Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Optimum nutrition of the pregnant ewe: A meta-analytic approach

A thesis presented in partial fulfillment of the requirements for the degree of

Doctorate of Philosophy

in

Animal Science

at Massey University, Manawatū, New Zealand.

Fernando Javier Roca Fraga

2017

Abstract

Formal systematic review guidelines and meta-analytic methods were used in the present study to achieve three main objectives. Firstly, literature on the effect of ewe nutrition during pregnancy on fetal and postnatal lamb growth was reviewed and effect sizes estimated for fetuses/lambs at three stages of their life: 1) late gestation fetal weight (LGFW), 2) lamb birth weight (BW) and 3) weaning weight (WW). Secondly, the contribution of experimental factors responsible for variation in study results was determined. Thirdly, a field trial was conducted to increase understanding in an area identified by the meta-analyses as requiring further experimentation. Overall, early- and mid-pregnancy undernutrition had no significant effect on LGFW (β [Early-pregnancy] = -0.0007, 95% Highest posterior density (HPD) = -0.26 to 0.28; β [Mid-pregnancy] = -0.07, 95% HPD = -0.27 to 0.16), BW (β [Early-pregnancy] = 0.01, 95% HPD = -0.36 to 0.34; β [Mid-pregnancy] = -0.02, 95% HPD = -0.36 to 0.33) and WW (β [first 100 days of pregnancy] = -0.008, 95% HPD = -0.42 to 0.18), suggesting that short to moderate periods of undernutrition in these stages are tolerated by ewes with limited impact on their offspring, when nutrition is re-established to pregnancy maintenance (PM) or above levels during late-pregnancy. Late-pregnancy undernutrition can significantly decrease LGFW and BW by up to 1.15 kg at birth, with residual effects at weaning resulting in weaned lambs that are up to 18% lighter than their control counterparts and thus, should be avoided. The present study also considered the effect of maternal above PM feeding on LGFW, BW and WW. The combined effects across these studies were variable, as few experiments investigated above PM feeding at each stage of pregnancy, and thus it was not possible to draw definitive conclusions. A field

i

experiment was undertaken to determine the effects of ad-libitum (AL) feeding at various

stages of pregnancy and for differing lengths of time on twin lamb BW and WW. Results

showed that providing ewes with AL feeding significantly (p<0.05) increased their live

weight and BCS, but did not increase (p>0.05) the BW or WW of their lambs relative to

their control counterparts. This study also suggested that AL feeding during late-pregnancy

may have negative consequences to the survival of twin lambs and requires further

examination. Thus, AL feeding is not justified as a management tool to increase twin lamb

BW and WW, when nutrition is adequate during lactation. The present study represents the

first meta-analytic approach examining the effect of changes in the ewe nutrition during

pregnancy on the growth of offspring at various developmental stages. Given the complex

interrelationship between nutrition of the pregnant ewe, her reproductive success, fetal

growth and development, and offspring post-natal performance, no single study can provide

a definitive understanding of responses to a particular treatment and there is value in

combining available experimental evidence to elucidate a more global picture. A meta-

analytic approach can find trends in combined data that would otherwise be overlooked

using traditional review methods and can also identify gaps in current knowledge.

Key words: meta-analysis, sheep, pregnancy, undernutrition, *ad-libitum*.

ii

Acknowledgements

I would like to take this opportunity to acknowledge and express my deep gratitude to my Massey University supervisors, Professors Paul Kenyon, Hugh Blair and Nicolas Lopez-Villalobos, for their support, patience and sound advice throughout my PhD and their continuing support both professionally and personally.

Special thanks to my University of New South Wales supervisor, Associate Professor Shinichi Nakagawa, for his mentorship and invaluable instruction in regards to meta-analytical methods and their application to my field of research.

I would also like to acknowledge the valuable input of Dr. Malgorzata Lagisz, whose contributions helped to shape the meta-analytic component of this thesis. In addition, I would like to extend my gratitude to Dean Burnham and Geoff Purchas for their technical support during my PhD experimental work.

Thanks to Gravida: National Centre for Growth and Development for providing funds for the research and my PhD Scholarship.

To my wife, whose constant encouragement and critical comment played an important role in my accomplishing this goal.



