Author/s:
Zamuner, Fernanda

Title:
Endocrine and metabolic status of dairy goats during the transition period

Date:
2020

Endocrine and metabolic status of dairy goats during the transition period

A thesis by

FERNANDA ZAMUNER

Submitted for the Degree of Doctor of Philosophy

Endocrine and metabolic status of dairy goats during the transition period

FERNANDA ZAMUNER

Bachelor of Science

ORCID ID: 0000-0002-9409-8779

Submitted in total fulfillment of the requirements for the degree of

Doctor of Philosophy – Agricultural Sciences

December 2020

School of Agriculture and Food

Faculty of Veterinary and Agricultural Sciences

The University of Melbourne

Parkville, Victoria

Australia

ABSTRACT

In dairy animals, the transition period, which spans from 3 weeks before to 3 weeks after parturition, is the most stressful time in their productive lives. This period is characterized by drastic physiological, metabolic, and endocrine adaptations to accommodate parturition and lactogenesis. Goats unable to adapt to this challenging time are more susceptible to infections and metabolic diseases, which might have a substantial impact on maternal health and productive efficiency beyond the transition period. An in-depth understanding of the biology of the transition period is essential for developing optimized strategies that could enhance milk yield without compromising herd health and welfare. While there is ample published information regarding the transition period in dairy cattle, to date, the endocrine and metabolic status of periparturient dairy goats has only been vaguely described in the literature.

Therefore, the overall goal of this dissertation was to expand on previous knowledge of hormonal and metabolic regulation of energy metabolism during the transition period and to explore factors that might aggravate the metabolic burden of pregnancy and lactation in periparturient dairy goats. Thus, a series of studies were conducted in a large commercial goat dairy farm in Australia to 1) determine the effects of month of kidding, parity number, and litter size on lactation curves of dairy goats raised in intensive systems; 2) characterize temporal variations in circulating levels of selected hormones and metabolites involved in energy balance regulation during the transition period; 3) investigate the effects of level of milk production, parity number and litter size on maternal metabolic profile; 4) determine whether higher plasma concentrations of markers of negative energy balance are associated with inferior productive performance; and 5) determine whether differential productivity is related to differences in nutrient partitioning between high- and low-yielding goats.

In the first part of this study, an analysis of the production data revealed that goats kidding in spring, in third/fourth parity, or carrying multiple fetuses produce more milk than their counterparts. Interestingly, although the month of kidding had the most significant impact on the shape of lactation curves, the magnitude of such impact increased with increasing parity number. Also, based on the concentration of key biomarkers of energy metabolism analyzed during this time, it was possible to conclude that nutritional deficit was increased with increasing milk yield, parity, and litter size (listed in order of importance) and that both pregnancy and lactation were less able to elicit lipomobilization in primiparous compared with multiparous goats. Further, the likelihood of early removal from the milking herd was significantly increased in goats with elevated blood levels of β-hydroxybutyrate (BHB). On the other hand, contrasting studies in dairy cows, a positive association was observed between blood levels of non-esterified fatty acids (NEFA) and milk yield.

Nevertheless, it was unclear what role, if any, the endocrine system played in the differential productivity in early lactation observed between high- and low-yielding goats. Therefore, in the second part of this study, goats of high and low milk yield were subjected to 3 metabolic challenges (glucose, insulin, and adrenocorticotropin hormone infusions) to determine if differential productivity is related to differences in some aspects of the regulation of nutrient partitioning in dairy goats. The results suggested that differences in milk yield, and overall production efficiency in early lactation, are primarily due to differences in insulin secretion and clearance rates rather than related to differences in peripheral tissue responsiveness to the effects of catabolic and anabolic hormones.

In summary, the research within this thesis provides the first comprehensive overview of both lactation performance and the metabolic status of Australian dairy goats. Collectively, the novel findings presented here contribute to further the current understanding of various aspects of the regulation of energy metabolism in periparturient dairy goats. Just as important, this

study also provides the local industry with robust and relevant information on the effects of several factors on the productive and metabolic responses of dairy goats during the transition period. Such information can assist with the optimization of farming practices and breeding plans, thereby accelerating increments in the national herd productivity.

DECLARATIONS

This declaration is to certify that:

i The thesis comprises only my original work towards the Ph.D.,

ii Due acknowledgment has been made in the text to all other material used,

iii The thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Fernanda Zamuner

Date: 06/12/2020

PREFACE

This thesis comprises seven chapters, including Introduction, Literature Review, General Discussion, and four experimental chapters (stand-alone scientific papers). I was the lead investigator in all these chapters. Sample collections, subsequent data analysis and interpretation, and writing of the manuscripts were my responsibility.

For all published work in this thesis, the respective co-authors have completed The University of Melbourne's co-author authorization form to certify that the candidate's contribution to the publication was greater than 50%. The candidate's principal supervisor, Professor Brian J. Leury has signed The University of Melbourne's declaration for a thesis with publication form. Manuscripts submitted or intended to be submitted for publication are formatted according to the respective journal's formatting requirements.

PUBLICATIONS ARRIVING FROM THIS THESIS

Manuscripts:

This thesis contains the following original manuscripts, where I was the lead author:

1. Full title: **Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia.**

Authors: Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury.

Status: Published by the Journal of Dairy Science on October 16, 2019

https://doi.org/10.3168/jds.2019-17051

2. Full title: **Endocrine and metabolic status of commercial dairy goats during the transition period.**

Authors: Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury.

Status: Published by the Journal of Dairy Science on March 27, 2020

https://doi.org/10.3168/jds.2019-18040

3. Full title: **Associations between non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and glucose in periparturient dairy goats.**

Authors: Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury.

Status: Published by the Journal of Dairy Science on April 21, 2020

https://doi.org/10.3168/jds.2019-17163

4. Full title: **Endocrine and metabolic responses to glucose, insulin, and adrenocorticotropin (ACTH) challenges in early lactation dairy goats of high and low milk yield.**

Authors: Zamuner, F., A.W.N. Cameron, E. K. Carpenter, B.J. Leury, K. DiGiacomo

Status: Published by the Journal of Dairy Science on November 18, 2020

https://doi.org/10.3168/jds.2020-18625

Conference Abstracts:

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2020. **Combined effects of month of kidding and stage of lactation on goat milk composition.** 71st Annual Meeting of the European Federation of Animal Science. Porto, Portugal. Oral presentation. Presenting author. (Accepted)

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2019. **Prevalence of hyperketonemia in periparturient dairy goats.** 70th Annual Meeting of the European Federation of Animal Science. Ghent, Belgium. Oral presentation. Presenting author.

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2018. **Impact of parity and number of fetuses on milk production and metabolic health in dairy goats.** Australian Association of Ruminant Nutritionists (AARN) Conference, Wagga Wagga, NSW, Australia. Oral presentation. Presenting author.

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2018**. Effects of parity and litter size on milk yield of commercial dairy goats in Australia.** 32nd Conference of the Australian Society of Animal Production, Wagga Wagga, NSW, Australia. Poster presentation. Presenting author.

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2018. **Changes in maternal blood beta-hydroxybutyrate and plasma non-esterified fatty acids concentrations in periparturient commercial dairy goats bearing single and twin fetuses.** 10th International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, France. Poster presentation. Presenting author.

Zamuner, F., K. DiGiacomo, A.W.N. Cameron, and B.J. Leury. 2018. **The effect of season of kidding on milk yield of commercial dairy goats in Australia.** 69th Annual Meeting of the European Federation of Animal Science. Dubrovnik, Croatia. Poster presentation. Presenting author.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my supervisors Prof. Brian Leury, Dr. Kristy DiGiacomo, and Dr. Alexander (Sandy) W.N. Cameron for their assistance, guidance, and supportive supervision through my doctoral studies. Thank you to all of you. It was an excellent experience for me to be around you guys. I would also like to thank Dr. Brendan Cullen for being an exceptional chair of my Ph.D. committee.

To Brian, my principal supervisor, thank you for the continuous support of my Ph.D. study. Your patience, motivation, and immense knowledge have given me more power and spirit to excel in research writing. I'm glad that even though, in the beginning, I told you about my weaknesses and doubts you looked past it and gave me a chance to be part of your research team and to lead this research project.

Kristy, I would like to thank you for your insightful suggestions, and your constant guidance and feedback on my work throughout my studies. Your help was instrumental in keeping me grounded and focused on what was really important to my research.

To Sandy, who convinced me that I should pursue my doctoral degree, thank you for your enthusiasm and encouragement. Your insight into addressing real-world problems was vital in inspiring me to think outside the box, from multiple perspectives, to form a comprehensive and objective critique.

I will forever be thankful to Sandy and July Cameron for their invaluable cooperation and assistance during my fieldwork. Sandy and July allowed me almost unlimited access to the Meredith Dairy infrastructure, animals, and equipment, they provided me with accommodation while I was in Meredith, and most importantly, they kindly engaged all the staff members at Meredith Dairy in helping me through my fieldwork.

I would like to thank all personnel of Meredith Dairy Ltd, especially Ann, Ashley, Eddie, Gustavo, Katherine, Lesley, Melissa, Molly, Murray, Paul, and Richard. I am also grateful to them for their friendship and the warmth they extended to me during my time in Meredith, and for always making me feel so welcome.

