Protein Nutrition and Fertility in Cattle

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1. SUMMARY

The objectives of the project were to determine the effects of dietary crude protein intake, on blood concentrations of ammonia and urea and on fertility, and on the possible biological mechanisms involved. Heifers were used in all experiments in order to avoid any confounding effects of lactation and, or, negative energy balance. The results are summarised as follows.

- Blood urea and ammonia increased linearly with increases in dietary urea. Fermentable carbohydrate, in the form of molassed sugar beet pulp (MSBP), was more effective than barley in reducing blood urea concentration. Plasma ammonia concentrations were more variable but MSBP was again more effective than barley in reducing these.

- Embryo survival rate was not affected by either dietary crude protein (CP) or fermentable energy intake irrespective of whether animals were fed a silage diet supplemented with feed grade urea or were grazing pasture with a high crude protein content. Despite generating systemic urea concentrations of up to 25 mmol/L and systemic ammonia concentrations of up to 242 µmol/L no significant relationship between these parameters and embryo survival rate was established.

- Consistent with these results on embryo survival, elevated blood urea or ammonia had little effect on the biochemical composition of oviduct fluid, the environment of the early developing embryo.

- It can be concluded that elevations in systemic concentrations of ammonia or urea *per se*, particularly of the magnitude observed under normal feeding conditions, are unlikely to impair embryo survival in cattle as a consequence of disruptions to the oviductal environment.

2. GENERAL INTRODUCTION

Intensive genetic selection for increased milk production, coupled with increased dry matter intake, has led to significant improvements in cow milk yield in recent decades, but there has been a decline in fertility. While the cause of the reduced fertility is probably multifactorial, a high intake of crude protein and the associated elevations in blood concentrations of ammonia and urea have been identified as possible contributors to the lower fertility recorded in some studies, though the published results are equivocal. In Ireland, seasonal milk production systems rely on maintaining high dry matter (DM) intake of pasture that is rich in nitrogen concentration. Such pasture typically has a concentration of crude protein (CP) in excess of 200g/kg DM, mostly in the form of rapidly rumen degradable protein (RDP). This high RDP is associated with reductions in soluble carbohydrate content of grass and this may further elevate systemic concentrations of ammonia and urea.

Objective

The overall objective of the project was to determine the effect of crude protein intake on conception rate. This involved four separate studies, the results of which are summarised in this report.

Study 1: Effect of rumen degradable protein and fermentable energy intake on systemic concentrations of urea and ammonia in cattle.

Objective

To measure the effect of increasing levels of dietary rumen degradable protein and dietary fermentable energy on systemic concentrations of ammonia and urea.

Materials and Methods

Experiment 1: Eighteen nulliparous beef heifers were individually fed grass silage to appetite. Feed grade urea (46%N) was used to increase the RDP fraction of the diet and the heifers were randomly assigned to one of four urea treatments as follows: Group 1 Control, 0.0g urea (n=5); Group 2, + 80g urea (n=5); Group 3, + 160g urea (n=4); Group 4, + 240g urea (n=4). Blood samples were taken at frequent intervals for measurement of plasma urea and ammonia.

Experiment 2 : A total of 80 nulliparous beef heifers were used in this experiment. Arising from the results of experiment 1, animals were fed the same grass silage based diet supplemented with either 0 (0U) or 240 (240U) g of dietary urea. Animals on each level of urea were then randomly supplemented with 1.5 or 3.0 kg of either rolled barley (B) or molassed sugar beet pulp (P), or received no supplementation (control), resulting in 10 dietary treatments (n = 8 heifers/treatment).

Results

The effect of dietary urea level on plasma urea and ammonia concentrations (experiment 1) is shown in Table 1. Both plasma urea and ammonia concentrations increased linearly ($P<0.05$) with increases in dietary urea. The effect of dietary fermentable carbohydrate level on systemic urea concentration (experiment 2) is shown in Figure 1.

Table 1. The effect of dietary urea on plasma urea and ammonia (mean ± s.e.)

Dietary urea (g/day)	urea mmol/L	ammonia mmol/L
0	3.97 ± 0.13	0.58 ± 0.05
80	4.97 ± 0.15	0.63 ± 0.05
160	6.67 ± 0.17	1.26 ± 0.09
240	7.52 ± 0.18	1.23 ± 0.07

MSBP at either 1.5 or 3.0 kg was more effective than barley at lowering systemic urea concentrations at all sample times in animals fed 240g urea. There was no significant effect of carbohydrate treatment on systemic ammonia in animals fed 0g urea, though 3.0 kg MSBP tended to cause a greater reduction than the other treatments.