Table of Contents

Abstracti
Acknowledgementsiii
Table of Contentsv
List of Tablesxiii
List of Figuresxvii
Chapter 1: Literature review 1
1.1 Preamble
1.2 Narrative, systematic and meta-analytic reviews
1.3 Meta-analysis – theory and practice
1.3.1 Formulation of the research problem12
1.3.2 Collection of research evidence
1.3.3 Evaluation and identification of relevant studies
1.3.4 Analyses and integration of evidence from individual studies
1.3.4.1 Selecting an effect size statistic
1.3.4.1.1 Calculation of the standardized mean difference
1.3.4.2 Estimating the overall effect across studies; the meta-analytic mean 18
1.3.4.2.1 Fixed-effect model
1.3.4.2.2 Random-effects model
1.3.4.2.3 Dealing with effect-size dependence
1.3.4.2.4 Multilevel meta-analytic models
1.3.5 Interpretation of the cumulative evidence

	1.3	.5.1	Quantifying and explaining heterogeneity	26
	1.3	.5.2	Publication bias	29
	1.3	.5.3	Sensitivity analysis	36
	1.3.6	Pres	sentation of meta-analysis methods and results	37
	1.4	Summ	ary and research objectives	39
	1.5 F	Refere	ences	40
Ch	apter 2:	Cha	inges in late gestation fetal weight via maternal nutrition:	meta-
	•		rom studies using adult sheep.	
			ct	
	2.2 I	ntrod	uction	55
	2.3 N	Materi	al and methods	58
	2.3.1	Lite	rature search and selection criteria	58
	2.3.2	Data	a extraction and coding	60
	2.3.3	Stat	istical analysis	61
	2.3	.3.1	Datasets	61
	2.3	.3.2	Meta-analysis and meta-regression	62
	2.3	.3.3	Assessment of heterogeneity	65
	2.3	.3.4	R ² estimation for meta-regression models	66
	2.3	.3.5	Publication bias	67
	2.3	.3.6	Sensitivity analysis	67
	2.4 F	Result	s	68
	2.4.1	Stuc	dy retrieval and selection strategy	68
	2.4.3	Und	lernutrition	70
	2.4.4	Ove	rnutrition	75
	245	Pub	lication bias	77

	2.4.	6 Sei	nsitivity analysis: lnRR as an alternative effect size	78
	2.5	Discu	ssion	79
	2.6	Conc	lusion	86
	2.7	Refer	ences	87
	2.8	Supp	lementary material for Chapter 2	93
	2.8.	1 Suj	pplementary references -list of excluded studies	93
	2.8.	2 Suj	pplementary references - list of included studies	127
	2.8.	3 Suj	pplementary tables	136
Cha	apter 3:	Me	eta-analysis of the role of pregnancy nutrition in adult multip	arous
ewe	es in det	termin	ing changes in lamb birth weight	147
	3.1	Abstr	act	149
	3.2	Introd	luction	151
	3.3	Mate	rials and methods	153
	3.3.	1 Sea	arch strategy and selection criteria	153
	3.3.	2 Da	ta extraction	155
	3.3.	3 Da	ta coding and datasets	155
	3.3.	4 Sta	tistical analysis	157
	3	.3.4.1	Meta-analysis	158
	3	.3.4.2	Heterogeneity	161
	3	.3.4.3	R ² estimation for meta-regression models	162
	3	.3.4.4	Publication bias	163
	3	.3.4.5	Sensitivity analysis	163
	3.4	Resul	ts	164
	3.4.	1 Stu	dy retrieval and selection strategy	164
	3.4.	2. Stu	dy characteristics and meta-analysis	166

3.4.2.1	Undernutrition	166
3.4.2.2	Overnutrition	181
3.4.3 Publ	lication bias	185
3.4.4 Sens	sitivity analysis	187
3.5 Discus	sion	188
3.6 Conclu	ision	194
3.7 Referen	nces	196
3.8 Supple	ementary material for Chapter 3	217
3.8.1 Supp	plementary methodology	217
3.8.2 Supp	plementary references – list of excluded studies	217
3.8.3 Supp	plementary tables	284
Chapter 4: Wea	ning weight is affected by maternal nutrition du	ring pregnancy – a
	tudies in adult sheep	
4.1 Abstra	ct	295
4.2 Introdu	action	297
4.3 Materia	al and methods	299
4.3.1 Sear	ch strategy and selection criteria	300
4.3.2 Data	extraction	301
4.3.3 Data	a coding and datasets	302
4.3.4 Stati	istical analysis	303
4.3.4.1	Heterogeneity	307
4.3.4.2	R ² for meta-regression models	308
4.3.4.3		
4.3.4.3	Publication bias	308
4.3.4.4	Sensitivity analysis	