My deep appreciation goes out to my colleagues Maree Cox, Shannon Holbrook, Leonie Walter, Evan Bittner, Emily Ruffles, Rachael Tromp, and Emerson Carpenter for excellent work during data collection, which was an invaluable contribution towards my Ph.D. Their timely help and friendship shall always be remembered.

I also owe my finest gratitude to all my friends in Melbourne. There are too many to name, but I would like to give a special thanks to Elias and Teresa for their kindness and hospitality. You guys were my family away from home. I also would like to thank my dearest friends, Paulo, Alvaro, Maria Ines, Viviana, and Thiago, whose love, care, and emotional support have kept me centered and given me the push I needed to get through the hard times.

I would like to say a heartfelt thank you to my family for providing me with moral and emotional support in my life. I must express my profound gratitude to my sister Daniela for her unconditional trust, endless patience, and for helping me in whatever way she could during this challenging period.

I gratefully acknowledge the funding sources that made my Ph.D. work possible. My work was supported by The Australian Government Research Training Program Scholarship, by the Faculty of Agriculture and Veterinary Science at The University of Melbourne, and by Meredith Dairy, Ltd.

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
~	Approximately
<	Less Than
>	Greater Than
≤	Less Than or Equal To
≥	Greater Than or Equal To
°C	Degrees C
µg	Microgram
µU	Milli Units
AcAc	Acetoacetate
AcCoA	Acetyl-Coa
ACT	Tricarboxylic Acid Cycle
ACTH	Adrenocorticotropin
ADG	Average Daily Gain
AIR	Acute Insulin Response to Glucose
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
AST	Adrenocorticotropin Stimulation Test
AUC	Area Under the Curve
BCF	Pancreatic β-Cell Function
BCS	Body Condition Score
BHB	B-Hydroxybutyrate

BOM	Bureau of Meteorology
BW	Body Weight
BWE	Body Weight Efficiency
Ca	Calcium
CI	Confidence Interval
cm	Centimeters
CMY	Cumulative Milk Yield
CP	Crude Protein
CR	Clearance Rate
CV	Coefficient of Variation
CI	Confidence Interval
d	Day
DI	Disposition Index
DIM	Days in Milk
DM	Dry Matter Intake
DMI	Dry Matter
e.g.,	Exempli Gratia
ECM	Energy-Corrected Milk
EMY	Total Milk Yield in Early Lactation
FE	Feed Efficiency
g	Gram
g	G-Force
G_B	Basal Glucose Concentration
GH	Growth Hormone
h	Hour

HMG	3-Hydroxy-3-Methylglutarate-Coa
HOMA	Homeostatic Assessment Model
HY	High Milk Yield
I_B	Basal Insulin Concentration
IGF-1	Insulin-Like Growth Factor 1
IR	Insulin Resistance
ITT	Insulin Tolerance Test
IU	International Units
IVGTT	Intravenous Glucose Tolerance Test
JUN	June
kg	Kilogram
L	Littre
LMP	Level of Milk Production
LMY	Total Milk Yield in Late Lactation
LS	Litter Size
LY	Low Milk Yield
MAR	March
ME	Metabolizable Energy
Milk-S	Individual milk produced the day before blood sampling
MJ	Mega Joules
MK	Month of Kidding
mL	Milliliter
m*M*	Millimolar
mmol	Millimolar
MMY	Total Milk Yield in Mid Lactation

MP	Metabolizable Protein
MY	Medium Milk Yield
N	Number of Observations
NDF	Neutral Detergent Fiber
NE	Net Energy
NEB	Negative Energy Balance
NEFA	Nonesterified Fatty Acid
ng	Nanogram
NOV	November
NP	Net Protein
NS	Not Significant
NSW	New South Wales
OCDE	Organization for Economic Co-Operation and Development
P	Phosphorus
P	P-Value
PN	Parity Number
PUN	Plasma Urea Nitrogen
r	Correlation
REML	Restricted Maximum Likelihood
RIA	Radioimmunoassay
RIRDC	Rural Industries Research and Development Corporation
SCC	Somatic Cell Count
SCS	Somatic Cell Score
SD	Standard Deviation
SE	Standard Error

SED	Standard Error of Differences
SEM	Standard Error of Means
SEP	September
S_G	Glucose Effectiveness
S_I	Insulin Sensitivity
$T_{1/2}$	Time to Reach Half-Life Concentration
T_{basal}	Time to Reach Basal Concentration
TCA	Tricarboxylic Acid Cycle
TMR	Total Mixed Ration
T_{peak}	Time to Reach Maximal Concentration
TS	Total Solids
U	Units
UK	United Kingdom
US	United States of America
VFA	Volatile Fatty Acids
VIC	Victoria
VLDL	Very-Low-Density Lipoprotein
vs.	Versus
wk	Week
wks	Weeks
yr	Year
yrs	Years

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1 – Introduction

1.1 Dairy Goat industry around the world

Over the last century, the rising demand for niche dairy products from increasingly wealthy urban consumers has created an unprecedented demand for goat dairy products worldwide. During the past decade (2007 to 2017), the global dairy goat population and total milk production increased 22% and 16%, respectively, reaching 218 million head and 18.7 million kg of goat milk produced in 2017 (Miller and Lu, 2019).

Most of the world's dairy goat population is found in Asian (52%) and African (39%) countries, followed by Europe (5%), Americas (3%), and Oceania (1%) (Miller and Lu, 2019). In developing countries, goat farming is often seen as a strategy for lifting people out of poverty and improving food security, while in developed countries, the dairy goat sector expansion is strongly linked to consumer's growing interest in specialty dairy products and goat's milk therapeutic properties (Escareño et al., 2012).

Europe, more specifically, the Mediterranean basin, has the most organized market for goat milk, and the largest source of information on dairy goat systems (Miller and Lu, 2019). Home to only 5% of the world goat population, Europe accounts for 15% of the global goat milk, and 35% of all goat cheese produced worldwide (Morales et al., 2019). The European dairy goat industry is very diverse but mainly market-oriented, and its production system resembles the dairy cow industry when it comes to the technologic level used on commercial farms, national programs for genetic improvement, and government subsidies or financial support to dairy goat farmers (Escareño et al., 2012).

New dairy goat industries are also thriving in other countries, such as China, New Zealand, United States (US), and Canada. Compared to the European industry, the North American dairy

goat sector is still considered "less mature" but it is expanding rapidly, showing a dramatic increase in the number of dairy goat farms registered over the last 20 years (Lu and Miller, 2019). In both the US and Canada, dairy goat farms are becoming larger and more commercialized, while research centers and producer associations are working together for the development of national research programs to advance the industry in several areas, such as education, research, and regulations for production, processing, and marketing of goat milk (Lu and Miller, 2019).

1.2 Australian Dairy Goat Industry

In Australia, goat dairying is regarded as an emerging industry, which makes a small but valuable contribution to the health and wealth of Australians (Foster, 2014). In 2016, the national herd (approximately 30,550 milking goats) produced 16.9 million liters of milk, most of it produced in Victoria (35%) and Queensland (25%), followed by South Australia (15%), New South Wales (15%), Tasmania (5%), and Western Australia (5%) (Zalcman and Cowled, 2018). In Australia, as much as 60% of goat milk produced is used for cheese making, 35% is sold as whole milk or yogurts, and around 5% is processed into powder and beauty products (Zalcman and Cowled, 2017, 2018).

Stimulated by the increasing popularity of specialty fresh dairy goat products among Australian consumers, the enterprise structure is fast-moving from small family-owned businesses to larger production scale and intensive husbandry systems (Celi and White, 2012; Zalcman and Cowled, 2017). Indeed, recent reports show that while the number of licensed farms and the average milk yield per head (300-d lactation) had a sluggish growth of less than 5% from 2009 to 2016, the national herd and total milk production increased over 60% during the same period (Stubbs and Abud, 2009; Zalcman and Cowled, 2018).

1.2.1 Current Challenges

Despite its impressive expansion, the Australian dairy goat industry still faces several challenges, including a generalized lack of evidence-based information in most areas of dairy goat husbandry (e.g., health, welfare, and production). Several authors have expressed their concerns about the fact that the enterprise expansion has not being paralleled by an increase in government investment in research and development of the dairy goat sector, which in its turn, could represent a severe limitation to the long-term viability of the Australian industry (Stubbs and Abud, 2009; Celi and White, 2012; Banks and Walkom, 2016; Zalcman and Cowled, 2018).

For instance, in recent decades, Australian dairy goat producers have thrived by exploring their main competitive advantage against European producers — producing fresh dairy products (e.g., fresh cheese, yogurt, whole milk, fermented milk, fresh cream) for the domestic market (Cameron, 2014). The drawback of fresh dairy products is their limited shelf life, requiring a constant year-round supply of milk to manufactures if shelf space at retailers' shops is to be maintained (Cameron, 2003). However, the national production of goat milk remains highly seasonal, and the lack of steady year-round milk supply is one of the major constraints in the Australian industry (Celi and White, 2012; Zalcman and Cowled, 2018).

