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Reproductive performance of northern Australia beef herds. 5. Factors influencing risk of non-pregnancy

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ABSTRACT

Context. Sound reproductive efficiency is a key determinant for the overall productivity and profitability of a beef breeding business. Failure of a cow to conceive results in either culling or the cost of carrying non-pregnant animals. **Aims.** This study aimed to determine and quantify the major factors associated with non-pregnancy in commercial beef breeding herds of northern Australia. **Methods.** A prospective population-based epidemiological study of the likelihood of non-pregnancy in cows after an annual mating in northern Australian beef breeding cows used data from 73 herds from four broad country types and 62 323 animal years; approximately 80 property-, management-group- and cow-level risk factors were considered. A multivariable model building process was employed to scrutinise the resulting dataset, so as to identify what herd management practices, nutritional, environmental, and individual cow factors were associated with non-pregnancy and estimate their magnitude of effect. **Key results.** Non-pregnancy was disproportionately high in the Northern Forest (32.1%), compared with the Northern Downs, Central Forest and Southern Forest where it was 17.1%, 16.0% and 13.2% respectively. Time of expected calving had the largest impact on occurrence of non-pregnancy. Parity also had a significant influence, with first-lactation cows typically having 5–12% higher non-pregnancy than did mature cows. Non-pregnancy decreased with an increasing body condition score at the branding/weaning muster for lactating cows. The difference in non-pregnancy when comparing availability of wet-season pasture phosphorus content and digestibility of pasture during the dry season was 13.2 and 10.2 percentage points respectively. **Conclusions.** This study demonstrated the substantial impact environment, herd management practices, nutrition and disease factors can have on the reproductive performance of females. **Implications.** To optimise the efficiency of females (through reducing the occurrence of non-pregnancy) under commercial conditions in northern Australia, production systems should support beef herds calving early in the production year, being in at least moderate body condition and having access to more digestible pastures that address the nutritional requirements for both protein and phosphorus. This indicates focus for management, especially in the Northern Forest where the likelihood of non-pregnancy was highest.

Keywords: agriculture, beef cattle, conception, fertility, north Australia, pregnancy, reproduction, tropics.

Introduction

Breeding is a major production activity for most beef cattle businesses in northern Australia (Bortolussi *et al.* 2005*a*). Therefore, reproductive performance is an important driver of the profitability and, thus, viability of beef breeding operations. Reproduction rate in northern Australia is typically low, with post-partum anoestrus being the main factor identified to limit production (Entwistle 1983).

Branding rates in northern Australia were recently reported as averaging 71% for the 10 years ending in 2011–12 (Martin *et al.* 2013). However, Australian Bureau of Agricultural and Resource Economics and Sciences' annual Australian Agricultural and Grazing Industries Survey data suggest differences in branding rates in the order of

20–30 percentage points among regions (Gleeson *et al*. 2012). Reproductive performance for important beef cattle production areas of northern Australia has previously been reviewed, with large variation within and among regions being highlighted by Entwistle (1983), Holroyd *et al*. (1989), Hasker (2000) and Burns *et al*. (2010). These studies highlighted that if causal factors of variation were identified, understood and quantified, targeted remedial management may moderate their impacts, increasing reproductive performance.

A number of property-, herd- and animal-level factors has been shown to influence annual pregnancy rates of beef breeding herds and include age, nutrition, time and duration of lactation and time of calving (Hasker 2000). The impact of most of these factors has been established in studies that have either not partitioned or controlled the effects of other extraneous factors and/or were conducted in non-commercial situations such as research stations. There are no known studies that have simultaneously assessed the relative importance of these factors in commercial beef herds of northern Australia. Therefore, it is not known whether the magnitude of effects drawn from the results of the more intensive controlled studies can be reasonably applied to the broader commercial beef breeding population of northern Australia.

A large prospective population-based epidemiological study was established to describe reproductive performance and productivity and identify the important determinants of these outcomes in commercial beef breeding herds across the major beef producing regions of Queensland, Northern Territory and Western Australia (McCosker 2016). This paper reports on the major factors associated with cows not becoming pregnant after a year in commercial northern Australian beef cows and quantifies the impact of factors determined as having significant influence on pregnancy rate.

Materials and methods

Study design and population

Data were collected as described in McCosker *et al.* (2022). In brief, a prospective population-based epidemiological study was conducted in which 78 beef breeding herds located across each of the major beef breeding regions of Queensland, the Northern Territory and northern Western Australia were monitored between 2008 and 2011 (Fig. 1). Collaborating beef businesses for this study were identified using several recruitment methods, including self-nomination, and were enrolled if herds and their management were considered representative and good cooperation was highly likely to be achieved. Participating properties were regionalised to country types as described in McCosker *et al.* (2022),