309
312
312
321
323
325
328
330
339
339
340
aintenance
aintenance g 371
g 371
g 371
g 371 373 375
g 371 373 375 376
g 371
g371373375376377
g371373375376377382
g 371 373 375 376 377 382 382 383
g371373375376377382383
g371373375376377382383383

	5.4.	3 Ewe body condition score	392
	5.4.	4 Gestation length	396
	5.4.	5 Lamb data	397
	5.5	Discussion	403
	5.6	Conclusion	407
	5.7	References	408
	5.8	Supplementary material for Chapter 5	413
Cł	napter 6:	General Discussion	415
	6.1	Introduction	417
	6.2	Summary of findings	418
	6.2.	1 Early- and mid-pregnancy undernutrition	419
	6.2.	2 Late-pregnancy undernutrition	420
	6.2.	3 Ewe nutrition above the requirements for pregnancy	421
	6.2.	4 Important moderator variables	422
	6	.2.4.1 Length of undernutrition	422
	6	.2.4.2 Litter size	423
	6	.2.4.3 Year of publication	423
	6	.2.4.4 Level of feeding of the control group	424
	6.3	Methodological considerations	426
	6.4	Limitations	429
	6.4.	1 Reporting details of primary studies	429
	6.4.	2 Conception rate, ewe live weight and BCS achieved in Chapter 5	431
	6.5	Practical implications for sheep grazing systems	432
	6.6	Future research consideration	434
	6.6	1 A meta-analysis guide for animal scientists	434

	6.6.2	Better and larger early- and mid-pregnancy undernutrition studies	435
	6.6.3	Can results from periconceptional undernutrition studies be replicated us	sing
	farm	-scale research?	436
	6.6.4	Is the magnitude of the effect of undernutrition similar for singletons	and
	twin	s relative to their potential size and can this be extended to triplets?	437
6	.7	Concluding remarks	438
6	.8	References	440

List of Tables

Table 1. Summary of methodological differences between narrative and meta-analytic
reviews (taken from Nakagawa and Poulin, 2012)
Table 2. Commonly used methods to detect and correct for publication bias and some of the
caveats in the listed methods. References are provided for the papers that propped the
methodology and also for those reporting on some of the caveats in the methods
Table 3. Main characteristics of the nutritional restrictions of the three undernutrition
subgroups (early-, mid- and late-pregnancy)
Table 4. Summary of papers used for meta-analysis in the early-pregnancy subgroup.
Liveweight (LW) change refers to the difference in ewe live weight between the
commencement of the nutritional treatment and that at the end of the nutritional treatment.
The energetic level of undernutrition (relative to maintenance) is shown when and as stated
by the authors. 169
Table 5. Summary of papers used for meta-analysis in the mid-pregnancy subgroup.
Liveweight (LW) change refers to the difference in ewe live weight between the
commencement of the nutritional treatment and that at the end of the nutritional treatment.
The energetic level of undernutrition (relative to maintenance) is shown as stated by the
authors. 172
Table 6. Summary of papers used for meta-analysis in the late-pregnancy subgroup.
Liveweight (LW) change refers to the difference in ewe live weight between the
commencement of the nutritional treatment and that at the end of the nutritional treatment
(including conceptus mass). In studies marked with ^{CF} , liveweight changes are the
difference between ewe live weight at the initiation of the nutritional treatment and the
post-lambing weight. In studies marked with ML, liveweight changes are the difference
between mating weight and post-lambing weight. The level of undernutrition (relative to
pregnancy maintenance. PM) is shown when and as stated by the authors.