Dividing the milking herd into multiple kidding seasons per year is an effective way to achieve year-round uniform milk production consistent with market needs (Shingu et al., 2002; Stubbs and Abud, 2009). Due to the seasonal reproductive pattern of goats, the management of multiple herds demands out-of-season breeding, which in turn requires significant additional input costs (e.g., hormones, light manipulation, extra labor costs), and increases farm management complexity (e.g., management of multiple milking herds and batches of kids) (Cameron, 2003). However, information on dairy systems based on multiple kidding seasons per year is very limited because both in Europe and North America, the two largest sources of

information on dairy goats, production systems are mostly based on seasonal kidding (once yearly) (Lu and Miller, 2019; Morales et al., 2019).

Moreover, many economically important performance traits are controlled by both genetic and nongenetic factors (Idowu and Adewumi, 2017), which makes information on individual traits (e.g., milk yield, milk composition, BW, BCS) and nongenetic factors (e.g., age at kidding, parity number, litter size, month (season) of kidding) a prerequisite for the development of a robust genetic improvement program (Barillet, 2007). On the other hand, accurate information on herd performance traits (e.g., average conception, mortality and culling rates, total milk yield, average lactation length) is crucial for the design of effective strategies for uniform distribution of annual milk production (Sahlu and Goetsch, 2005).

Previous studies have demonstrated that parity number and litter size can significantly affect milk fat and protein concentrations, yield, and somatic cell count (SCC) (Carnicella et al., 2008; Goetsch et al., 2011; Granado et al., 2014; Lérias et al., 2014). Also, goat milk yield and physicochemical characteristics are highly influenced by the stage of lactation and climatic conditions (e.g., photoperiod, air temperature, relative humidity) (Granado et al., 2014; Clark and García, 2017; Arnal et al., 2018; Kljajevic et al., 2018). Thus, to estimate the genetic factors contributing to milk yield, nongenetic sources of variance must also be considered, because such factors may suppress the animal's true genetic ability, thereby compromising the effectiveness of breeding programs (Djemali and Berger, 1992). However, to date, no study has explored the potential interaction between photoperiod, parity, and litter size on milk yield and composition of commercial dairy goats managed in multiple kidding seasons per year.

Furthermore, in an attempt to meet the rising demand for goat dairy products, Australian farmers are progressively adopting intensive husbandry methods and investing in genetic selection for increased milk yields (Celi and White, 2012; Zalcman and Cowled, 2017), which in turn are likely to increase the incidence of metabolic diseases (e.g., ketosis, fatty liver,

mastitis, metritis, milk fever) as a consequence of intensified metabolic burden in high-yielding goats (Celi et al., 2008; Solaiman, 2010). Nevertheless, information on the metabolic status of transitioning dairy goats remains scarce.

Therefore, studies characterizing both the productive performance and the metabolic status of Australian dairy goats, particularly of those raised in intensive husbandry systems and managed in multiple kidding seasons per year, are warranted. Such characterizations would provide farmers, and professionals in the field, with invaluable information for the development of optimized herd management and breeding plans that could enhance milk yield without compromising herd health and welfare.

1.3 Overview of Study Objectives

The overall goal of this thesis was to expand on previous knowledge of hormonal and metabolic regulation of energy metabolism during the transition period in dairy goats. The main specific objectives were:

- To estimate the relative influence of month of kidding, parity number, and litter size on goats' productive performance throughout lactation.

- To explore the temporal changes of blood biochemical parameters around parturition, and to describe how the level of milk production, parity number, and litter size can affect the maternal regulation of energy metabolism during the transition period.

- To evaluate potential associations between the excessive mobilization of body fat and adverse outcomes, such as milk loss and increased likelihood of early removal of goats from the milking herd.

- To examine underlying differences in the endocrine and metabolic regulation of energy metabolism in early lactation dairy goats of high and low milk yield.

In order to accomplish these research objectives, the remainder of this thesis is structured as follows:

Chapter 2 discusses the relevant literature concerning mechanisms involved in the regulation of energy metabolism during late pregnancy and early lactation in dairy goats.

Chapter 3 describes how kidding season, parity, and litter size influence the overall production performance of commercial dairy goats in Australia.

Chapter 4 characterizes the changes in endocrine and metabolic profiles over the transition period and describes how such changes differ according to the level of milk production, parity, and litter size of the goats.

Chapter 5 describes the temporal relationships between non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and glucose in periparturient dairy goats.

Chapter 6 investigates underlying differences in endocrine and metabolic regulation of energy metabolism in dairy goats of high and low milk yield.

Chapter 7 summarizes the major contributions of the research described in this thesis to the progress of our understanding of mechanisms regulating the energy metabolism of transition goats and outlines future research that might be of value to the Australian dairy goat industry.

1.4 References

Arnal, M., C. Robert-Granié, and H. Larroque. 2018. Diversity of dairy goat lactation curves in France. J. Dairy Sci., 101 (12):11040-11051. https://doi.org/10.3168/jds.2018-14980

Banks, R. G., and S. Walkom. 2016. Scoping study for genetic evaluation of Australian dairy goats. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 02/01/2018. https://www.agrifutures.com.au/product/scoping-study-for-genetic-evaluation-of-australian-dairy-goats/

Barillet, F. 2007. Genetic improvement for dairy production in sheep and goats. Small Rum. Res., 70 (1):60-75. https://doi.org/10.1016/j.smallrumres.2007.01.004

Cameron, A. W. N. 2003. Increase in autumn and winter milk production in dairy goats. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 13/04/2018. https://www.agrifutures.com.au/wp-content/uploads/publications/03-105.pdf

Cameron, A. W. N. 2014. Optimising genetics, reproduction, and nutrition of dairy sheep and goats. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 20/09/2018. https://www.agrifutures.com.au/product/optimising-genetics-reproduction-and-nutrition-of-dairy-sheep-and-goats/

Carnicella, D., M. Dario, M. C. C. Ayres, V. Laudadio, and C. Dario. 2008. The effect of diet, parity, year, and number of kids on milk yield and milk composition in Maltese goat. Small Rum. Res., 77 (1):71-74. https://doi.org/10.1016/j.smallrumres.2008.02.006

Celi, P., A. Di Trana, and S. Claps. 2008. Effects of perinatal nutrition on lactational performance, metabolic and hormonal profiles of dairy goats and respective kids. Small Rum. Res., 79 (2-3):129-136. https://doi.org/10.1016/j.smallrumres.2008.07.010

Celi, P., and P. J. White. 2012. Dairy goat farming in Australia: current challenges and future developments. Paper presented at the First Asia Dairy Goat Conference, Kuala Lumpur, Malaysia. http://www.fao.org/3/i2891e/i2891e00.htm

Clark, S., and M. B. M. García. 2017. A 100-year review: Advances in goat milk research. J. Dairy Sci., 100 (12):10026-10044. https://doi.org/10.3168/jds.2017-13287

Djemali, M., and P. J. Berger. 1992. Yield and reproduction characteristics of Friesian cattle under North African conditions. J. Dairy Sci., 75 (12):3568-3575. https://doi.org/10.3168/jds.S0022-0302(92)78133-8

Escareño, L., H. Salinas-Gonzalez, M. Wurzinger, L. Iñiguez, J. Sölkner, and C. Meza-Herrera. 2012. Dairy goat production systems: status quo, perspectives and challenges. Trop. Anim. Health Prod., 45 (1):17-34. https://doi.org/10.1007/s11250-012-0246-6

Foster, M. 2014. Emerging animal and plant industries: Their value to Australia. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 13/04/2020. https://www.agrifutures.com.au/product/emerging-animal-and-plant-industries-their-value-to-australia/

Goetsch, A. L., S. S. Zeng, and T. A. Gipson. 2011. Factors affecting goat milk production and quality. Small Rum. Res., 101 (1-3):55-63. https://doi.org/10.1016/j.smallrumres.2011.09.025

Granado, R. J., M. S. Rodríguez, C. Arce, and V. R. Estévez. 2014. Factors affecting somatic cell count in dairy goats: a review. Span. J. Agric. Res. (1):133-150. http://dx.doi.org/10.5424/sjar/2014121-3803

Haenlein, G. F. W. 2001. Past, present, and future perspectives of small ruminant dairy research. J. Dairy Sci., 84 (9):2097-2115. https://doi.org/10.3168/jds.S0022-0302(01)74655-3

Idowu, S. T., and O. O. Adewumi. 2017. Genetic and nongenetic factors affecting yield and milk composition in goats. J. Adv. Dairy Res., 5 (175):1-4. https://doi.org/0.4172/2329-888X.1000175

Kljajevic, N. V., I. B. Tomasevic, Z. N. Miloradovic, A. Nedeljkovic, J. B. Miocinovic, and S. T. Jovanovic. 2018. Seasonal variations of Saanen goat milk composition and the impact of climatic conditions. J. Food Sci. Technol., 55 (1):299-303. https://doi.org/10.1007/s13197-017-2938-4

Lérias, J. R., L. E. Hernández-Castellano, A. Suárez-Trujillo, N. Castro, A. Pourlis, and A. M. Almeida. 2014. The mammary gland in small ruminants: major morphological and functional events underlying milk production–a review. J. Dairy Res., 81 (3):304-318. https://doi.org/10.1017/S0022029914000235

Lu, C. D., and B. A. Miller. 2019. Current status, challenges and prospects for dairy goat production in the Americas. Asian-Australas. J. Anim. Sci., 32 (8):1244. https://doi.org/10.5713/ajas.19.0256