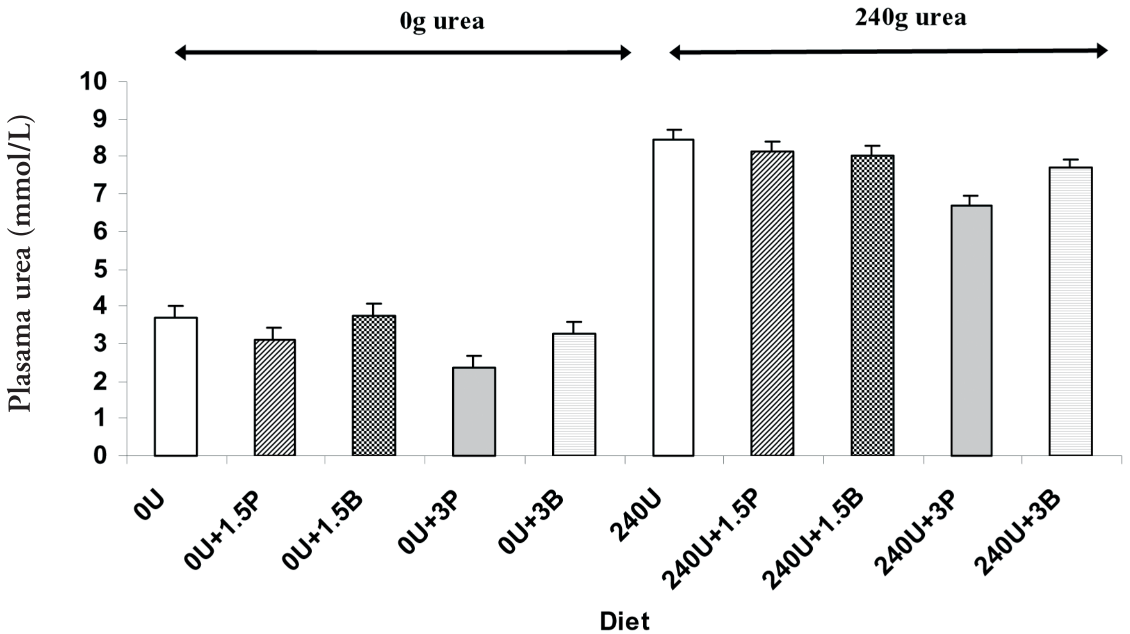

Figure 1 The effect of dietary urea and source (Barley;B v MSBP;P) and level (1.5 kg v 3.0 kg) of carbohydrate on plasma concentrations of urea.

Discussion

The main objective was to develop a dietary model to consistently elevate and maintain systemic concentrations of ammonia and urea for use in studies on reproductive performance (Studies 2 & 3 of this report). Both systemic urea and ammonia concentrations increased linearly with increases in dietary urea. Fermentable carbohydrate, in the form of MSBP, was more effective than barley in reducing plasma urea concentration in animals fed 240 g urea day $^{-1}$. Plasma ammonia concentrations were more variable but MSBP tended to be more effective than barley in reducing these also. The mean systemic urea concentrations achieved on the high urea diet (7.5 + 0.18 mmol/L) is higher than the threshold systemic urea concentrations of 5.7 and 6.8 mmol urea/l that have been published for Holstein heifers and cows, respectively, above which significant reductions in pregnancy rates have been reported. The systemic urea and ammonia concentrations achieved in the study summarised here allowed an accurate assessment to be made of the impact of elevated systemic concentrations of both ammonia and urea on embryo survival rate in the later studies.

Study 2: Effect of rumen degradable protein with or without fermentable energy supplementation on blood metabolites and embryo survival in cattle: indoor feeding study.

Objective

To examine the effect of feeding a high rumen degradable protein (RDP) diet, with or without supplemental fermentable energy, on blood metabolite concentrations and on embryo survival rate in heifers.

Materials and Methods

Animals and experimental design

A total of 162 nulliparous beef heifers were used in this study. The animals were randomly assigned to a 2 x 2 factorial designed experiment where grass silage based diets, fed indoors, were supplemented with either zero (0U) or 240 (240U) grams of feed grade urea and with either zero (0P) or 3 (3P) kg DM of molassed sugar beet pulp (MSBP) per day. Heifers were oestrous synchronised and were artificially inseminated by one operator using semen from one bull. Heifers were allowed a 10-day transition period to become accustomed to their respective diets before the commencement of the experimental period. Heifers remained on their respective diets until day 40 after artificial insemination. Blood samples were collected at appropriate times to measure metabolites and progesterone.

Results

Dietary treatment and plasma metabolite and progesterone concentration

The effect of dietary treatment on plasma concentration of metabolites and progesterone is shown in Table 2. Animals receiving the dietary urea supplement had higher plasma concentrations of urea and ammonia ($P < 0.001$). MSBP supplementation reduced plasma concentrations of urea in animals fed the high urea diet ($P < 0.001$) and also in the animals that did not receive urea ($P < 0.001$). Plasma concentration of ammonia was not affected by MSBP supplementation ($P > 0.05$) and there was no interaction between urea treatment and MSBP supplementation on plasma concentration of ammonia ($P > 0.05$).