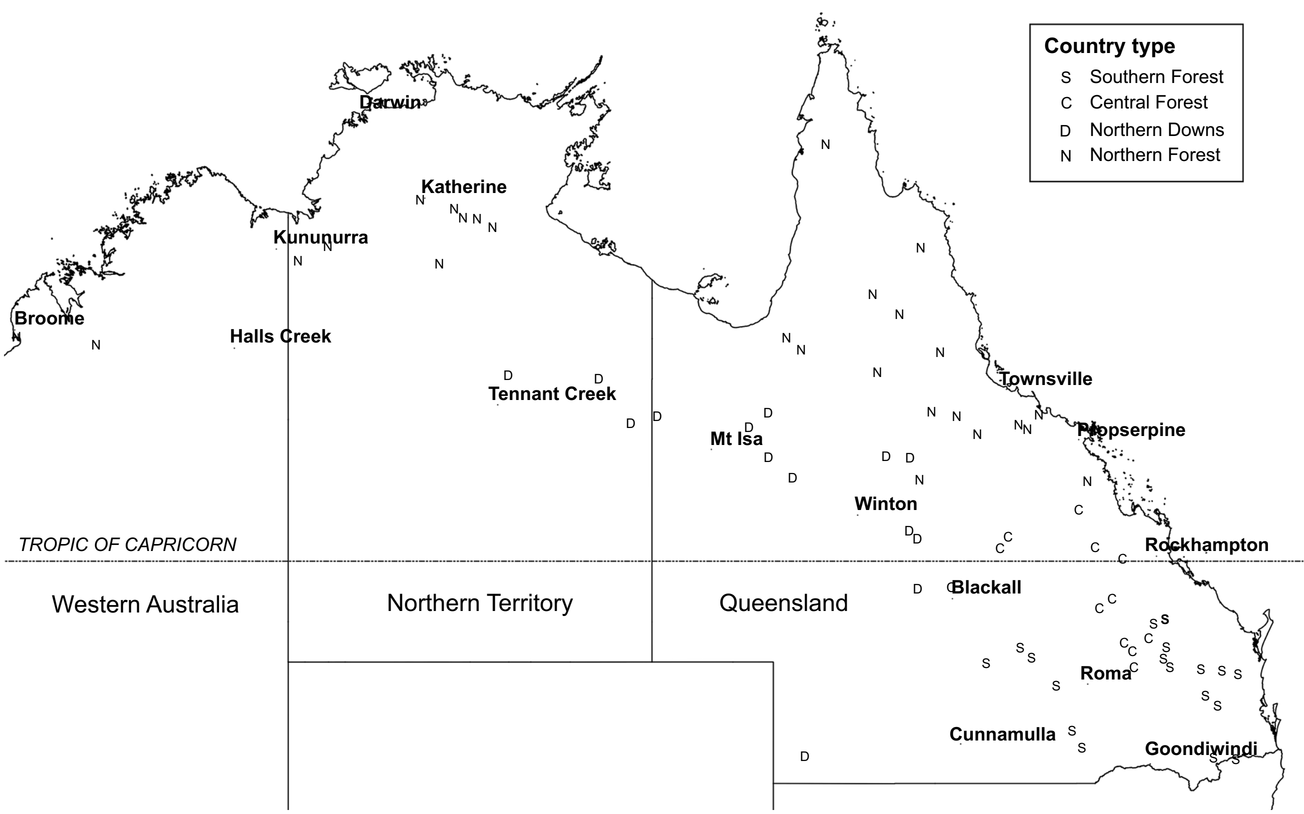

Fig. 1. Location of cooperating properties by country type.

which defined the central and south-eastern subtropical regions of Queensland as either Southern Forest or Central Forest, by either being outside or within the northern brigalow belt respectively. Properties located in the northern parts of Queensland, Northern Territory and northern Western Australia were discerned as either Northern Downs (predominantly large treeless black soils plains) or Northern Forest (largely forested with soils of low fertility).

Herds and cattle

This study focussed on pregnancy in cows and not heifers, with each participating business usually enrolling two management groups of females, enabling study of first-lactation, and mature and aged cows. The number of females per management group were mostly within the size of 100 and 500 cows. In groups of females larger than 500 cows, a representative subset of 300 cows was enrolled.

The monitoring of animal performance was supported by each individual animal data identified using a National Livestock Identification System (NLIS) ear tag and the use of commercially available crush-side animal data recording systems (e.g. BeefLink™, AgInfoLink). A visual management tag displaying a unique five-digit identification number was also attached to the animal in most situations, to identify that the animal was enrolled into the study and to establish data linkages to historical performance data in the event of the NLIS tag requiring replacing due to either being missing or not functioning.

Animals were described at their first muster with information on estimated *Bos indicus* content, year of weaning (year brand) and hip height (Fordyce *et al.* 2013) being recorded. Performance and explanatory data were recorded twice a year for each cow enrolled into the project, at the main branding or weaning muster and at the pregnancy diagnosis muster, which, on average, occurred 3.8 months later. At each muster, body condition score (BSC; Gaden *et al.* 2005) and lactation status were visually assessed and recorded. Liveweight of cattle was captured wherever possible and was for approximately 77% of mobs. Pregnancy status was recorded for all cows at or near the last annual weaning muster in June–October. Fetal age was estimated by rectal palpation for all pregnant cows; the mean interval from pregnancy diagnosis to predicted calving was 4.4 months. The estimated month of calving was based on fetal ageing, date of pregnancy diagnosis and an assumed gestation length of 287 days (Casas *et al.* 2011). The animal's status within the herd was recorded as either kept or culled at each muster.

Potential risk factors assessed and laboratorial analysis

Candidate resource (e.g. property area, herd size, average rainfall) and herd management factors (e.g. culling and selection policies, mating management, provision of supplements, weaning and vaccination policies) were derived from data obtained from a face-to-face survey of herd managers at the commencement of the study and have been fully described in the associated paper McCosker *et al.* (2020; Table 1). Paddock factors (area, distances to water) were calculated using the ArcGIS version 9.3 Environmental Systems Research Institute Inc. mapping software program.

Environmental and weather conditions were measured using interpolated data derived using the GPS location of a paddock or homestead (https://www.longpaddock.qld.gov.au/silo/datadrill/index.php; Bureau of Meteorology, Australia, accessed 15 October 2020).

The nutritional quality of the diet selected by the enrolled mobs was assessed as described in McCosker *et al.* (2022). Briefly, faecal samples were collected by herd managers in January, March, May, August and November and analysed using near-infrared reflectance spectroscopy to estimate dry-matter digestibility (DMD) and crude protein (CP; Dixon 2007; Dixon and Coates 2009). The faecal phosphorus concentrations (FecP) of samples were assessed using wet-chemistry techniques (Zarcinas *et al.* 1987). Dietary ME content was estimated from faecal near infrared reflectance spectroscopy (F.NIRS) prediction of DMD, by using equation 1.12A in Freer *et al.* (2007) ($ME = 0.172 \times DMD - 1.707$). In a few instances, sampling coincided with a mustering event and a representative sample was collected per rectum from multiple cows within the mob or a sample was not able to be collected in the scheduled month, so a sample was collected the following month. The predicted dietary attributes (DMD, CP, FecP) were aggregated and summarised to derive several features, including the ratios of DMD:CP and FecP:ME, describing each study mobs' dietary quality during the wet and dry seasons. Established threshold values (8 and 10) for the ratio DMD:CP were used to assess the risk of performance being restricted by insufficient available protein relative to energy (Dixon 2007), whereas the ratio of FecP:ME characterised the availability of dietary P. Threshold indicator values of 390, 420 and 460 for FecP:ME were applied to create risk factors representing the proposed requirements of lactating 400 kg breeders producing 5 L milk/day (Jackson *et al.* 2012).

Deriving the outcome non-pregnancy

Non-pregnancy was defined as whether cows were confirmed pregnant or not in a mating year. In continuously mated herds, conceptions estimated to have occurred after 1 September each year were ascribed to the following annual reproductive cycle. This matched when calves from pregnancies were weaned and contributed to the annual calf crop. There were occasions where non-pregnancy for animals over a year was misclassified but retrospectively ascribed as pregnant during data-checking procedures, which included cross-referencing pregnancy status with subsequent lactation.