Miller, B. A., and C. D. Lu. 2019. Current status of global dairy goat production: an overview. Asian-Australas. J. Anim. Sci., 32 (8):1219. https://doi.org/10.5713/ajas.19.0253

Morales, F. A. R., J. M. C. Genís, and Y. M. Guerrero. 2019. Current status, challenges and the way forward for dairy goat production in Europe. Asian-Australas. J. Anim. Sci., 32 (8):1256. https://doi.org/10.5713/ajas.19.0327

Sahlu, T., and A. L. Goetsch. 2005. A foresight on goat research. Small Rum. Res., 60 (1-2):7-12. https://doi.org/10.1016/j.smallrumres.2005.06.002

Shingu, H., K. Hodate, S. Kushibiki, Y. Ueda, A. Watanabe, M. Shinoda, and M. Matsumoto. 2002. Breed differences in growth hormone and insulin secretion between lactating Japanese Black cows (beef type) and Holstein cows (dairy type). Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 132 (4):493-504. https://doi.org/10.1016/s1532-0456(02)00115-1

Solaiman, S. G. 2010. Goat science and production. John Wiley & Sons. Singapore. Accessed on 18/01/2020 https://www.wiley.com/en-es/Goat+Science+and+Production-p-9780813809366

Stubbs, A. K., and G. L. Abud. 2009. Farming and marketing goat and sheep milk products. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 13/04/2020. https://www.agrifutures.com.au/wp-content/uploads/ publications/08-207.pdf

Zalcman, E., and B. Cowled. 2017. The Australian dairy goat industry an assessment of the population and farm gate value. Rural Industries Research and Development Corporation (RIRDC). Canberra, Australia. Accessed on 13/04/2020. https://www.agrifutures.com.au/product/the-australian-dairy-goat-industry-an-assessment-of-the-population-and-farm-gate-value/

Zalcman, E., and B. Cowled. 2018. Farmer survey to assess the size of the Australian dairy goat industry. Aust. Vet. J., 96 (9):341-345. https://doi.org/10.1111/avj.12734

Chapter 2 - Literature Review

2.1 Introduction

This chapter reviews the current and historical scientific literature concerning some of the primary factors affecting the metabolic and endocrine status of dairy goats during the transition period. This review will also cover mechanisms involved in the regulation of energy metabolism and its impacts on goats' physiological parameters and productive performance. Although the focus of this review will be on periparturient dairy goats, aspects from other species will be discussed when appropriate, particularly when goat specific data is lacking.

2.2 The Transition Period

The period of three weeks before and three weeks after parturition is defined as the transition period (Wankhade et al., 2017). In dairy goats, as in dairy cows, this period is characterized by increased nutritional requirements and reduced voluntary dry matter intake (DMI) (Cannas and Pulina, 2008; Lean et al., 2013; Idamokoro et al., 2017), which often result in severe or prolonged negative energy balance (NEB) and, consequently, higher risk of production disease [e.g., gestational and lactational ketosis, hepatic lipidosis (fatty liver), hypocalcemia (milk fever)] (Cannas and Pulina, 2008; Lean et al., 2013; Matthews, 2016).

2.2.1 Voluntary dry matter intake in transition dairy goats

In goats, the decline in DMI usually begins around four weeks before partition, progressively declining as pregnancy advances, sometimes reaching as much as a 30% reduction during the last week prepartum (Idamokoro et al., 2017). Some of the primary factors responsible for the reduced DMI in transition goats are physical compression of the rumen by

the growing uterus, increased degree of fatness, and sex hormones (Forbes, 2007; Cannas and Pulina, 2008).

Small ruminants are particularly susceptible to a marked reduction in voluntary intake in late pregnancy because contrasting cattle that generally only give birth to single offspring, goats, and sheep often give birth to twins, triplets, and occasionally quadruplets (Lérias et al., 2014). Increasing litter size is associated with greater physical restriction to rumen extension and with increasing levels of estrogen and mammogenic hormones, which negatively impact voluntary feed intake (Forbes, 2007). Additionally, goats can store over 50% of their body fat reserves as visceral fat (Jibir et al., 2013). Thus, given that an increased abdominal fat mass, alongside the ever-expanding uterus, will further limit rumen expansion, over-conditioned pregnant goats carrying multiple kids might show an even steeper decrease in DMI during the last month of pregnancy (Lima et al., 2012).

2.2.2 Energy requirements of transition dairy goats

The daily metabolizable energy (ME) and metabolizable protein (MP) required for maintenance in dairy goats is approximately 0.5 MJ ME and 2.2 g MP per kg of $BW^{0.75}$ (Cannas and Pulina, 2008). According to these authors, the nutrient requirements for pregnancy can reach up to 5.4 MJ ME/d and 58 g MP/d in the last month of gestation. Assuming the maintenance requirements of a 70-kg goat (12 MJ ME/d and 53 g MP/d), pregnancy would increase maternal ME and MP requirements by 45% and 109% (above maintenance), respectively.

Moreover, in goats, the net requirement for pregnancy is directly related to litter size (NRC, 2007). According to Härter et al. (2016), twin pregnancy increases the daily net energy (NE) requirements by 31% and the daily net protein (NP) requirements by 38%. Given that increasing litter size reduces voluntary intake at the same time that it increases nutrient

requirements for fetal development, multiple pregnancies are likely to represent a bigger metabolic challenge to the pregnant dam (Cannas and Pulina, 2008; Matthews, 2016).

The onset of lactation can represent an even more drastic increase in nutrient demands. The average nutritional cost of goat milk production is approximately 5MJ ME and 45 g MP per kg of milk with 3.5% fat (Cannas and Pulina, 2008). Assuming the maintenance requirement of a 70-kg goat, the production of 4 kg milk would increase daily requirements for ME and MP by 2 and 3 folds, respectively. Therefore, given the high prolificacy and improved milk production of modern dairy goats, commercial goats might be at risk of developing metabolic complications, both ante- and postpartum (Cannas and Pulina, 2008).

2.3 Endocrine and Metabolic Status During the Transition Period

Endocrine and metabolic responses to NEB are primarily aimed at providing alternative energy sources (for maternal metabolism) via mobilization of endogenous substrates, thereby sparing glucose and amino acids for fetal growth and, later, for lactation (Chilliard et al., 2000). Body fuels consist of carbohydrates, amino acids, and fats (Herdt, 1988). Shifts in the use and conservation of these fuels are primarily based on the availability and supply of glucose, NEFA, and ketone bodies (Bauman, 2000).

The endocrine system is responsible for regulating interactions among metabolic fuels and fuel-using organs and tissues, partitioning nutrients according to different tissues' priorities for available nutrients (e.g., growth, pregnancy, lactation) (Herdt, 2000). There are a plethora of hormones at play in the long-term regulation of nutrient partition. However, insulin, insulin-like growth factor (IGF-1), placental lactogen (PL), growth hormone (GH), prolactin, and glucocorticoids are fuel-regulatory hormones with a crucial role in the adaptation to NEB in the peripartum (Bauman, 2000; Herdt, 2000). These physiological adaptations modify the concentration of several biochemical parameters in the bloodstream, which make the use of hormonal and metabolic profiles an essential tool for (1) evaluation of individual and herd

nutritional status, (2) early detection of metabolic problems and production diseases, and (3) identification of metabolically superior animals (Van Saun, 2009).

However, a complex interplay of biological and environmental effects influence physiological functions and affects the nature and extent of maternal adaptations to support pregnancy and lactation (Bell, 1995; Herdt, 2000). For instance, previous studies in dairy goats have revealed that hormonal and metabolic profiles of dairy goats can be significantly affected by several nongenetic factors including nutritional state (Celi et al., 2008), environmental conditions (Lacasse et al., 2016; Pehlivan et al., 2018), parity (Magistrelli and Rosi, 2014; Radin et al., 2015), litter size (Khan and Ludri, 2002; Castagnino et al., 2015), and diseases (Albay et al., 2014; Marutsova and Binev, 2017). Therefore, while the characterization of endocrine and metabolic changes in transition goats is complex, such characterization can contribute to a more integrated understanding of the energy metabolism in dairy goats, which is essential for developing optimized strategies that could enhance milk yield without compromising herd health and welfare.

2.4 Metabolic Fuels and Fuel Regulatory Hormones

2.4.1 Glucose

In ruminants, as in any other mammal, pregnancy and lactation impose a drastic increase in the demand for glucose because glucose is a primary nutrient for conceptus growth and milk synthesis (Bell and Bauman, 1997). However, in ruminants, pregastric fermentation converts most of the dietary carbohydrates to volatile fatty acids (VFA), leaving as little as 5 to 20% of the consumed carbohydrates to be digested in the small intestine (Bell and Bauman, 1997). Given the limited ability to convert dietary carbohydrates into glucose, ruminants are dependent upon hepatic and renal gluconeogenesis to provide for as much as 90 to 100% of the glucose needed for metabolism (Larsen and Kristensen, 2013). The major gluconeogenic

precursors are propionate (60-74%), lactate (16-26%), amino acids (11-16%), valerate, isobutyrate (5%–6%), and glycerol (0.5-3.0%) (De Koster and Opsomer, 2013; Larsen and Kristensen, 2013). The relative contribution of endogenous and exogenous substrates to blood glucose levels changes according to the physiological stage, feed intake, tissue mobilization, and energy balance of the animal (De Koster and Opsomer, 2013).