Insulin was not affected by either dietary urea or MSBP treatment ($P > 0.05$). The effect of dietary treatment on plasma concentration of progesterone is shown in Table 2. There was no effect of either urea or MSBP treatment on plasma concentrations of progesterone ($P > 0.05$).

Plasma urea was linearly related to plasma concentration of ammonia and the relationship is described by the equation: Urea (mmol/L) = 5.26 + 0.032(mmol/L ammonia) ($P < 0.001$; $R^2=0.26$). There was no relationship between plasma concentrations of urea or ammonia and plasma concentrations of glucose, insulin or progesterone ($P > 0.05$).

Dietary treatment and embryo survival

Embryo survival rate (Table 2) was not affected by dietary urea or by MSBP supplementation ($P > 0.05$). There was no difference ($P > 0.05$), within treatment, in embryo survival rate recorded at 30 and 40 days after AI. There was no relationship, on any sample day or on all days combined, between plasma concentrations of ammonia, urea, insulin or glucose and embryo survival rate ($P > 0.05$). Embryo survival rate within interquartile concentrations of plasma urea on day 7 after AI is shown in Figure 2. There was no relationship between plasma concentrations of progesterone measured on day 7 of the pre- or days 7 and 14 of the post-AI oestrous cycles and subsequent embryo survival rate ($P > 0.05$).

Table 2. The effect of dietary treatment on mean plasma urea, ammonia, glucose, insulin and progesterone concentrations and on embryo survival

Variable	Urea		Max s.e.	Supplement		Max s.e.	Main effects		
	0U	240U		0P	3P		Urea	Supplement	Urea x Supplement
Urea (mmol/l)	4.03	9.15	0.214	7.16	6.01	0.217	***	***	
Ammonia (µmol/l)	21.83	79.06	4.826	51.63	49.56	4.915	***		
Glucose (mmol/l)	3.98	4.18	0.042	4.11	4.04	0.043			
Insulin (µIU/ml)	15.97	18.40	1.293	17.18	17.20	1.323			
Progesterone (ng/ml)	7.70	8.61	0.425	8.43	7.88	0.431			
Total heifers	87	75		83	79				
Total Pregnant	56	45		57	44				
Embryo survival	0.64	0.60		0.69	0.56				

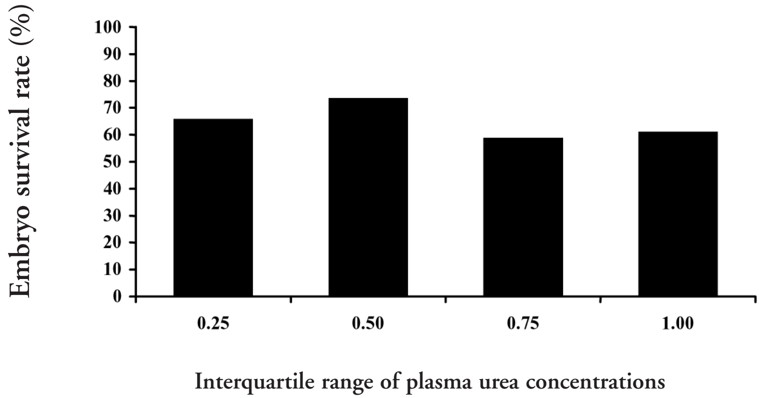

Figure 2. Embryo survival rate within quartile concentrations of plasma urea on day 7 after AI. Plasma urea concentrations within each quartile are: 1.52 to 3.70, 3.701 to 5.50, 5.501 to 9.07 and >9.071 mmol/l for the lowest to the highest quartile of animals.

Discussion

Embryo survival rate averaged 62% across all dietary treatments and was not affected by either CP or fermentable carbohydrate intake. The results clearly show that high dietary RDP increased systemic ammonia and urea while supplementation with fermentable carbohydrate decreased urea, but did not significantly alter systemic ammonia. Despite generating systemic urea concentrations up to 25 mmol/L and systemic ammonia concentrations up to 242 μmol/L, embryo survival rate was not compromised. To our knowledge, this is the first study to report normal *in vivo* embryo survival simultaneous with such elevated systemic concentrations of urea. Supplementation with fermentable energy (3 kg MSBP) significantly reduced systemic urea but not systemic ammonia. However, as embryo survival rate was not affected by either ammonia or urea, predictably it was not affected by the addition of the fermentable energy.

Neither dietary CP nor systemic concentrations of ammonia or urea were associated with systemic progesterone, either in the pre- or post-AI oestrous cycles, consistent with the results of study 3 of this report. Systemic glucose was not affected by CP intake and was not related to systemic urea or ammonia, consistent with other reports for dairy cows. There was no evidence of an association between systemic glucose and embryo survival. However, the variation in blood concentrations of glucose recorded was quite small and the range may not have been sufficiently large to demonstrate any relationship with embryo viability. As in the case of glucose, there is variability among reports documenting the relationship between protein intake and systemic insulin. In this study, we found no relationship between dietary treatment and systemic concentrations of insulin nor was insulin related to embryo survival.