Table 7. Summary of papers used for meta-analysis in the overnutrition dataset. Differences
in live weight (LW) gains were calculated as the difference of LW gain/loss between the
control and overfed groups between the commencement of the nutritional treatment and
that at the end of the nutritional treatment (including conceptus mass). In studies marked
with CF, differences in LW gain were calculated as the difference in ewe live weight
between the initiation of the nutritional treatment and the post-lambing weight. In studies
marked with $^{\mathrm{ML}}$, differences in LW gain were calculated as the difference between mating
weight and post-lambing weight. The level of overnutrition (relative to pregnancy
maintenance, PM; as pasture height; or ewe body condition score, BCS) is shown when and
as stated by the authors

Table 9. Results from the meta-analytic and meta-regression models in the undernutrition dataset. Estimates and highest posterior density intervals (95% HPD) in bold were considered statistically significant.

Table 10. Summary of the statistical results of meta-analytic and meta-regression models in two subgroups from the undernutrition dataset. Period 1 refers to studies with nutritional manipulations ending within the first 100 days of pregnancy. Period 2 include studies undertaken in late-pregnancy or that extended to late-pregnancy.95% highest posterior density (HPD) intervals excluding zero are considered statistically significant (in bold text).

......319

Table 11. Pre- and post-grazing herbage masses of pregnancy maintenance (PM) and ad-
libitum (AL) herbage allowances (least squares means ± SEM) offered to ewes during
early-pregnancy (pregnancy day 1 (P1) - P50), mid-pregnancy (P51-P100) and late-
pregnancy (P101-140)
Table 12. Chemical composition and metabolizable energy expressed on a dry matter (DM)
basis of pasture grab samples collected throughout pregnancy from the pregnancy
maintenance (PM) and <i>ad-libitum</i> (AL) nutritional treatments (least squares means \pm SEM).
Table 13. Effect of ewe nutritional treatment on ewe live weight (kg) prior to artificial
insemination (AI; at P-2) and in pregnancy (least squares means \pm SEM)
Table 14. Effect of ewe nutritional treatment on their live weights (kg) in lactation (least
squares means \pm SEM). 391
Table 15. Effect of ewe nutritional treatment on ewe body condition scores (BCS) prior to
artificial insemination (AI, at P-2) and in pregnancy (least squares means \pm SEM) 393
Table 16. Effect of ewe nutritional treatment during pregnancy on ewe body condition
scores (BCS) in lactation (least squares means ± SEM)
Table 17. Effect of ewe nutritional treatment on ewe gestation length (least squares means \pm
SEM) and the proportion of ewes lambed by day 149
Table 18. Effect of ewe nutritional treatment on lamb birth weight, crown-rump-length
(CRL), chest circumference (CC), front (FL) and rear (RL) leg length and total litter weight
(least squares means \pm SEM)
Table 19. Effects of ewe nutritional, with gestation length as a covariate on lamb birth
weight, crown-rump-length (CRL), chest circumference (CC), front (FL) and rear (RL) leg
length and total litter weight (least squares means ± SEM)
Table 20. Effect of ewe nutritional treatment during pregnancy on lamb live weights (kg)
during lactation (least squares means \pm SEM). 401

Table 21. Effect of ewe nutritional treatments during pregnancy on lamb weaning weight
total litter weight at weaning (least squares means \pm SEM) and lamb survival402
Table 22. Birth weight data from nutritional studies in sheep in which three nutritional
scenarios have been studied: pregnancy maintenance (PM), above pregnancy maintenance
(APM) and undernutrition (UN). The difference in birth weight of the undernutrition groups
was compared against PM and APM groups
Table 23. Statistical power of some undernutrition studies that varied in the size of
estimated effects and sample sizes. The sample size (n) for each group necessary to achieve
a 50% and 90% power was also estimated using Cohen (1988) guidelines