Table 1. Cow- and herd-level risk factors for cows at the risk of non-pregnancy that were considered during unconditional assessment and were considered in the multivariable model building process

Herd management	
Property management experience of manager	Culling rate of breeding females
Reported size of the herd	Culling age of breeding females
Size of management group at pregnancy diagnosis size of management group at pregnancy diagnosis	Mating management
Bull selection policy	Botulism vaccination policy
Annual bull management policy	Leptospirosis vaccination policy
	Bulls vaccinated against bovine ephemeral fever
Environment	
Year observed	Cumulative number of days temperature–humidity index exceeded 71 during month of calving
Timing of wet-season onset	Cumulative number of days temperature–humidity index exceeded 79 during month of calving
Wet-season duration	Average temperature–humidity index during month of calving
Cumulative number of days maximum temperature exceeded 32°C during month of calving	
Cumulative number of days maximum temperature exceeded 39°C during month of calving	
Nutrition	
Minimum dry-season biomass	Provision of supplemental nitrogen
Average dry-season crude protein (CP)	Provision of supplemental phosphorus
Average dry-season dry-matter digestibility (DMD)	Average ratio of faecal phosphorus to dietary metabolisable energy during wet season
Average dry-season DMD:CP	Proportion of the paddock grazed which was ≤2.5 km from permanent water around time of calving
Average wet-season CP	
Average wet-season DMD	
Average wet-season DMD:CP ratio	
Animal	
Cow age class	Body condition score (BCS) at the pregnancy diagnosis muster
Percentage *Bos indicus* of heifers and cows	BCS at the branding or weaning muster
Estimated period of calving	BCS change between pregnancy diagnosis and branding or weaning musters
Liveweight at the pregnancy diagnosis muster	
Hip height	

Data management and statistical analyses

Data were managed using a relational database (Microsoft Access 2010 for Windows; Microsoft Corporation, Redmond, WA, USA) and a spreadsheet system (Microsoft Excel 2010 for Windows; Microsoft Corporation). All statistical analyses were performed using the StataIC® (versions 11 and 12 for windows; Stata Corporation, TX, USA) software, with one animal's annual (September–August) pregnancy status as the unit of analysis.

Screening of risk factors (Table 1) for inclusion in the multivariable-model building process was based on associations between potential risk factors and non-pregnancy by using a random-effects logistic regression model with Stata's xtlogit command, fitting herd as a random effect. The overall significance of risk factors was assessed using Wald-test P-values. Risk factors were retained for consideration in the multivariable-model building process if their association with the outcome was significant at $P \leq 0.20$ (Dohoo *et al.* 2009). A liberal P-value was used to reduce the risk of important predictors not being considered in the multivariable-model building process when the effects of some variables become evidence once potential confounding or distorting variables are controlled.

The assumption of linearity of continuous variables in the logit were evaluated by inspecting partial residual graphs following herd-adjusted logistic regression models fitting the continuous variables as the main effect of non-pregnancy using Stata's lpartr command (Hilbe 2009). Continuous variables that appeared to fail the assumption of linearity were categorised into two or more categories. Wherever possible, continuous variables were categorised using established threshold values. However, in some cases, where these were not found to be discriminatory, cut points were determined by changes in the slope of cubic splines fitted to partial residual plots.

Examination of pairwise Spearman correlations was used to identify pairs of risk factors that were highly correlated ($r \geq 0.90$; Dohoo *et al.* 2009). Where pairs of risk factors were highly correlated, one risk factor was selected for inclusion in the multivariable-model building process on the basis of biological plausibility, fewer missing values and Akaike's and Schwarz's Bayesian information criteria estimates. Putative risk factors that had an excessive amount (≥40%) of missing values were not considered for inclusion in the multivariable-model building process.

A multivariable model was built using a backwards elimination process. Commencing with all significant ($P \leq 0.20$) risk factors on screening being added to a starting model, non-significant variables with the highest *P*-value were dropped one at a time. This process was continued until only significant ($P \leq 0.05$) variables remained in an interim model. With the exception of those variables with a high degree of missing values, all risk factors previously eliminated during the model building process were again reconsidered, one at a time, for inclusion into the interim model. The predictor 'country type' was forced into all interim models due to specific interest in the effects of region that were being represented by 'country type'. An appraisal of effects of potential confounding variables was completed by individually including each variable into the candidate model and assessing changes in the measure of association for statistically significant variables. Confounding was considered important when odds ratios for statistically significant variables changed by >20–30% (Dohoo *et al.* 2009) and the variable was included in the final main-effects model. All potential interactions between pairs of risk factors remaining in the interim model were considered one at a time and were retained in the final model if their association was significant ($P \leq 0.05$) and their effects were biologically plausible.

The overall goodness-of-fit of the multivariable model was assessed using Hosmer–Lemshow goodness-of-fit tables and statistics (Hosmer *et al.* 2013). Outliers were identified by an analysis of the residuals and were omitted if they were found to be erroneous or having an undue effect on the model.

Following fitting the final multivariable model, average marginal effects of risk factors were computed using Stata's margins postestimation command. Differences between estimated marginal means across levels of each risk factor or interaction term were estimated and statistically compared using non-linear combinations of estimators and pairwise comparisons respectively.

Population-attributable fractions

Using a logistic regression model fitting only main explanatory factors that were contained in the multivariable model, the population-attributable fractions were estimated using Newson (2010) Stata command punafcc.

Effects of risk factors not contained in the final model

Two risk factors (hip height; average change in liveweight for management group between pregnancy diagnosis and the subsequent branding/weaning muster) did not progress into the multivariable-modelling process because of the occurrence of ≥40% incomplete records. A further six risk factors were not considered in the multivariable-model building process as they were not significant by using the liberal *P*-value of 0.2 and included the following: number of days exceeding 40°C during the estimated month of calving; average dietary CP content during the wet season; provision of supplemental phosphorus (P); mustering inefficiency; age cows are routinely culled; and pestivirus vaccination policy. The effects of these variables were assessed by adding them one at a time to the final multivariable model.

Ethical clearance

The University of Queensland Animal Ethics Committee approved the conduct of the present research per Certificates SVS/756/08/MLA and SVS/729/07/MLA.

Results

Description of study population

The starting dataset contained observations from 73 herds relating to 62 323 animal years from cows deliberately mated to bulls with valid records for pregnancy status. Using a null model, the population-averaged prevalence of non-pregnancy was estimated as 20.4% (95% confidence interval [CI], 17.3–23.6%) of cows per year. Most of the 14 883 observed cases of non-pregnancy were within the Northern Forest (57%) or Northern Downs (22%). The overall likelihood of non-pregnancy in herds for Northern Forest, Northern Downs, Central Forest and Southern Forest was estimated at 32.1% (95% CI, 26.7–37.6%), 17.1% (95% CI, 11.7–22.6%), 16.0% (95% CI, 11.4–20.7%) and 13.2% (95% CI, 9.7–16.8%) respectively. The percentage of variation in non-pregnancy explained by differences at the herd level in the null model was estimated to be 23.9%.