Study 3: Effect of pasture crude protein with or without fermentable energy supplementation on blood metabolites and embryo survival in cattle: outdoor grazing study.

Objective

To determine the effects of grazing pastures either low or high in crude protein concentration and, supplementation with a readily fermentable energy source on systemic concentrations of ammonia, urea, insulin, glucose and progesterone and on embryo development and survival.

Materials and Methods

Oestrus synchronised, crossbred heifers (n=175) were randomly assigned to one of four pasture-based dietary treatments in a 2 x 2 factorial study carried out over two years. Animals were randomly allocated to either high (85 kg N / Ha; HN) or low (0.0 kg N / Ha; LN) nitrogen fertilized pastures and within pasture treatments were then randomly allocated to receive either 0 or 3.0 kg (+3P) DM of molassed sugar-beet pulp (MSBP) per head per day. Blood concentrations of ammonia, urea, insulin, glucose and progesterone were measured at appropriate times. Heifers were artificially inseminated (AI) and pregnancy diagnosis was carried out by ultrasonography 30 days after AI. Embryo survival was determined initially by ultrasonography on Day 30 after AI. Subgroups of animals diagnosed pregnant, in Year 1, n=38 and Year 2, n=38 were randomly assigned within treatment to slaughter and conceptus recovery at 40 days after AI.

Results

Herbage nutrient composition
The effect of pasture nitrogen treatment on nutrient composition of the herbage is shown in Table 3. There was no effect of year on any of the pasture variables measured. The HN pastures had a higher herbage CP concentration than LN pastures. The estimated ME concentration of the herbage was also higher in HN pastures. Herbage DMD was unaffected

by N treatment. Water-soluble carbohydrate was lower in the HN than in the LN pastures (Table 3).

Dietary treatment and blood metabolites and progesterone

The relationship between pasture CP, MSBP supplementation and plasma urea concentration for all sample days is presented in Figure 3. Plasma concentration of urea was linearly related to pasture crude protein concentration and was the strongest determinant of plasma concentration of urea, regardless of supplementation. The relationship between pasture CP, MSBP supplementation and plasma ammonia concentration for all sample days is shown in Figure 4. There was a weak but positive linear relationship between pasture CP concentration and plasma ammonia concentration in the unsupplemented animals and no relationship in the supplemented animals. The effect of dietary treatment on blood insulin and glucose concentrations is shown in Table 4. Blood glucose concentrations were not affected by pasture N treatment and were not related to pasture CP concentration. Glucose concentrations were higher in the animals supplemented with MSBP. Blood insulin was not affected by pasture N treatment or related to pasture concentration of CP, nor was insulin affected by supplementation with MSBP. There was no relationship between blood urea or ammonia and blood glucose or insulin.

The effect of diet on plasma concentrations of progesterone is presented in Table 4. Progesterone was not affected by either pasture N treatment or by supplementation with MSBP. There was no relationship between any of the metabolites measured and blood progesterone.

Dietary treatment and embryo survival and development

Embryo survival rate measured at Day 30 of gestation was similar for both years and the data were combined for analysis. The effect of dietary treatment on embryo survival rate is shown in Table 4. Embryo survival was high overall (71%) and not affected by pasture nitrogen treatment, pasture CP or WSC concentration or by supplementation with MSBP. The effect of dietary treatment on conceptus development at Day 40 of gestation is presented in Table 4.

There was no treatment effect on any of the conceptus measurements. There was no embryo loss between pregnancy diagnosis carried out at Day 30 and again at Day 40 of gestation.