The liver plays a crucial role in maintaining energy homeostasis as it acts as a metabolic hub by coordinating metabolism with other tissues and constantly adjusting internal metabolic activities in response to changes in nutrient availability (e.g., glucose, lipid, and amino acid; **Figure 2.1**) (Herdt, 2000; Rui, 2011). In addition to increased hepatic gluconeogenesis, increased muscle proteolysis and decreased protein catabolism in the liver, increased NEFA mobilization from adipose tissue, and increased peripheral tissue utilization of NEFA and BHB, are some of the other major metabolic adaptations to NEB (Herdt, 1988; Bell, 1995; Herdt, 2000).

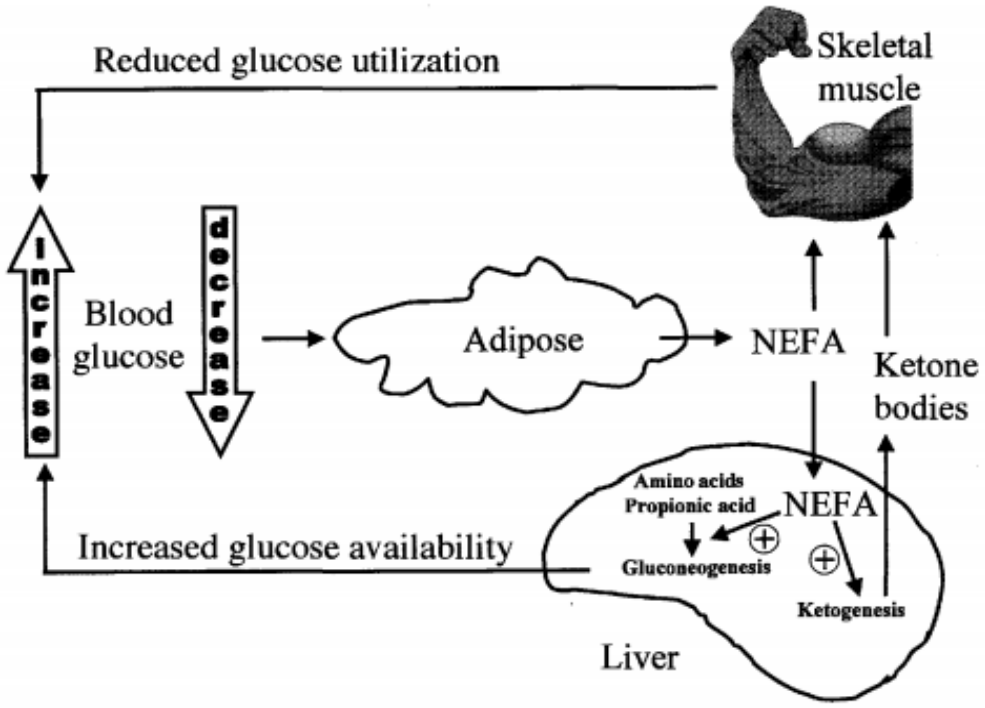

Figure 2.1. Metabolite-mediated feedback control of energy homeostasis. Sourced from Herdt (2000).

The combined effects of these metabolic adjustments result in increased glucose and amino acid availability for fetal growth and milk synthesis, while the maternal tissues become more reliant on NEFA and ketones (Bell, 1995). However, the fetal-placental unit and the mammary gland are potent drains for blood glucose and amino acids, and despite those adaptative mechanisms, glucose concentration often drops in periparturient ruminants (Herdt, 2000; Harðarson and Ingvartsen, 2005; Lima et al., 2012).

2.4.2 Non-Esterified Fatty Acids (NEFA)

The reduction in blood glucose increases the glucagon/insulin ratio, which triggers adipose tissue mobilization and NEFA release in the bloodstream (Herdt, 2000). The circulating NEFA can then be used as an alternative energy source to glucose by various tissues, including the mammary gland, liver, spleen, and muscle (Bell and Bauman, 1997). Due to the glucose-sparing effect, increased concentrations of NEFA contribute to rebalancing fuel availability by, indirectly, raising blood glucose concentration and initiating negative feedback loops to regulate the amount of lipolysis (Herdt, 2000).

The liver extracts a large portion of circulating NEFA because of its high blood flow and high NEFA extraction efficiency (Herdt, 1988; Emery et al., 1992). As shown in **Figure 2.2**, once inside the liver, NEFA can follow four pathways: (1) complete oxidation in the tricarboxylic acid cycle to produce adenosine triphosphate (ATP); (2) transport out of the liver in very-low-density lipoproteins (VLDL); (3) transformation to ketone bodies via β-oxidation; (4) storage in the liver as triglycerides (Ospina et al., 2013).

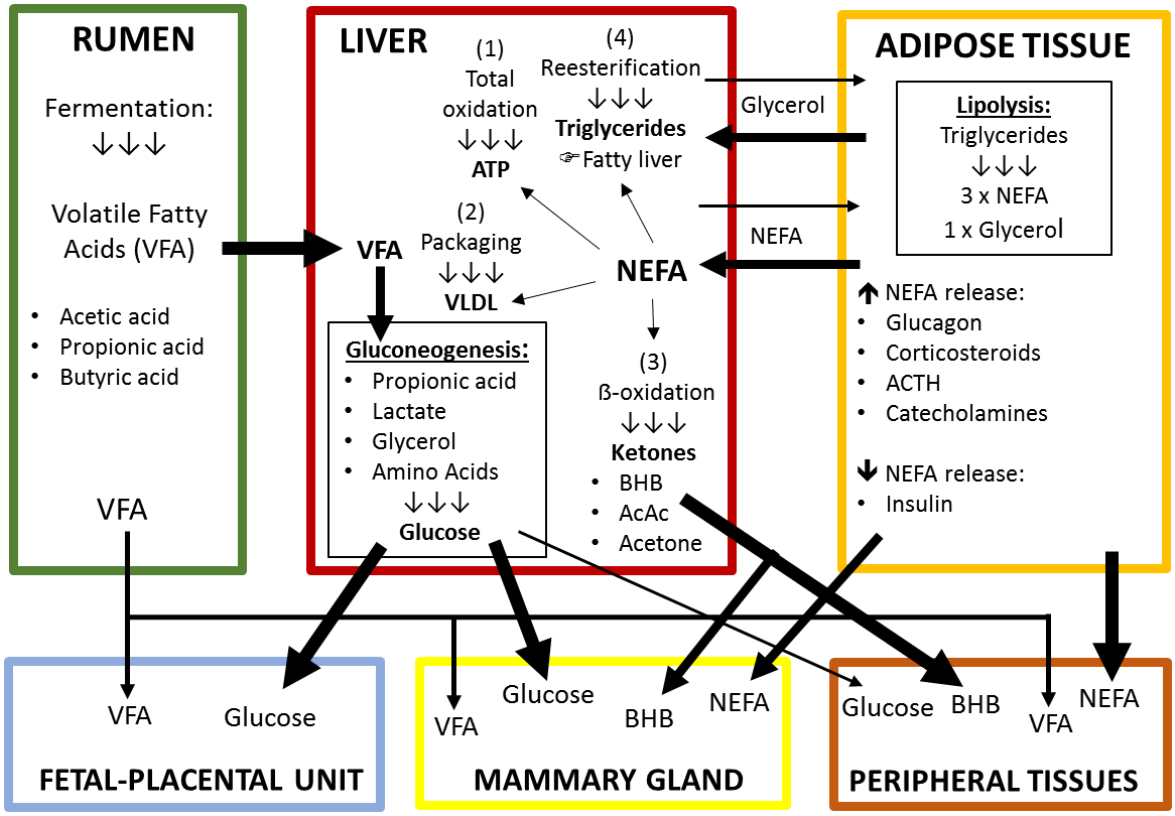

Figure 2.2. The relationships between energy demands, energy reserves, and metabolic fuels. The thickness of arrows indicates the importance of the metabolite or tissue in production or use during negative energy balance. NEFA = non-esterified NEFA, BHB = β-hydroxybutyrate, ACTH = adrenocorticotropic hormone, ATP = adenosine triphosphate, AcAc = acetoacetate, VLDL = very-low-density lipoprotein. Modified from Ospina et al. (2013)

During severe or prolonged NEB, increasing amounts of NEFA are released into the bloodstream (Chilliard et al., 2000), which results in excessive hepatic NEFA uptake (Herdt, 1988; Emery et al., 1992). However, the ruminant liver capacity to oxidize NEFA completely (route 1; **Figure 2.2**) or to export triglycerides in VLDL (route 2; **Figure 2.2**) is limited (Emery et al., 1992). Although the liver can partially oxidize large amounts of NEFA via β-oxidation (route 3; **Figure 2.2**), when the rate of NEFA uptake exceeds the hepatic capacity for NEFA oxidation plus VLDL synthesis, the surplus of NEFA is stored in the liver as triglyceride (route 4; **Figure 2.2**) (Harðarson and Ingvartsen, 2005; Rui, 2011). Therefore, excessive adipose

mobilization may result in hepatic accumulation of triglycerides, a process known as hepatic steatosis (fatty liver) (Herdt, 2000).