List of Figures

Figure 1. The expected proportion of significant results as a function of sample size and effect size (adapted from Hedges and Olkin, 1985)
Figure 2. Number of meta-analysis publications in PubMed since 1990. Journal articles were search using the search string: meta-analysis OR meta-analy* OR meta-analy* OR meta-analy* as publication type.
Figure 3. Schematic representation of the fixed-effect model (reprinted from Borenstein e al. 2010). • represents the common mean ($\theta = 100$) across studies, i.e. the true effect magnitude. \blacksquare represents the observed mean for each particular study, and V_I and V_I represents the variance of the mean in study 1 and 2 in relation to θ .
Figure 4. Schematic representation of the random-effects model (reprinted from Borenstein et al. 2010). For each study, the sample mean is represented by a full circle (\bullet). In this case the true effect is not fixed and so it varies from Study 1 to Study 2 since they were samples from the distribution at the bottom where μ represents the mean of the distribution of true effects with variance $\tau 2$. The observed mean (\blacksquare) in Study 1 and 2 differ from the true mean (\bullet) of each particular population because they use a finite number of individuals to estimate the effect.
Figure 5. Schematic representation of a multilevel meta-analytic model (adapted from Nakagawa et al. 2017)
Figure 6. Examples of hypothetical a) symmetrical and b) asymmetrical funnel plots, where the horizontal axis represents the treatment effects estimated from individual studies and the vertical axis represents some measure of study size (e.g. standard error on a reverse scale that places larger studies on top of the graph). Solid line represents zero effect Dashed line represents the meta-analytic mean (Based on Sterne et al. 2005). More details
in the text

rigure 7. Hypothetical examples of the results of a trim-and-fill method: a) an asymmetrical
funnel plot suggesting possible publication bias due to missing studies in the bottom-right
of the plot, b) the "filled" and more symmetrical funnel plot including the imputed studies
as mirror images of those on the left hand side (open circles). Solid line represents zero
effect. Dashed line represents the meta-analytic mean a) before and b) after adjusting for
publication bias
Figure 8. Four-phase diagram depicting the flow of information through the different
phases of a systematic review (adapted from Moher et al. 2009)38
Figure 9. A Preferred Reporting Items for systematic Reviews and Meta-Analysis
(PRISMA) flow diagram. The number of studies in each particular category is shown in
parenthesis. The number of effect sizes (ES) is shown for each dataset69
Figure 10. Forest plot of estimates from the meta-regression of the undernutrition dataset.
Posterior means for each period of pregnancy (intercepts) and "total days" (slope) are
represented by circles. Horizontal lines represent 95% highest posterior density (HDP)
intervals. Zero effect is shown as a vertical dashed line. Statistically significant effects are
considered those whose 95% HDP do not cross zero
Figure 11. Forest plot of estimates from the meta-regression of the overnutrition dataset.
Posterior means for each litter size (intercepts) and "total days" (slope) are represented by
circles. Horizontal lines represent 95% highest posterior density (HDP) intervals. Zero
effect is shown as a vertical dashed line. Statistically significant effects are considered
those whose 95% HDP do not cross zero76
Figure 12. Funnel plots of raw data (a and c) and the residuals and sampling error effects (b
and d) from full models in the undernutrition (a and b) and overnutrition (c and d) datasets.
Overall posterior (meta-analytic) means are shown as solid lines and zero effect sizes are
shown as dashed lines
Figure 13. Four-phase flow diagram following the Preferred Reporting Items for Systematic
Review and Meta-Analyses (PRISMA) statement. The number of studies in each category

is shown in parenthesis. The number of effect-sizes (E.S.) are given for each dataset.165

Figure 14. Forest plot of estimates from the meta-regression of the undernutrition dataset.
Posterior means for each period of pregnancy (intercepts) are represented by circles.
Horizontal lines represent 95% highest posterior density (HDP) intervals. Zero effect is
shown as a vertical dashed line. Statistically significant effects are considered those whose
95% HDP do not cross zero. 167
Figure 15. Bubble plot depicting the differences in birth weight responses to maternal
undernutrition starting before or at mating (day 0, shown in dashed line). Bubbles represent
the effect sizes for experiments undertaken in singleton- (red) and twin-bearing ewes
(grey). Differences in bubble circumference demonstrate differences in the precision
(1/standard error) between studies
Figure 16. Line what manner thing the matrix and manipulations for each absorbation in the
Figure 16. Line plot representing the nutritional manipulations for each observation in the
mid-pregnancy subgroup. Lines of different colour represent studies using a control groups
fed to meet their pregnancy maintenance requirements (PM; in green) or a control groups
fed above PM requirements (in grey), respectively
Figure 17. Line plot representing the nutritional manipulations for each observation in the
late-pregnancy subgroup. Black and green lines represent nutritional manipulations
undertaken indoors or outdoors, respectively
Figure 18. Line plot representing the nutritional manipulations for each observation in the
overnutrition dataset. Green and black lines represent studies with nutritional manipulations
undertaken outdoors and indoors, respectively
Figure 19. Funnel plot of effect sizes in the overnutrition dataset plotted against their
precision (1/standard error). Red and grey bubbles are effect sizes for studies undertaken
indoors and outdoors, respectively. Differences in bubble circumference accentuate the
differences in sample size between studies. 184
Figure 20. Funnel plots of raw data (a and c) and the residuals and sampling error effects (b
and d) from full models in the undernutrition (a and b) and overnutrition (c and d) datasets.
Overall posterior (meta-analytic) means are shown as solid lines and zero effect is shown as