Multivariable-model results

An explanatory multivariable model represented 73% (55) and 55% (32 382) of herds and animal years respectively, that were included in the starting dataset (Tables 2, 3). The remainder of the records had a missing value for at least one of the risk factors contained in the final model. On average, each herd and individual cow contributed information relating to 589 (range 54–5210) and 1.3 (range 1–3) animal years respectively, in the final model. The proportion of total variance explained by the variance among herds was 18.4%. The final multivariable model explained 28.1% of the variance at the property level, estimated using an intercept-only model.

The final model had an acceptable ability to discriminate between those cows that were non-pregnant and those that were not, with 69.9% correct predictions, while the area under the receiver-operating curve was 0.76 (95% CI, 0.76–0.77). Sensitivity was high (>0.90) at low probability cut points (<0.1), while specificity was high (>0.90) at probability cut points >0.5. The fixed part of final multivariable model fitted the data only partially well, with there being fewer cases of non-pregnancy than expected at lower probabilities. The *P*-value for the Hosmer–Lemeshow goodness-of-fit statistic was <0.001, indicating a poor fit. Potential outlier and influential data points were evaluated and an inspection of the covariate values showed that all values were plausible, and, as a result, all observations were retained in the analysis.

The variable representing the reproductive outcome for the previous year interacted both with BCS at the branding/weaning muster and cow age class. Generally, non-pregnancy in lactating cows was 8–15% for those calving in July–September, compared with 25–37% for those calving in February–March (Table 3). However, the effect of BCS was more evident in lactating cows, with similar occurrence of non-pregnancy being observed for different BCS categories ($P < 0.001$; Table 2). The difference in pregnancy between BCS ≤ 2 and BCS = 3 at the branding/weaning muster for cows calving in July–September (about 7–8 months earlier) was 6.0% (95% CI, 2.7–9.3%; $P < 0.01$), which was lower than the 29.8% difference (95% CI, 23.1–36.4%; $P < 0.01$) in those calving in February–March.

The occurrence of non-pregnancy for cows in BCS ≤ 2 at the branding/weaning muster was 7.1% (95% CI, 2.6–11.6%; $P < 0.01$), 12.3% (95% CI, 8.4–16.1%; $P < 0.01$) and 16.2% (95% CI, 10.9–21.4%; $P < 0.01$) greater for first-lactation, mature and aged cows respectively, than it was for BCS 3. However, minimal differences for non-pregnancy among levels of greater BCS occurred in all age classes. However, the occurrence of non-pregnancy was slightly higher for cows in the greatest category of BCS ($P < 0.001$; Table 3).

In cows calving between July and March, 5–12% higher non-pregnancy was observed in first-lactation cows than in mature and aged lactating cows ($P < 0.05$). In contrast, mature and aged cows calving in April and May had ~20 percentage point lower occurrence of non-pregnancy than did their first-lactation cow counterparts ($P < 0.05$). Occurrence of non-pregnancy was <10% for cows that did not lactate during the year. Within cows that did not lactate during the year, non-pregnancy was 5–8% greater ($P < 0.01$) in cows that experienced fetal or calf loss the previous year. Non-pregnancy increased in each of the 3 years, relative to the previous year, of the study ($P < 0.05$).

The effects of the variable summarising the protein content of wet-season pasture on non-pregnancy significantly interacted both with country type and the wet-season mob-level indicator FecP:ME. Cows grazing protein-inadequate wet-season pastures (DMD:CP ≥ 8) in the Northern Forest and Downs were associated with 10.6% (95% CI 4.5–16.6%; $P < 0.01$) and 16.8% (95% CI, 11.7–21.9%; $P < 0.01$) percentage point greater occurrence of non-pregnancy respectively, than were cows grazing protein-adequate pastures. In contrast, this risk factor was associated with an 8.3% (95% CI, 3.2–13.4%; $P < 0.01$) and 11.7% (95% CI, 5.3–18.0%; $P < 0.01$) lesser chance of non-pregnancy respectively, in the Southern and Central Forest. The impact of reduced wet-season dietary P intake, indicated by FecP:ME of <500, was greater when pastures contained adequate protein, with 10.9% (95% CI, 8.2–13.7%, $P < 0.01$) lesser chance of non-pregnancies than the 7.7% (95% CI, 3.2–12.2%, $P < 0.01$) when wet-season dietary protein was inadequate. In the Northern Forest average wet season FecP:ME ratio of <500 was associated with 13.2% (95% CI, 8.0–18.5%, $P < 0.01$) greater occurrence of non-pregnancy, but this association was not evident in each of the other country types. Cows that grazed dry-season pastures with <55% DMD had a 10.2% (95% CI, 6.6–13.8%, $P < 0.01$) greater chance of non-pregnancy than did those cows that grazed more digestible pastures.

Population-attributable fraction

The final model used to estimate population-attributable fraction (PAF) omitted interaction terms. Models with dummy-coded interaction terms were tested, with moderate changes to the estimated proportional reduction in non-pregnancy evident, although the overall ranking of risk factors was comparable. Emphasis is suggested on the likely relative importance of different risk factors rather than the absolute PAF estimates presented (Table 4). The analysis determined that reproductive history and average dry-season DMD are top-order determinants of the likelihood of non-pregnancy. BCS measured at the branding/weaning muster and risk of P deficiency were intermediate in their impact, with the lowest-order impacts from country type, cow age class and production year.

Table 2. The final multivariable logistic regression model summarising herd-adjusted associations between risk factors and the odds of non-pregnancy in commercial beef cows of northern Australia, with adjusted odds ratio (OR), 95% confidence intervals and *P*-value.