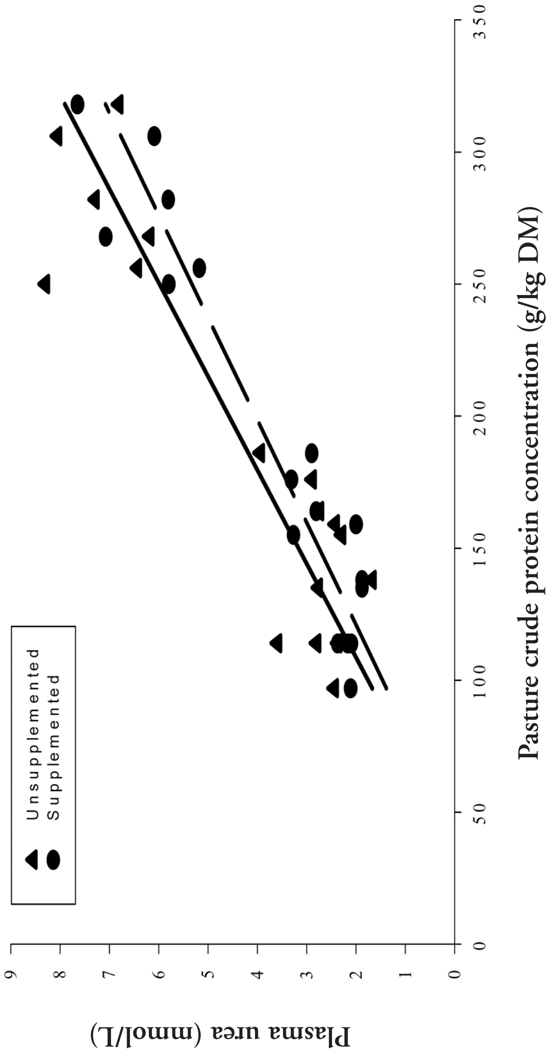

Figure 3. Relationship between pasture crude protein concentration, MSBP supplementation and plasma urea. Mean concentrations of urea for supplemented and unsupplemented animals are presented. Broken and solid lines represent regression lines for supplemented and unsupplemented animals, respectively.

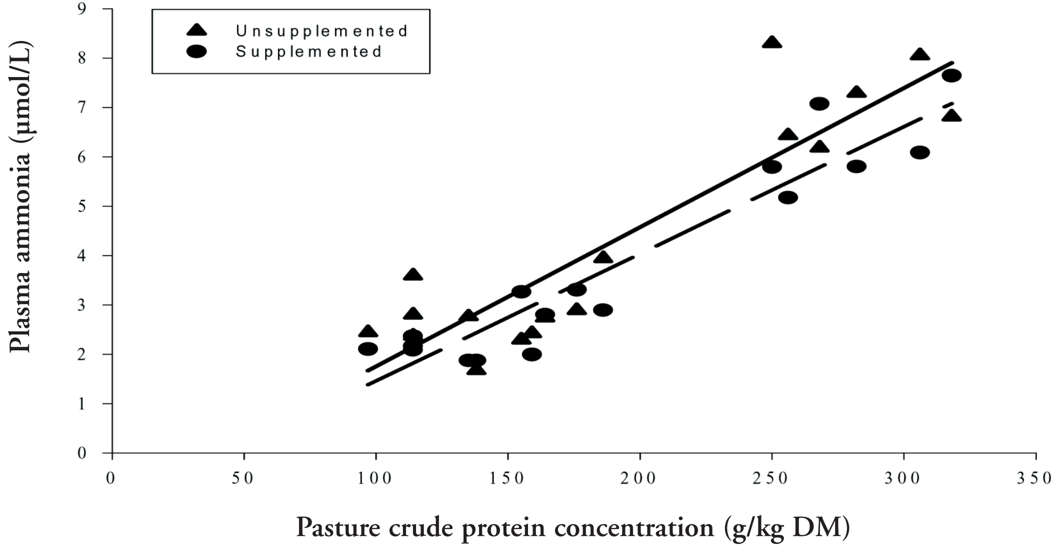

Figure 4. Relationship between pasture crude protein concentration, MSBP supplementation and plasma ammonia. Mean ammonia concentrations for supplemented and unsupplemented animals are presented and the respective pasture CP concentration on the day of blood sampling. Broken and solid lines represent regression lines for supplemented and unsupplemented animals respectively.

Table 3. Effect of nitrogen treatment on nutrient composition of herbage				
	HN	LN	Max s.e.	Significance
CP (g/kg DM)	231.77	127.74	11.832	***
Estimated ME (Mj/kg DM)	10.96	10.46	0.092	**
DMD (g/kg)	750.44	730.12	29.985	
WSC (g/kg DM)	25.09	33.40	0.943	**

Progesterone and metabolite concentrations and embryo survival

There were no relationships between plasma concentrations of ammonia, urea, insulin or glucose and embryo survival rate. Plasma urea concentrations were categorized and the relationship between embryo survival rate within categories is presented in Figure 5. Progesterone concentration was higher on Day 7 post-AI in heifers that became pregnant than in those that failed to become pregnant. Embryo survival within interquartile ranges of plasma progesterone on day 7 after AI is presented in Figure 6.

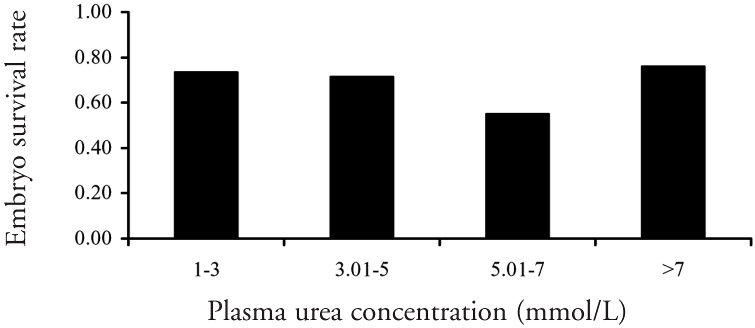

Figure 5. Relationships between mean plasma concentration of urea and embryo survival rate.