In addition to the harmful effects of hepatic steatosis on liver function (e.g., reduced gluconeogenic and ureogenic hepatic capacity) (Ospina et al., 2013), several studies in dairy cows have reported associations between elevated NEFA concentrations and pronounced inflammatory reactions, increased oxidative stress, and impaired immune responsiveness, which consequently leads to a higher occurrence of production diseases (e.g., displaced abomasum, retained placenta, milk fever, ketosis, metritis, mastitis) (Overton et al., 2017; Wankhade et al., 2017). Elevated NEFA concentration has also been related to decreased feed intake, milk production, and reproductive capacity in dairy cows (Wankhade et al., 2017).

Over the last 20 years, several research groups have investigated the use of NEFA as a marker of excessive NEB, and antepartum NEFA ≥ 0.3-0.5 mmol/L, and postpartum NEFA ≥ 0.7-1.0 mmol/L thresholds have been established to predict the risk of NEB-related diseases and milk loss in transition dairy cows (Ospina et al., 2013; Overton et al., 2017). In contrast to the extensive information on dairy cows, only limited information is available on transitioning dairy goats, and specific NEFA reference values have not been established for goats (Radin et al., 2015).

Nevertheless, studies conducted in dairy goats have shown that during the first month of lactation, well-fed multiparous goats can mobilize similar amounts of body fat of nonlactating nonpregnant goats being fed only 25% of maintenance requirements (Dunshea et al., 1988; Dunshea et al., 1990). These findings reinforce the importance of preventing excessive lipid mobilization in transition dairy goats because elevated blood NEFA is closely associated with increased lipolysis of milkfat and the occurrence of rancid and tart flavors in goat milk (Eknæs et al., 2009; Park et al., 2017). Therefore, the prompt identification and mitigation of excessive

NEB is not only a matter of animal welfare but could also be essential for ensuring a consistently high-quality milk supply.

2.4.3 Ketone Bodies

The term ketone bodies refer to three molecules; acetoacetate (AcAc), acetone, and BHB, with BHB, the most abundant of the three (Fukao et al., 2014). Exogenous and endogenous ketogenic precursors can be used for ketone production (Lean et al., 1992; Herdt, 2000). In ruminants, butyric acid is the primary exogenous ketone precursor because it is readily converted into AcAc and BHB during absorption across the rumen epithelium (Figure 1.3) (Lean et al., 1992).

The primary source of endogenous ketogenic precursors is the NEFA mobilized from adipose tissue (Lean et al., 1992). There are two steps for complete hepatic oxidation of NEFA. First, NEFA is cleaved in several molecules of acetyl-CoA (β-oxidation), and then acetyl-CoA is oxidized to CO2 in the tricarboxylic acid cycle (TCA) (Lehninger, 2017). The complete oxidation of acetyl-CoA in the TCA necessitates a minimal input of oxaloacetate or oxaloacetate precursors (Lean et al., 1992; Lehninger, 2017). However, during starvation, much of the oxaloacetate available is used for glucose synthesis via gluconeogenesis, thereby slowing down TCA reactions and resulting in an accumulation of acetyl-CoA in the liver (Lehninger, 2017). By converting acetyl-CoA into ketones, the liver can export a great deal of the absorbed NEFA, in the form of AcAc and BHB, at the same time that it frees up CoA for continued fatty acid oxidation (Herdt, 2000; Lehninger, 2017).

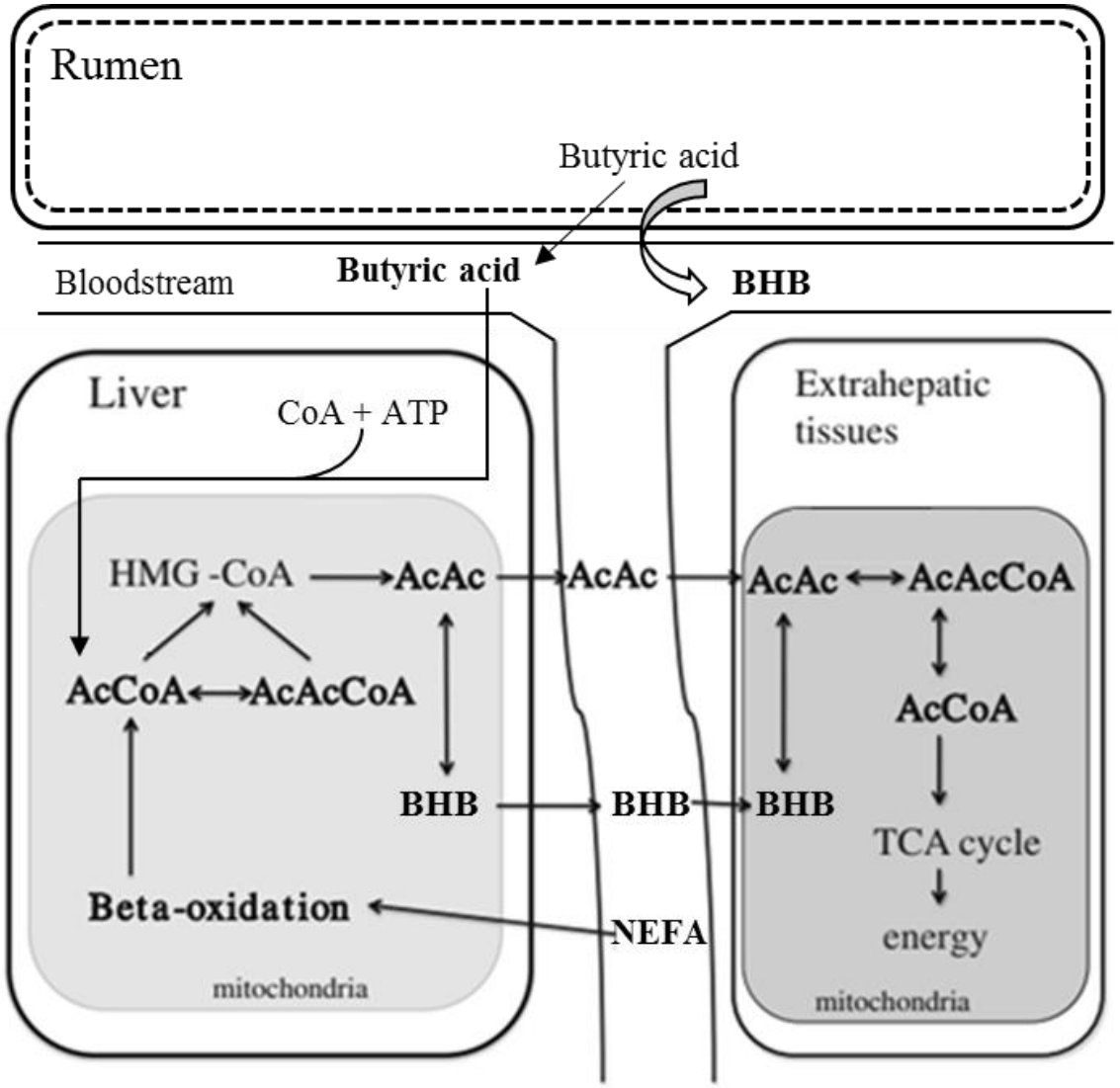

Figure 2.3. Summary of ketone body metabolism. The liver converts butyric acid and NEFA into ketone bodies (BHB and AcAc) that diffuse from liver mitochondria to the circulation and then to extrahepatic tissues for use in energy production or synthesis. NEFA = non-esterified fatty acids, BHB = β-hydroxybutyrate, AcAc = acetoacetate, TCA = tricarboxylic acid cycle, HMG = 3-hydroxy-3-methylglutarate-CoA, CoA = Coenzyme A, AcCoA = acetyl-CoA, AcAc-CoA = acetoacetyl-CoA, ATP = adenosine triphosphate. Modified from Fukao et al. (2014)

In comparison with NEFA, ketones are quite soluble in blood, can diffuse freely across cell membranes and thus not requiring binding proteins for cellular absorption, and are not highly toxic if unbound (Lean et al., 1992). Circulating ketones are readily available for uptake by

extrahepatic tissues (e.g., heart, kidney, skeletal muscle, placenta, and the lactating mammary gland) to be used as an energy substrate, thereby sparing glucose oxidation in these tissues (Lehninger, 2017). Additionally, according to Lean et al. (1992), ketone bodies are not only an alternate energy source, but they may have an essential role in inhibiting protein degradation, given that ketone bodies oxidation compensate for the energy deficit, ensuring that protein resources are not rapidly depleted during periods of increased demand for endogenous gluconeogenic precursors. Another beneficial effect of ketone bodies, alongside with NEFA, provide an essential substrate for milk fat synthesis (Herdt, 2000).

Nevertheless, in cases of excessive lipolysis, ketone synthesis frequently exceeds the peripheral tissue uptake capacity, which might result in an elevated concentration of ketones (hyperketonemia) in the bloodstream, milk, and urine (Doré et al., 2015). Hyperketonemia, when accompanied by clinical symptoms, is usually referred to as pregnancy toxemia or gestational ketosis in pregnant animals, and as lactational ketosis after delivery. Some of the clinical manifestations of ketosis are reduced feed intake, reduced milk yield, bodyweight loss, and impairment of the central nervous system (e.g., staggering, lack of coordination, blindness) (Rook, 2000).