Variable	Coefficient	s.e.	Adjusted OR	OR 95% CI		*P*-value
				Lower	Upper	
Country type						**<0.001**
Northern Downs	Ref					
Southern Forest	−1.63	0.53	0.52	0.23	1.17	0.12
Central Forest	−0.65	0.41	2.48	1.23	5.01	0.01
Northern Forest	0.91	0.36	0.2	0.07	0.56	<0.01
Year observed						**<0.001**
2008–09	Ref					
2009–10	0.25	0.12	1.28	1.02	1.62	0.03
2019–11	0.54	0.13	1.72	1.33	2.21	<0.01
Cow age						**<0.001**
First-lactation cows	0.99	0.11	2.68	2.16	3.33	<0.01
Mature cows	Ref					
Aged cows	−0.08	0.11	0.92	0.74	1.15	0.47
Pregnancy and time of calving for previous mating						**<0.001**
July–September	−0.37	0.14	0.69	0.53	0.9	<0.01
October–November	Ref					
December–January	0.28	0.09	1.33	1.11	1.59	<0.01
February–March	1.27	0.11	3.55	2.85	4.43	<0.01
April–June	2.17	0.14	8.74	6.62	11.54	<0.01
Pregnant	1.49	0.12	4.45	3.49	5.67	<0.01
Non-pregnant	−1.16	0.16	0.31	0.23	0.43	<0.01
Pregnant, failed to rear calf	−0.08	0.22	0.93	0.6	1.42	0.73
Average dry-season DMD[A]						**<0.001**
<55%	Ref					
≥55%	−0.8	0.14	0.45	0.35	0.59	<0.01
Average wet-season FecP:ME[C]						**0.01**
<500 mg P:1 MJ ME	Ref					
≥500 mg P:1 MJ ME	0.54	0.21	1.71	1.13	2.6	0.01
Average wet- season DMD:CP[C]						**0.25**
>8:1	Ref					
≤8:1	−0.16	0.14	0.85	0.65	1.12	0.25
BCS at branding/weaning muster[B]						**<0.001**
1–2	1.42	0.1	4.13	3.38	5.04	<0.01
2.5	0.84	0.09	2.33	1.95	2.77	<0.01
3	Ref					
3.5	−0.31	0.12	0.73	0.58	0.93	0.01
4–5	−0.03	0.15	0.97	0.73	1.29	0.82
Interaction: average wet season FecP:ME[C] × average wet season DMD:CP						**<0.001**
≥500 mg P:1 MJ ME: ≤8:1	−1.46	0.16	0.23	0.17	0.32	<0.01
Interaction: country type × average wet season FP:ME[C]						**<0.001**
Southern Forest: ≥500 mg P:1 MJ ME	0.56	0.2	1.75	1.17	2.61	<0.01
Central Forest: ≥500 mg P:1 MJ ME	0.26	0.17	1.3	0.94	1.81	0.12

(*Continued on next page*)

Table 2. (*Continued*).

Variable	Coefficient	s.e.	Adjusted OR	OR 95% CI		*P*-value
				Lower	Upper	
Northern Forest: ≥500 mg P:1 MJME	−0.59	0.21	0.55	0.37	0.83	<0.01
Interaction: pregnancy and time of calving for previous mating × cow age						**<0.001**
First-lactation cows						
July–September	0.09	0.14	1.1	0.83	1.45	0.51
December–January	0.03	0.11	1.03	0.83	1.27	0.81
February–March	−0.06	0.16	0.94	0.69	1.28	0.7
April–June	−1.46	0.35	0.23	0.12	0.47	<0.01
Pregnant	−0.13	0.24	0.88	0.55	1.42	0.6
Non-pregnant	−0.77	0.2	0.46	0.31	0.68	<0.01
Pregnant, failed to rear calf	−0.37	0.21	0.69	0.46	1.03	0.07
Aged cows						
July–September	0.39	0.19	1.48	1.02	2.15	0.04
December–January	−0.09	0.11	0.91	0.73	1.14	0.42
February–March	0.1	0.14	1.1	0.84	1.45	0.48
April–June	0.04	0.2	1.04	0.71	1.54	0.83
Pregnant	0.46	0.15	1.58	1.18	2.12	<0.01
Non-pregnant	0.81	0.18	2.24	1.59	3.17	<0.01
Pregnant, failed to rear calf	0.45	0.22	1.57	1.02	2.4	0.04
Interaction: Country type × average wet season DMD:CP[D]						**<0.001**
Central Forest: ≤8:1	1.85	0.4	5.4	3.41	8.54	<0.01
Northern Forest: ≤8:1	1.69	0.23	0.9	0.64	1.28	0.56
Southern Forest: ≤8:1	−0.1	0.18	6.37	2.92	13.9	<0.01
Interaction: pregnancy and time of calving for previous mating × BCS at branding/weaning muster						**<0.001**
July–September						
BCS 1–2	−0.55	0.18	0.58	0.4	0.83	<0.01
BCS 2.5	−0.27	0.15	0.76	0.56	1.03	0.08
BCS 3.5	0.09	0.21	1.09	0.72	1.65	0.67
BCS 4–5	0.02	0.3	1.02	0.57	1.83	0.94
December–January						
BCS 1–2	0.16	0.12	1.17	0.92	1.49	0.19
BCS 2.5	0.11	0.11	1.12	0.9	1.4	0.31
BCS 3.5	0.27	0.15	1.3	0.98	1.74	0.07
BCS 4–5	0.15	0.17	1.16	0.82	1.63	0.4
February–March						
BCS 1–2	0.04	0.17	1.04	0.75	1.44	0.82
BCS 2.5	−0.08	0.15	0.92	0.68	1.23	0.57
BCS 3.5	0.26	0.18	1.3	0.92	1.84	0.13
BCS 4–5	−0.05	0.25	0.95	0.58	1.56	0.84
April–June						
BCS 1–2	−0.9	0.33	0.41	0.21	0.78	<0.01
BCS 2.5	0.44	0.28	1.56	0.9	2.7	0.11
BCS 3.5	0.55	0.22	1.74	1.13	2.68	0.01

(*Continued on next page*)

Table 2. (*Continued*).

Variable	Coefficient	s.e.	Adjusted OR	OR 95% CI		*P*-value
				Lower	Upper	
BCS 4–5	1.22	0.37	3.39	1.65	6.97	<0.01
Pregnant						
BCS 1–2	−1.51	0.19	0.22	0.15	0.32	<0.01
BCS 2.5	−0.44	0.17	0.64	0.46	0.89	<0.01
BCS 3.5	1.03	0.19	2.8	1.94	4.06	<0.01
BCS 4–5	1.23	0.21	3.41	2.27	5.12	<0.01
Non-pregnant						
BCS 1–2	−0.71	0.44	0.49	0.21	1.16	0.11
BCS 2.5	−0.12	0.3	0.88	0.49	1.6	0.69
BCS 3.5	−0.09	0.2	0.91	0.61	1.35	0.64
BCS 4–5	0.53	0.21	1.7	1.13	2.55	0.01
Pregnant, failed to rear calf						
BCS 1–2	0.15	0.4	1.16	0.53	2.55	0.71
BCS 2.5	−0.55	0.37	0.58	0.28	1.19	0.14
BCS 3.5	−0.01	0.26	0.99	0.59	1.66	0.98
BCS 4–5	0.2	0.26	1.22	0.74	2.01	0.45
Interaction: cow age × BCS at branding/weaning muster[C]						<0.001
First-lactation cows						
BCS 1–2	−0.55	0.13	0.58	0.45	0.74	<0.01
BCS 2.5	−0.16	0.11	0.85	0.68	1.06	0.15
BCS 3.5	−0.43	0.14	0.65	0.49	0.85	<0.01
BCS 4–5	−0.65	0.16	0.52	0.38	0.71	<0.01
Aged cows						
BCS 1–2	0.12	0.12	1.12	0.89	1.43	0.34
BCS 2.5	−0.08	0.11	0.92	0.74	1.15	0.47
BCS 3.5	0.15	0.12	1.16	0.91	1.47	0.22
BCS 4–5	−0.15	0.15	0.86	0.65	1.15	0.31
Intercept	−2.57	0.33				