Table 4. The effect of pasture N treatment and fermentable carbohydrate supplementation on mean plasma concentrations of metabolites and progesterone and on embryo survival and development in heifers

Variable	N treatment			Supplement			Main effects	
	High	Low	Max s.e.	0P	3P	Max s.e.	Nitrogen	MSBP
Urea (mmol/L)	6.64	2.81	0.162	5.07	4.37	0.166	***	**
Ammonia (mmol/L)	25.19	17.88	1.333	22.75	20.35	1.328	*	
Insulin (mIU/ml)	16.01	14.48	0.862	14.97	15.53	0.868		
Glucose (mmol/L)	3.99	3.95	0.044	3.91	4.04	0.044		*
Progesterone (ng/ml)	8.79	8.61	0.326	8.91	8.50	0.330		
Embryos								
Embryo survival	0.68	0.74		0.65	0.76			
Embryo Wt. (g)	1.28	1.22	0.039	1.23	1.27	0.042		
Crown-Rump (mm)	32.28	30.90	0.740	31.40	31.78	0.758		
Amn. Vol. (ml)	3.12	2.93	0.162	2.95	3.10	0.164		
Allan. Vol. (ml)	108.03	104.57	3.840	106.80	105.80	3.811		

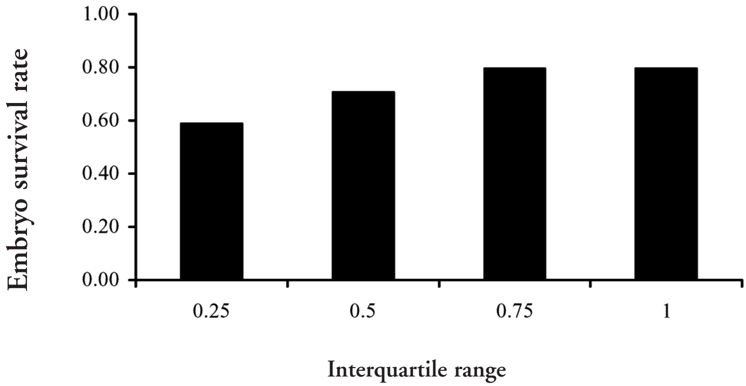

Figure 6. Embryo survival rate within quartile ranges of progesterone

Discussion

The results presented here clearly show that intake of pasture with a high crude protein (CP) concentration significantly increased systemic ammonia and urea in heifers and that supplementation with fermentable carbohydrate decreased plasma urea, with a non-significant decrease in plasma ammonia. Embryo survival rate across all dietary treatments was high, (mean = 71%) within a range of 65 to 82% and was not affected by either CP or fermentable carbohydrate intake or by the systemic concentrations of either ammonia or urea. Despite intakes of pasture with a mean CP concentration of over 230 g/kg DM there was no effect on embryo survival rate or development. We are not aware of similar pasture-based studies that allow direct comparison with our results. This is the first reported study where pasture was manipulated to achieve high and low CP concentrations in order to determine the effect on systemic concentrations of ammonia and urea and the effect of these in turn on embryo survival and on various measurements of embryo development.

Study 4: Effect of elevated systemic concentrations of ammonia and urea on the biochemical composition of oviduct fluid in cattle

Objective

To establish the effects of elevated systemic concentrations of ammonia and urea on the biochemical composition of oviduct fluid, the environment in which the early embryo develops, in order to gain a better understanding of the possible mechanisms involved in putative protein mediated infertility in cattle.

Materials and Methods

Twenty-five nulliparous crossbred beef heifers were used in this study. In order to elevate systemic concentrations, ammonia and urea were infused into the jugular veneous system. Heifers were randomly allocated to one of three infusion treatments; Saline (S; Control), Urea (U) or ammonium chloride (A) carried out on one of two days (Days 2 or 8 after oestrus; oestrus = Day 0) of the oestrous cycle in a 2 x 3 factorial designed study as follows. Oviduct fluid recovery was carried out during mid ventral laparotomy under general anesthesia. The volume of fluid was recorded and the fluid was then centrifuged, snap frozen in liquid nitrogen (-196°C) and stored at -80° C until the analyses were carried out. Blood was sampled for determination of plasma concentrations of ammonia, urea, glucose, insulin progesterone, lactate, calcium, potassium, magnesium and sodium. Plasma and oviduct fluid concentrations of urea, ammonia, glucose and lactate were determined using an autoanalyser (COBAS Mira). Plasma insulin and progesterone were measured by RIA. Cation concentrations were measured using a Dionex 2000 ion chromatograph fitted with a CS12 cation exchange column and a self-regenerating cation suppressor operated in auto-regeneration mode.