In summary, NEFA concentration reflects the magnitude of mobilization of body fat, while BHB reflects the completeness of fat oxidization in the liver, providing an indirect measure of hepatic saturation with NEFA (LeBlanc, 2010). Thus, due to a close association between changes in plasma concentrations of NEFA and BHB with shifts in the use of energy reserves, NEFA and BHB are the two most useful metabolic parameters for monitoring the metabolic health of dairy animals in the transition period (Overton et al., 2017).

2.4.4 Plasma Urea Nitrogen

Associated with changes in carbohydrate and lipid metabolism, changes in protein metabolism also occur during the transition period. Activation of tissue protein breakdown is

another maternal mechanism to compensate for deficient dietary amino acid supply. Protein catabolism favors gluconeogenesis by supplying the liver with gluconeogenic precursors, at the same time that increases amino acids availability for fetal development and milk synthesis (Herdt, 1988; Bell, 1995; Lean et al., 2013).

Enhanced proteolysis results in an elevation of plasma urea nitrogen (PUN) and creatinine concentrations, which, in turn, can be used as an extra tool in NEB diagnosis (Van Saun, 2009). However, alterations in PUN should be carefully analyzed, as PUN concentration can be influenced by several different factors, such as dietary protein and carbohydrate intake and rumen degradability, protein intake relative to requirements, liver and kidney function, muscle tissue breakdown, etc. (Van Saun, 2009), but also by physiological and hormonal changes inherent to the transition period (Brun-Bellut, 1997).

2.4.5 Insulin

Insulin is produced by pancreatic β-cells in the islets of Langerhans and is considered the primary anabolic hormone controlling intermediary metabolism (Wilcox, 2005). Insulin is a key player in the regulation of nutrient homeostasis because its acts simultaneously in several target tissues (e.g., liver, muscle, adipose), promoting uptake and storage of metabolic fuels (carbohydrates, lipids, and amino acids) within cells, and stimulating glycogenesis, lipogenesis, and protein synthesis, and inhibiting gluconeogenesis, glycogenolysis, and lipolysis (Petersen and Shulman, 2018). Insulin secretion is stimulated by energetic substrates, with glucose being the most important, followed by NEFA and ketone bodies, and its secretion is inhibited by hypoglycemia, norepinephrine, somatostatin, and prostaglandins (Wilcox, 2005).

The action of insulin starts with binding to the receptors located on the plasma membrane of virtually all mammalian cells (Watanabe et al., 1998). However, the magnitude of the biological response of a given tissue will vary according to the number of insulin receptors, the

receptor-binding affinity for insulin, as well as tissue sensitivity to insulin regarding intracellular signaling and gene expression (Sasaki, 2002). Additionally, the sensitivity of peripheral tissues to insulin can be affected by several factors such as elevated NEFA or BHB levels, obesity, inflammation (Petersen and Shulman, 2018), reproductive hormones (e.g., estrogen, progesterone, prolactin), and counterregulatory hormones (e.g., glucagon, catecholamines, cortisol, GH) (Bell and Bauman, 1997).

Insulin also plays a vital role in several physiological functions such as gene expression, protein degradation, cell differentiation, survival, and proliferation, and organ development (Tokarz et al., 2018). However, the mechanisms of insulin action on glucose uptake are the most studied action of insulin in ruminants (Sasaki, 2002). Most cells capture glucose by facilitated diffusion via glucose-transporters (GLUT). There are 14 types of GLUTs, all of them having a specific tissue distribution and responsiveness to insulin (**Figure 2.4**) (Tokarz et al., 2018). For instance, GLUT-1, which is independent of insulin, is the predominant glucose transporter in the placenta and lactating mammary gland, and GLUT-2, also insulin-independent, is highly expressed in the liver (Zhao and Keating, 2007). Whereas GLUT-4, the only insulin-dependent glucose transporter, is widely distributed among cells of skeletal and cardiac muscle, adipose tissue, and endothelial cells (De Koster and Opsomer, 2013).

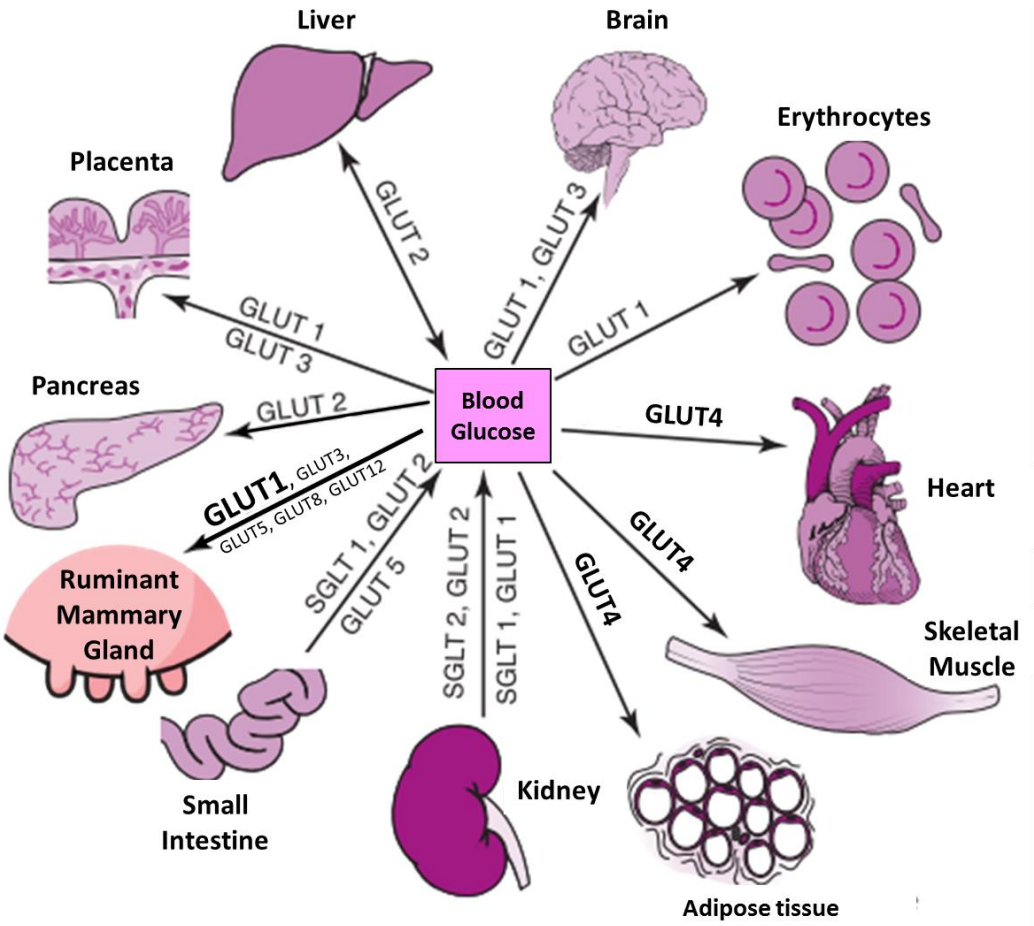

Figure 2.4. Predominant glucose transporters in several ruminant tissues. Modified from Engelking (2015)

The tissue-specific distribution of different types of GLUT is the key to insulins' influence on nutrient partitioning between and within major organs and tissues (Bell and Bauman, 1997). For instance, quantitatively, the greatest glucose-consuming tissues are the skeletal muscles, the mammary gland, and the pregnant uterus (De Koster and Opsomer, 2013). Thus, because peripheral tissues are highly responsive to insulin regulation of glucose uptake, while mammary and placental cells are freely permeable to glucose, a state of reduced insulin concentration will favor the partitioning of nutrients away from storage and toward fetal development and milk synthesis (Baumgard et al., 2017).

Indeed, studies in lactating goats have reinforced the importance of insulin in the coordination of tissue metabolism. Nielsen et al. (2001) demonstrated that although mammary

uptake of glucose was not directly affected by insulin, insulin infusion increased mammary blood flow, thereby increasing glucose supply and uptake rate by the udder. Whereas, Bequette et al. (2001) reported that insulin infusion did not stimulate glucose or amino acid removal and/or transport activity in hind-leg tissues, suggesting altered insulin sensitivity in non-mammary tissues.

2.4.5.1 Insulin Sensitivity and Resistance

Insulin resistance refers to reduced insulin responsiveness to glucose (e.g., reduced insulin secretion by pancreatic β-cells) and/or reduced sensitivity of target tissues such as liver, muscle, and adipose tissues to the metabolic actions of insulin (De Koster and Opsomer, 2013). Peripartum insulin resistance is an evolutionary adaptation of mammals to ensure that nutrient supply to placental and mammary cells is prioritized over maternal reserves, thereby favoring offspring survivor and postnatal development (Bell and Ehrhardt, 2000; Kampmann et al., 2019).