Random effect		95% CI	
		Lower	Lower
Level 2 (property)	0.862	0.7	1.06
rho (ICC)	0.184	0.13	0.25

Data drawn from 32 382 annual production years involving 24 736 individual cows from 55 herds. Values in bold in the *P*-value columnvalues are generalised Wald-test *P*-values; others are Wald-test values.
[A]Relates to the 3–4 months leading up to the determination of non-pregnancy outcome.
[B]Conducted approximately 3–4 months prior to the pregnancy diagnosis muster.
[C]Relates approximately to the December to April period.
BCS, body condition score; FecP:ME, ratio of faecal phosphorus to metabolisable energy; DMD:CP, ratio of dry matter digestibility to dietary crude protein.

Herd-adjusted univariable associations

The proportion of the paddock within <2.5 km of water at the time of calving showed a possible trend in that there was a progressive increase in the occurrence of non-pregnancy as the proportion of the paddock within 2.5 km of water occupied during mating decreased. Several other variables (including: hip height, measurements of liveweight at various time points, temperature at previous calving and mating, average dietary CP content during the wet season; provision of supplemental P; mustering inefficiency; age cows are routinely culled; and pestivirus vaccination policy) were added to the final multivariable model one at a time

Table 3. Predicted marginal means for cows non-pregnant for risk factors and interactions among risk factors in northern Australian beef herds.

Variable	*n*	Mean (%)	95% CI of mean	
			Lower	Upper
Year observed				
2009	2138	11.9	8.4	15.5
2010	19 546	14.8	11.3	18.3
2011	10 698	18.8	14.5	23.2
Average dry-season DMD[A]				
<55	28 054	20.8	16.2	25.4
≥55	4328	10.6	7.5	13.7
Average wet-season FP:ME[C] × average wet-season DMD:CP[C]				
DMD:CP > 8:1	8772	11.7	8.1	15.4
<500 mg P:1 MJME				
≥500 mg P:1 MJME	1295	19.4	13.9	24.9
DMD:CP ≤ 8:1				
<500 mg P:1 MJME	12 494	10.1	7.5	12.7
≥500 mg P:1 MJME	9821	21.1	16.3	25.8
Country type × average wet-season FecP:ME[C]				
Southern Forest				
<500 mg P:1 MJME	2431	7.9	3.2	12.6
≥500 mg P:1 MJME	3928	11.0	5.0	17.0
Central Forest				
<500 mg P:1 MJME	7963	17.3	9.7	24.8
≥500 mg P:1 MJME	3066	18.4	10.6	26.1
Northern Downs				
<500 mg P:1 MJME	1849	14.7	7.9	21.5
≥500 mg P:1 MJME	2473	12.5	6.1	18.9
Northern Forest				
<500 mg P:1 MJME	9023	28.9	20.3	37.5
≥500 mg P:1 MJME	1649	15.7	9.3	22.1
Cow age × pregnancy and time of calving for previous mating				
First-lactation cows				
July–September	1472	14.8	11.1	18.4
October–November	1997	10.2	7.3	13.1
December–January	1350	21.3	16.3	26.3
February–March	407	37.4	28.8	46.1
April–June	48	31.5	16.4	46.6
Pregnant	109	41.9	29.7	54.1
Non-pregnant	776	2.3	1.3	3.2
Pregnant, failed to rear calf	588	9.6	6.1	13.2
Mature cows	1581	8.5	6.3	10.7
July–September				
October–November	5097	5.2	3.6	6.9
December–January	5694	12.4	9.3	15.4

(*Continued on next column*)

Table 3. (*Continued*).

Variable	*n*	Mean (%)	95% CI of mean	
			Lower	Upper
February–March	1650	25.4	19.6	31.2
April–June	548	51.3	42.1	60.5
Pregnant	1288	30.5	24.1	36.8
Non-pregnant	2463	2.6	1.7	3.5
Pregnant, failed to rear calf	814	7.6	4.9	10.3
Aged cows				
July–September	520	7.9	5.6	10.2
October–November	1442	7.1	4.4	9.8
December–January	2214	10.7	7.8	13.6
February–March	709	25.9	19.5	32.3
April–June	230	50.6	39.7	61.5
Pregnant	449	39.1	31.0	47.3
Non-pregnant	658	5.3	3.3	7.3
Pregnant, failed to rear calf	278	10.7	6.4	15.1
Cow age × BCS at branding/weaning muster[B]				
First-lactation cows				
BCS 1–2	1052	23.1	16.7	29.4
BCS 2.5	1357	25.1	18.9	31.3
BCS 3	1762	16.0	11.7	20.2
BCS 3.5	1485	10.5	7.4	13.6
BCS 4–5	1091	12.6	8.6	16.5
Mature cows				
BCS 1–2	1877	21.3	15.8	26.7
BCS 2.5	3162	17.1	12.7	21.4
BCS 3	6571	9.0	6.6	11.4
BCS 3.5	4342	8.6	6.3	10.9
BCS 4–5	3183	12.6	9.2	16.0
Aged cows				
BCS 1–2	835	26.9	19.9	33.8
BCS 2.5	1089	18.7	13.6	23.7
BCS 3	2310	10.7	7.7	13.7
BCS 3.5	1408	11.7	8.4	15.0
BCS 4–5	858	13.1	9.1	17.1
Country type × average wet-season DMD:CP[C]				
Southern Forest				
DMD:CP > 8:1	142	6.0	1.4	10.6
DMD:CP ≤ 8:1	4180	14.3	7.9	20.6
Central Forest				
DMD:CP > 8:1	621	12.7	6.2	19.2
DMD:CP ≤ 8:1	5738	24.4	15.2	33.6
Northern Downs				
DMD:CP > 8:1	3415	19.7	10.4	29.0
DMD:CP ≤ 8:1	7614	9.1	4.6	13.7

(*Continued on next page*)

Table 3. (*Continued*).