Results

The effects of infusion treatment on plasma concentrations of insulin, glucose, ammonia and urea is presented in Figure 7. The effect of infusion treatment on oviduct fluid metabolite concentrations is presented in Table 5. The plasma and oviduct concentrations of urea, ammonia, glucose, lactate and magnesium were all positively related. There was no association between plasma and oviduct concentrations of calcium, potassium or sodium. Plasma calcium was positively associated with oviductal concentrations of glucose ($P < 0.05$). Oviduct calcium concentration was positively associated ($P < 0.05$) with plasma magnesium and sodium but negatively associated ($P < 0.001$) with plasma urea. There was no relationship between the plasma concentration of progesterone measured on either Day 2 or Day 8 and the plasma or oviductal concentrations of any metabolite or ion measured ($P > 0.05$).

Table 5. Effect of infusion treatment and day of oestrous cycle on the biochemical composition of oviduct fluid (± s.e.)

	Treatment			Day		Treatment x Day	Treatment	Day
	Control	Urea	NH4Cl	2	8			
Urea (mmol/l)	5.91 ± 0.350	14.01 ± 0.350	8.27 ± 0.369	9.55 ± 0.291	9.25 ± 0.291		***	
Ammonia (µmol/l)	451.94 ± 174.050	416.54 ± 119.770	1187.08 ± 118.059	815.38 ± 96.170	555.19 ± 105.610		***	p=0.08
Glucose (mmol/l)	2.95 ± 0.509	2.12 ± 0.468	2.83 ± 0.493	2.17 ± 0.389	3.10 ± 0.412			
Lactate (mmol/l)	10.02 ± 1.806	4.99 ± 1.806	7.98 ± 1.904	6.36 ± 1.502	8.98 ± 1.502			
K+ (mM)	3.48 ± 0.661	3.26 ± 0.559	3.84 ± 0.559	3.78 ± 0.441	3.26 ± 1.527			
Na+ (mM)	185.00 ± 24.948	179.85 ± 21.058	162.36 ± 21.085	147.47 ± 16.632	203.997 ± 19.879			*
Mg2+ (mM)	0.40 ± 0.060	0.33 ± 0.050	0.35 ± 0.050	0.36 ± 0.040	0.35 ± 0.047			
Ca2+ (mM)	0.80 ± 0.078	0.51 ± 0.066	0.68 ± 0.066	0.72 ± 0.052	0.61 ± 0.062		*	

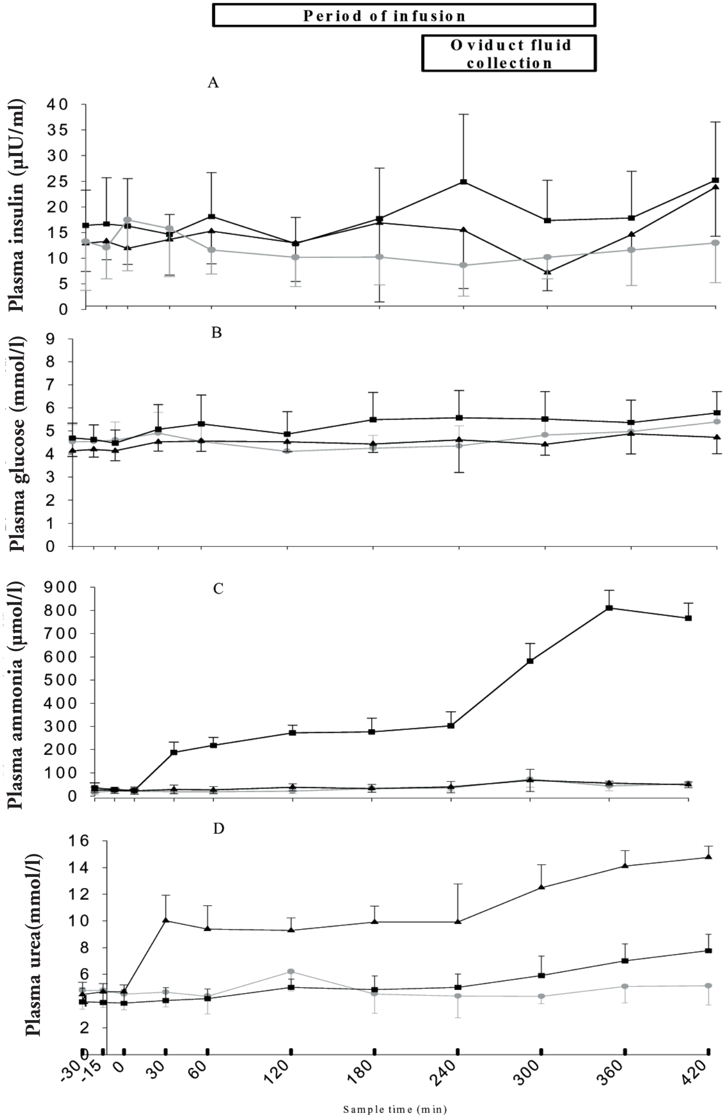

Figure 7. Effect of infusion treatment on mean plasma concentrations of insulin (A), glucose (B), ammonia (C) and urea (D).

