM. However, the conditions under which biuret ammonia release is measured may have dramatic effects on what the release profile may look like. Research would suggest that like SBM 30% (or more) of the nitrogen in biuret may bypass the rumen. Unfortunately, any biuret that bypasses the rumen, even if absorbed is wasted since it is excreted in urine. Biuret is not recycled in the cow. Consequently, research is needed to determine the true protein value of biuret in dairy rations.

Most of the research surrounding biuret was performed 50 to 60 years ago and minimal work has been performed with lactating dairy cows. An adaptation period is required to allow ruminal micro-organisms to develop maximal biuretolytic activity. Research has not been conducted with the higher protein levels fed to modern dairy cows, so we do not know what the adaptation and development of biuretolytic activity looks like in dairy cows. This is important to understand, particularly regarding early lactation cows. If not properly adapted these animals could be subjected to inadequate RDP at a time where microbial efficiency is critical to performance. Managing biuret feeding may be further complicated by feeding management schemes on dairies. Rapid de-adaptation and long re-adaptation occurs if biuret is removed at any point in time from diets for more than a few days. Consequently, the efficacy of biuret as an NPN source would be compromised for some period after it's reintroduction.

References

Altona, R. E. and H. I. MacKenzie. 1964. Observations on cyanuric acid as a source of non-protein nitrogen for sheep. J. S. African. Vet. Med. Ass. 35:203

Bartle, S. J., P. A. Ludden, and M. S. Kerley. 1998. Ruminal nitrogen release from biuret, urea, and soybean meal. J. Anim. Sci.76 (Suppl. 1):347. (Abstr.)

Clark, R., E. L. Barrett and J. H. Kellerman. 1965. A comparison between nitrogen retention from urea biuret, triuret and cyanuric acid by sheep on a low protein roughage diet. J. S. African Vet. Med. Ass. 36:79.

Farlin, S. D., U. S. Garrigus, and E. E. Hatfield. 1968. Changes in metabolism of biuret during adjustment to a biuret-supplemented diet. J. Anim. Sci. 27:785-789.

Fonnesbeck, P. V., L. C. Kearl, and L. E. Harris. 1975. Feed grade biuret as a protein replacement for ruminants: A Review. J. Anim. Sci. 40:1150–1184.

Miyazaki A., T. Yoshikawa, S. Kinoshita and R. Kawashima. 1974. Evaluation of triuret (carbonyl-diurea) as a source of nitrogen for ruminants. J-Stage 45:281-286

Schroder, H. H. E. 1970. Pathways for the elimination of biuret in sheep. J. Agr. Sci. Camb. 75:231.

Schroder, H. H. E. and F. M. C. Gilchrist. 1969. Adaptation of the bovine ruminal flora to biuret. J. Agr. Sci. Camb. 72:1.

Tiwari, A. D., F. N. Owens, and U. S. Garrigus. 1973a. Metabolic pathway of biuret degradation and formation of a biuret-complex in the rumen. J. Anim. Sci. 37:1390-1395.

Tiwari, A. D., F. N. Owens, and U. S. Garrigus. 1973b. Metabolism of biuret by ruminants: *In vivo* and *in vitro* studies and the role of protozoa in biuretolysis. J. Anim. Sci. 37:1396-1402.

Waite, R., M. E. Castle, J. N. Watson and A. D. Drysdale, 1968. Biuret and urea in concentrates for milking cows. J. Dairy Res. 35:191.