Variable	*n*	Mean (%)	95% CI of mean	
			Lower	Upper
Northern Forest				
DMD:CP > 8:1	5889	31.1	21.7	40.5
DMD:CP ≤ 8:1	4783	14.3	9.0	19.7
Pregnancy and time of calving for previous mating × BCS at branding/weaning muster[B]				
July–September				
BCS 1–2	477	12.5	8.4	16.6
BCS 2.5	875	10.1	7.1	13.2
BCS 3	1271	6.5	4.4	8.5
BCS 3.5	687	4.8	2.9	6.7
BCS 4–5	263	5.0	2.4	7.6
October–November				
BCS 1–2	1145	23.4	18.0	28.7
BCS 2.5	1661	15.5	11.7	19.3
BCS 3	3139	7.9	5.8	10.0
BCS 3.5	1621	5.4	3.7	7.0
BCS 4–5	970	5.9	3.9	8.0
December–January				
BCS 1–2	1266	31.7	25.2	38.2
BCS 2.5	1451	21.1	16.2	25.9
BCS 3	3576	10.0	7.3	12.7
BCS 3.5	1919	8.8	6.3	11.3
BCS 4–5	1046	8.7	5.9	11.4
February–March				
BCS 1–2	495	53.3	44.4	62.2
BCS 2.5	510	37.7	29.8	45.7
BCS 3	1055	23.5	17.7	29.3
BCS 3.5	537	21.0	15.1	27.0
BCS 4–5	169	17.8	10.7	24.8
April–June				
BCS 1–2	58	40.5	24.1	56.9
BCS 2.5	218	60.9	47.5	74.4
BCS 3	308	31.8	23.0	40.6
BCS 3.5	181	35.0	24.5	45.5
BCS 4–5	61	53.9	36.3	71.5
Pregnant				
BCS 1–2	242	25.2	17.4	32.9
BCS 2.5	374	36.9	28.2	45.5
BCS 3	508	29.8	22.1	37.4
BCS 3.5	360	44.1	34.8	53.4
BCS 4–5	362	51.7	42.3	61.0
Non-pregnant				
BCS 1–2	44	4.5	0.8	8.2
BCS 2.5	183	4.9	2.1	7.6
BCS 3	828	2.6	1.7	3.6
BCS 3.5	1442	1.6	1.0	2.2
BCS 4–5	1400	3.3	2.2	4.4
Pregnant, failed to rear calf				
BCS 1–2	37	25.3	11.4	39.1
BCS 2.5	85	9.2	3.7	14.7
BCS 3	209	7.5	4.3	10.7
BCS 3.5	488	5.1	3.2	7.0
BCS 4–5	861	6.8	4.6	9.0

(*Continued on next column*)

[A]Relates to the 3–4 months leading up to the determination of non-pregnancy outcome.
[B]Conducted approximately 3–4 months prior to the pregnancy diagnosis muster.
[C]Relates approximately to the December to April period.
BCS, body condition score; FP:ME, ratio of faecal phosphorus to metabolisable energy; DMD:CP, ratio of dry matter digestibility to dietary crude protein.

Table 4. Estimated population-attributable fraction of non-pregnancy for risk factors contained in the full multivariable model.

Variable	PAF	95% CI	
		Lower	Upper
Previous reproductive outcome	76.1%	73.2%	78.6%
Average DMD during the dry season	62.1%	54.8%	68.2%
BCS at the weaning/branding muster	38.3%	34.1%	42.3%
Average FecP:ME ratio during the wet season	34.2%	29.4%	38.6%
Average DMD:CP ratio during the wet season	13.0%	9.4%	16.4%
Year observed	6.4%	4.2%	8.5%
Cow age cohort	6.0%	0.1%	11.6%
Country type	3.5%	−63.0%	42.9%

as described above and, in all cases, the findings suggested either no apparent effect or confounding with other variables.

Discussion

This study is the first population-based epidemiologic study to quantify the risk of non-pregnancy in commercial beef herds across northern Australia and determined the associated risk factors, namely, time of calving, lactation and pre- and post-partum nutrition. A 15–19% percentage point higher occurrence of non-pregnancy was observed in the Northern Forest than in more fertile country types, reflecting the extreme environmental and nutritional challenges faced by beef producers in this country type (Ash *et al.* 1997; Bortolussi *et al.* 2005*b*). The effect of geographical location on reproduction performance is well established in the literature (Entwistle 1983; O'Rourke *et al.* 1992; Bortolussi *et al.* 2005*c*; Gleeson *et al.* 2012). If incidence of fetal and calf loss across the country types averages at 9.5% (McGowan *et al.* 2014), one potential estimate of average weaning rates in each of the country types is 61%, 75%, 76% and 79% for Northern Forest, Northern Downs, Central Forest and Southern Forest respectively. This indicates that maintaining a self-replacing herd would not be possible in the Northern Forest if cows were routinely culled (permanently removed from the herd) for non-pregnancy each year.

In the present study, the population-attributable fraction statistic was used to compare the relative importance of risk factors. This statistic represents the theoretical proportional reduction in negative outcomes that would be achieved by eliminating the exposure(s) of interest from the population, while distributions of other risk factors in the population remained unchanged. Its estimation therefore is dependent on the prevalence and the strength of association for the risk factor(s) of interest, meaning that exposure of a large proportion of the cattle population to a small risk is likely to yield a greater impact on performance than is exposure to a large risk on a small proportion.

The results from this analysis in the present study reaffirmed that reproductive outcome from the previous mating, including time of calving, is a top-order determinant for likelihood of non-pregnancy, which is consistent with results reported by O'Rourke *et al.* (1991*a*). Cows calving earlier had a lesser change of non-pregnancy, having more time to return to normal ovarian activity after calving, and the best opportunity to respond to improving pasture quality and quantity following first rains of the season (Nicholls *et al.* 1982). Although all non-lactating cows had low non-pregnancy rates, it was 4–5% lesser in those failing to conceive in the previous year than in those that were pregnant but failed to rear a calf. This indicates adverse effects of pregnancy and potentially early lactation, depending on when calf loss occurred.