Discussion

The results presented here are the first to report the relationship between elevated systemic concentrations of ammonia and urea and the composition of oviduct fluid in cattle. Furthermore, the infusion protocol employed, enabled an examination of the discrete effects of ammonia and urea on the metabolite and ionic composition of oviductal fluid. The infusion of ammonium chloride and of urea into the systemic circulation significantly elevated the oviductal concentrations of ammonia and urea respectively. While the oviductal concentration of urea was strongly reflective of the systemic concentrations, the relationship between systemic and oviductal concentrations of ammonia, although still highly significant, was somewhat weaker, with ammonia being higher in the oviduct than blood plasma. Despite generating systemic and oviductal concentrations of ammonia in excess of 820 µmol/l and 1180 µmol/l respectively and of urea of up to 14 mmol/l, with the exception of calcium, we found no effect of elevating systemic ammonia or urea and any of the other components of oviduct fluid measured. Furthermore, with the exception of sodium we found no difference between days 2 or 8 of the oestrous cycle, in the concentrations of any of the constituents of oviduct fluid measured, nor was there any interaction between treatment and day of collection on the concentration of any constituent measured.

These results are consistent with the results of studies 2 and 3 that showed no effect of elevated systemic urea and, or, ammonia on embryo survival rate.

3. OVERALL CONCLUSIONS

Systemic urea and ammonia concentrations increase linearly with increases in dietary urea. Fermentable carbohydrate, in the form of MSBP, is more effective than barley in reducing plasma urea concentration. Embryo survival rate is not affected by either CP or fermentable carbohydrate intake, irrespective of whether animals are fed a silage diet supplemented with feed grade urea or are grazing pasture with a high crude protein content. Furthermore, despite generating systemic urea concentrations up to 25 mmol/L and systemic ammonia concentrations up to 242 µmol/L no significant relationship between these parameters and embryo survival is evident.

Neither dietary CP nor systemic concentrations of ammonia or urea are significantly associated with systemic progesterone, though a positive relationship between progesterone on Day 7 after AI and subsequent embryo survival rate is evident. Systemic glucose and insulin are not affected by CP intake and not related to systemic urea or ammonia and do not appear to affect embryo survival rate. There is no relationship between elevated systemic concentrations of ammonia and urea and the biochemical composition of oviduct fluid in cattle, with the exception of calcium.

From these four studies it can be concluded that elevations in systemic concentrations of ammonia or urea *per se*, particularly of the magnitude observed under normal feeding conditions, are unlikely to impair embryo survival in cattle as a consequence of disruptions to the oviductal environment.

4. PUBLICATIONS ARISING FROM THIS PROJECT

1. Kenny, D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 1999. The effect of rumen degradable protein (RDP) and fermentable carbohydrate intake on systemic concentrations of urea and ammonia in cattle. In Proceedings of the Agricultural Research Forum. pp. 37-38, and the Irish Journal of Agricultural and Food Research. pp. 270-271 (Abstr.).

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3. Kenny, D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2000. Protein and fermentable carbohydrate intake: Embryo survival and development in cattle. Theriogenology. 53 (1): 284 (Abstr.).

4. Kenny D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2001. Protein nutrition and fertility in cattle. In Proceedings of the Agricultural Research Forum 2001 p. 41.

5. Kenny D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2002. Effect of elevated systemic concentrations of ammonia and urea on the biochemical composition of oviduct fluid in cattle. In Proceedings of the Agricultural Research Forum 2001.

6. Kenny, D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2001. The effect of crude protein and fermentable carbohydrate intake on blood metabolite concentrations and fertility in beef heifers. In Fertility in the High Producing Dairy Cow, British Society of Animal Science, Occasional Publication No. 26 (2) 375-379.

7. Kenny D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2001. The effect of crude protein and fermentable carbohydrate intake on blood metabolite concentrations and fertility in beef heifers. In Fertility in the High Producing Dairy Cow, British Society of Animal Science, Occasional Publication No. 26 (2) 375-379.

8. Kenny D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2001. Effect of rumen degradable protein with or without fermentable carbohydrate supplementation on systemic concentrations of ammonia and urea in cattle. (in press: Irish J. Agr. and Food Res).

9. Kenny D.A., Boland, M.P., Diskin, M.G. and Sreenan J.M. 2002. Effect of rumen degradable protein with or without fermentable carbohydrate supplementation on blood metabolites and embryo survival in cattle. Animal Science (in press :publication, June 2002).

10. Kenny, D.A., Humpherson, P.G., Leese, H.J., Morris, D.G., Diskin, M.G. Tomos, A.D. and Sreenan, J.M. 2002. The effect of elevated systemic concentrations of ammonia and urea on the metabolite and ionic composition of oviduct fluid in cattle. Biology of Reproduction (in press:Expected publication date May 2002).