dashed lines. Observations marked in black, green and red in the undernutrition funnel plots
(a and b) depict observations in studies undertaken in early-, mid- and late-pregnancy186
Figure 21. Flow diagram of studies identified, excluded and included in the present study.
The number of studies in each category are shown in parenthesis. The number of effect-
sizes (E.S.) are given for each dataset
Figure 22. Line plot representing the undernutrition periods used across experiments. Green
lines represent effects measured with nutritional manipulations ending before day 100 of
pregnancy, whilst black lines represent effects measured with nutritional manipulations
during late-pregnancy or nutritional manipulations that extended to late-pregnancy. Dotted
line demarcates day 100 of pregnancy
Figure 23. Bubble plot of effect-sizes in studies with nutritional manipulations ending
before day 100 of pregnancy. Red and grey bubbles represent studies undertaken in
singletons and twins, respectively. Differences in bubble circumference represent the
precision (1/SE) of the study
Figure 24. Bubble plot of effect-sizes against the length of nutritional manipulations of
studies undertaken in late-pregnancy or that extended into late-pregnancy. Red and grey
bubbles represent studies undertaken in singletons and twins, respectively. Differences in
bubble circumference represent the precision (1/SE) of the study320
Figure 25. Line plot representing the overnutrition periods used across experiments. Red
and grey lines represent studies undertaken in singletons and twins, respectively321
Figure 26. Forest plot of effect-sizes (ES) extracted from the overnutrition studies and the
overall effect estimated using random-effects meta-analysis. The raw ES for each study is
represented by a dot (•) their estimated standard errors (SE of Hedges' g). The overall
effect (♠) is the posterior mean and 95% highest posterior density (HPD) interval from the
meta-analytic model. 322
Figure 27. Funnel plots of raw (a and c) and the residuals and sampling error effects from

meta-regression models (b) in the undernutrition (a and b) and overnutrition (c only)

datasets. Overall posterior (meta-analytic) means are shown as red solid lines and zero
effect is shown as dashed lines. Observations in green and black in the undernutrition
funnel plots depict studies undertaken in Period 1 (nutritional manipulations during the first
100 days of pregnancy) and Period 2 (nutritional manipulations including late-pregnancy).
324

Figure 28. Schematic representation of the six nutritional treatments used in this study. AL0-50 = maternal *ad-libitum* (AL) feeding from artificial insemination (AI) to P50; AL50-100 = maternal AL feeding from P50 to P100; AL100-140 = maternal AL feeding from P100 to P140; AL0-100 = maternal AL feeding from AI to P100; AL0-140 = maternal AL feeding from AI to P140; Control = pregnancy maintenance from AI to P140. Outside the aforementioned AL feeding periods, ewes were offered herbage at pregnancy maintenance levels.

Figure 30. Body condition score (BCS) trajectory of the ewes in the six nutritional treatments throughout pregnancy. Each nutritional treatment is represented by a different line and colour: AL0-50, early–pregnancy ad-libitum (AL) feeding from pregnancy day 1 (P1) to P50 (---; blue); AL50-100, mid–pregnancy AL feeding P51-P100 (— —; green); AL100-140, late–pregnancy AL feeding P101-P140 (— • • - -; purple); AL0-100, early-and mid-pregnancy AL feeding P1-P100 (—••-••; red); T140, pregnancy AL feeding P1-P140 (— , black). Outside the aforementioned AL feeding periods ewes were offered the same pasture allowance as the control treatment.