The increased risk of non-pregnancy for all first-lactation cows with the exception of those that calved between April and June, compared with lactating mature and aged cows, is consistent with existing scientific literature (O'Rourke *et al.* 1991*b*; Schatz and Hearnden 2008; Burns *et al.* 2010) and is explained by the increased nutritional demands associated with lactation. The absence of this effect for cows that calved between April and June is potentially explained by differential weaning strategies between first-lactation and multiparous cows; that is, calves from first-lactation cows weaned earlier so that the duration of lactation is reduced and additional time is provided for first-lactation cows to grow and achieve pregnancy.

The large and consistent effect of nutritional risk factors on risk of non-pregnancy was demonstrated by the large number contained in the final model, including country type, BCS measured at the branding/weaning muster, inadequate wet-season pasture protein, lower P content of the diet and low digestibility of dry-season pasture. There are numerous other studies where positive curvilinear or linear relationships between nutritional indicators, such as body condition, and pregnancy rate have been demonstrated (Rae *et al.* 1993; Jolly *et al.* 1996; Dixon 1998; Wettemann *et al.* 2003; Schatz and Hearnden 2008). The odds of non-pregnancy were 2.3 times greater at BCS of 2.5 and 4.1 times greater at BCS of ≤2.0 than at BCS of 3 measured at the branding/weaning muster in the present study. These findings are comparable to those reported by Waldner and García Guerra (2013) who stated odds ratios of 1.3–1.8 at BCS 2.5 and 3.5–4.2 at BCS 2.0 when referenced to BCS 3.0 measured at the pregnancy diagnosis muster. Mature cows performed better within most BCS categories than did both aged cows (1.0–2.4%) and first-lactation cows (1.8–8.0%), but there was no age effect for cows in the highest BCS category. The biological mechanism explaining the increased likelihood of non-pregnancy for first-lactation cows is thought to be explained by the increased nutritional demands of having to support both lactation and maternal growth (Entwistle 1983). These findings validated recommendations, especially mating, weaning and diet management strategies, aimed at maintaining cows in BCS 3 or greater.

The importance of good nutrition during both wet and dry seasons to achieve high annual pregnancy rates was emphasised in the present study by the higher likelihood of non-pregnancy for cows that experienced low available energy during the dry season (between weaning and pregnancy diagnosis) and diets low in protein and P during the wet season. Apart from DMD during the dry season, the magnitude of effect for each of these risk factors was dependent on country type. The 10.6% and 16.8% fewer pregnancies when cows grazed low-protein wet-season pastures (DMD to CP ratio of >8:1) in the Northern Downs and Northern Forest respectively, than in cows grazing protein-adequate pastures is consistent with demonstrated improvements in pregnancy rates from the provision of supplemental non-protein nitrogen during the wet season reported by McCosker *et al.* (1991). It is unclear why the direction of effect for wet-season protein adequacy was reversed in Southern and Central Forest country types; however, it may be partially explained by differences in distribution and timing of calving and lactation, as management usually concentrates calving into the late dry season and early wet season in these country types. The increased supply of protein during early lactation favours partitioning of available nutrients to mammary secretion (Oldham 1984), thus increasing the risk of negative energy balance which can reduce the ability of cows to cycle during lactation (Roche *et al.* 2013).

Negative energy balance is associated with down-regulation of folliculogenesis, which can take up to 6 months between primoidial follicle recruitment and pre-ovulatory follicle, resulting in increased likelihood of anovulation

(Scaramuzzi *et al.* 2011). This is consistent with the 10.2% fewer pregnancies across country types in cows that grazed pastures with DMD of <55% (low available ME) than in those that grazed pastures with higher energy availability during the dry season when pastures are senesced. This effect occurred even when weaning, which mostly coincides with the commencement of the dry season, and provides the opportunity for conception under continuously mating.

This study used the ratio of FecP to ME as a mob-level indicator of the dietary P content, which at the time of implementing this research was recommended by extension personnel as a practical tool to determine the likely response of cattle to P supplementation (Jackson *et al.* 2012). Recommendations based on findings from research activities conducted since suggest that some degree of caution should now be exercised when interpreting the strength of association for this risk factor in the current study, until further validation of Fec:ME is performed and until it has been determined under what specific conditions is this a useful indicator of P intake, such as when cattle have been consuming a similar diet for a reasonable length of time. Under such conditions, which is potentially the case in some grazing situations, FecP:ME has been reported to provide some indication of P intake (Quigley *et al.* 2015) and this is how this parameter has been considered in the current study. The 13.2% greater chance of non-pregnancy in the Northern Forest where dietary P content of wet-season pasture was potentially low observed in the current study is consistent with reports by McCosker *et al.* (1991) and Hart and Michell (1965) where cows were determined to be P deficient from grazing pastures low in P content. The lack of a similar effect in other regions may be explained by their generally higher soil P status (Jackson 2012). The association between wet-season pasture protein content and FecP:ME is also largely consistent with current knowledge that there is limited benefit to the provision of dietary P when the available protein supply is limited (Jackson 2012). Despite the uncertainty around the validity FecP:ME, the observed association in the current study provides further evidence that herds grazing potentially P-deficient pastures throughout northern Australia should be managed accordingly to alleviate the risk of P efficiency, which has suggested to be not the case for many cattle in northern Australia (Niethe 2009; Dixon *et al.* 2011).

Non-pregnancy varying by 2.9–6.9% among production years represents seasonal differences not accounted for by other risk factors and is consistent with previous studies in a tropical system with a unimodal rainfall pattern (O'Rourke 1994). Research sites in this study experienced periods of extended dry and high-rainfall events that varied in incidence among regions as the study covered a huge area. It has previously been recommended that studies of systems subject to seasonal variation in northern Australia continue for 6–8 years (Taylor and Tulloch 1985). Despite this, production year did not modify the impact of other risk factors, demonstrating the consistency of their effects, and that the study period used in this research was sufficient.

The study population of properties and herds in this research were not randomly selected, and as the owners were more likely to have represented higher-performance herds, annual pregnancy rates may have been slightly higher than across all northern Australian herds at the time. However, just as year had no impact on risk-factor effects, there is no evidence that the effects of risk factors were different in the research population from those in the overall northern Australian herd. Despite all efforts to comprehensively record all primary risk factors for annual pregnancy, the residual intra-class correlation estimate (0.18) in the selected model indicated there is still a relatively large amount of variance at the property level, some of which may be possible to explain.

Conclusions

This study demonstrated that the major risk factors associated with lower annual pregnancy rate in northern Australian beef herds are previous reproductive outcome and nutritionally associated. The major expression of the latter was through BCS, the effects of macro-nutrient adequacy in both wet- and dry-season pastures and that much lower annual pregnancy rates occur in the Northern Forest than in more-fertile country types in northern Australia.

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