As mentioned previously, lipolysis is increased in the peripartum to meet maternal energy requirements for maintenance and production. Considering that mobilization of body fat reserves elevates circulating concentrations of NEFA and that NEFA downregulates lipolysis by stimulating insulin secretion, a state of insulin resistance is essential for the maintenance of exaggerated lipolysis necessary to spare glucose during NEB (Bell, 1995; Sonagra, 2014). Also, in an insulin-resistant-state, the liver is less responsive to increases in insulin concentration, meaning that rises in circulating glucose will not trigger a hepatic metabolic shift towards glucose utilization (De Koster and Opsomer, 2013).

Several excellent reviews have summarized the physiology of insulin resistance in humans (Wilcox, 2005; Petersen and Shulman, 2018; Kampmann et al., 2019), and in dairy cows (De Koster and Opsomer, 2013; Cincović et al., 2018). However, despite early studies on mechanisms of insulin resistance in dairy goats (Debras et al., 1989) and ewes (Guesnet et al.,

1991), and later investigations on the effects of exogenous insulin on nutrient homeostasis in small ruminants (Bequette et al., 2001; Nielsen et al., 2001; Duehlmeier et al., 2013), the biological mechanisms responsible for changes in whole-body responsiveness/sensitivity to insulin in periparturient goats and ewes are still poorly understood.

2.4.5.2 Measures of Insulin Sensitivity in ruminants

2.4.5.2.1 Hyperinsulinemic-euglycemic clamp (HEC)

The hyperinsulinemic-euglycemic clamp (HEC) technique is the gold standard for the estimation of whole-body insulin sensitivity and insulin secretion (Singh and Saxena, 2010). This method consists of the administration of insulin at a constant rate to raise plasma insulin concentrations to a steady-state level while glucose is intravenously infused at variable rates to maintain euglycemia. The rate of glucose infusion necessary to maintain euglycemia directly correlates with the rate of glucose uptake by body tissues; thus, the HEC test allows for measurement of tissue sensitivity to insulin (De Koster and Opsomer, 2013). However, this method is time-consuming, experimentally demanding, expensive, and impractical when large scale epidemiological studies are involved (Singh and Saxena, 2010; Cincović et al., 2018)

2.4.5.2.2 Intravenous glucose tolerance test (IVGTT)

Compared to HEC, the IVGTT requires far less labor, sampling, and equipment to measure insulin sensitivity. It involves the intravenous administration of a glucose bolus, followed by sequential blood samples taken at regular intervals over 3 hours (De Koster and Opsomer, 2013). Variations in insulin levels during an IVGTT are the result of pancreatic insulin secretion in response to glucose bolus and the rate of hepatic and renal clearance of insulin, while variations in glucose levels are derived from the glucose bolus, endogenous glucose production in liver and kidneys, and glucose uptake by the insulin-sensitive tissues (e.g., muscle and adipose cells) and insulin-independent tissues (e.g., mammary gland, gravid uterus,

brain, kidneys) (De Koster and Opsomer, 2013). By using data obtained during an IVGTT, it is possible to calculate several parameters of glucose-insulin kinetics (e.g., clearance rates, time to reach half-maximal and basal concentration, area under the curve), which enables estimation of insulin sensitivity and secretion with reasonable accuracy (Hahn et al., 2011).

2.4.5.2.3 Modeling glucose and insulin responses to IVGTT

The use of a mathematical model to describe the dynamic relationship between glucose and insulin in controlling glucose production and disposal in the body was first formulated and introduced by Bergman et al. (1979). The latest Windows-based version of the Bergman minimal model (MINMOD Millennium) uses data from either an IVGTT or a modified IVGTT (where 20 min after the glucose injection, an insulin bolus is infused) to estimate several parameters of glucose and insulin kinetics. The ability of insulin to stimulate glucose uptake and to inhibit the endogenous production of glucose (SI), the insulin-independent glucose disappearance rate (SG), the acute insulin response (AIR) in terms of glucose-induced insulin release by pancreatic β-cells, and disposition index (DI), which represents the ability of the islet cells to compensate for insulin resistance, are some of the indices that are output by the model (Boston et al., 2003).

MINMOD software has been validated for the estimation of insulin sensitivity in humans and dogs after showing good agreement between MINMOD-calculated parameters and measures derived from the HEC test in those species (De Koster et al., 2016). Although the method has not been validated for use in ruminants, several researchers have used MINMOD for estimation of insulin sensitivity in dairy cows (Marett et al., 2015; De Koster et al., 2016; Bogaert et al., 2018), ewes (Williams et al., 2004), and goats (Souza et al., 2020).

2.4.5.2.4 Surrogate Indexes

The need for cheaper and less laborious methods to estimate insulin sensitivity (compared to HEC and IVGTT) has driven the development of several surrogate indexes for insulin resistance in humans (Singh and Saxena, 2010), most of which uses fasting blood samples with measures of insulin, glucose and sometimes NEFA and BHB, and are calculated as follows:

Homeostasis model assessment (HOMA) = glucose (mmol/L) × insulin (uU/mL)

Homeostasis model of insulin resistance (HOMA-IR) = [glucose (mmol/L) × insulin (uU/mL)] / 22.5

Quantitative insulin sensitivity check index (QUICKI) = 1 / [$\log_{10}$ glucose (mg/dL) + $\log_{10}$ insulin (uU/mL)]

Revised quantitative insulin sensitivity check index (RQUICKI) = 1 / [$\log_{10}$ glucose (mg/dL) + $\log_{10}$ insulin (uU/mL) + $\log_{10}$ NEFA (mmol/L)]

RQUICKI including BHB (RQUICKI-BHB) = 1 / [$\log_{10}$ glucose (mg/dL) + $\log_{10}$ insulin (uU/mL) + $\log_{10}$ NEFA (mmol/L) + $\log_{10}$ BHB (mmol/L)]

In general terms, insulin-resistant individuals are expected to have elevated insulin, increased HOMA-IR, and decreased QUICKI, RQUICKI, RQUICKI-BHB (De Koster and Opsomer, 2013). Because RQUICKI and RQUICKI-BHB indices include concentrations of NEFA, and NEFA and BHB, respectively, they are considered more robust indices for the assessment of insulin resistance (Mann et al., 2016; Marinković et al., 2019). Although such surrogate indexes have been used as a measure of insulin sensitivity/resistance in dairy cows (De Koster et al., 2016; Mann et al., 2016; Marinković et al., 2019) and in dairy goats (Cai et al., 2018), all of these indexes have been developed and validated in humans; thus the use of surrogate indexes for the determination of insulin resistance in ruminants is questionable (De Koster et al., 2016).

2.4.6 Growth Hormone (GH)

Growth hormone also called somatotropin, is a calorigenic hormone synthesized and secreted by the anterior pituitary gland under the action of a dual system of hypothalamic hormones, GH-releasing factor (GRF) (stimulatory) and somatostatin (SS) (inhibitory) released from the hypothalamus (Bauman and Vernon, 1993). Growth hormone and insulin are the two key players in the regulation of nutrient homeostasis in periparturient ruminants (Kim, 2014). Each hormone affects the hierarchy of nutrient partitioning in an opposite way; while insulin promotes nutrient storage, GH promotes mobilization of nutrients from body reserves by stimulating lipolysis, hyperglycemia, gluconeogenesis, mammogenesis, and galactopoiesis, and inhibiting lipogenesis and glucose uptake (Baumgard et al., 2017).

The initial step in the action of GH is binding to GH receptors (GHR) that are present on the plasma membrane of target cells. Because GHR mediates GH biological actions on target tissues by transducing the GH signal across the cell membrane, GH responses will vary according to GHR abundance and binding affinity for GH of each given tissue (Chakraborty et al., 2016). Although GHR is expressed in the outer membrane of cells throughout the body (Chakraborty et al., 2016), the two primary direct targets of GH are the adipocyte and the hepatocyte (Bauman and Vernon, 1993), while indirect effects of GH in several extrahepatic tissues (e.g., mammary, adipose, muscle, pancreas) are mediated by IGF-I actions (Rhonda et al., 2018).

Several comprehensive reviews have provided in-depth coverage of mechanisms by which GH affects nutrient partitioning and productive efficiency in dairy ruminants, with particular emphasis on mammogenic and galactopoietic properties of GH (Bauman and Vernon, 1993; Etherton and Bauman, 1998). In a review of the metabolic effects of GH administration in ruminants, Capuco and Akers (2011) listed some of the main metabolic responses to exogenous GH stimulation, such as increased lipolysis and gluconeogenesis, reduced glucose

uptake, reduced the ability of insulin to stimulate lipogenesis and to inhibit gluconeogenesis, increased mammary blood flow and secretory capacity (**Figure 2.5)**. Likewise, studies in goats have shown that treatment with exogenous GH increases milk yield and the percentage of fat and lactose in milk (Disenhausat et al., 1995; Chadio et al., 2000), and delayed mammary involution, thereby improving lactation persistency (Baldi et al., 2002).

However, the positive effects of GH on overall lactational performance appears to be, at least in part, mediated by IGF-1 action (Napso et al., 2018), which contributes to enhanced milk production by promoting hepatic glucose output, inducing insulin resistance and blood flow to the mammary gland, reducing apoptosis rate and stimulating proliferation of mammary epithelial cells (Cohick, 1998; Etherton and Bauman, 1998).

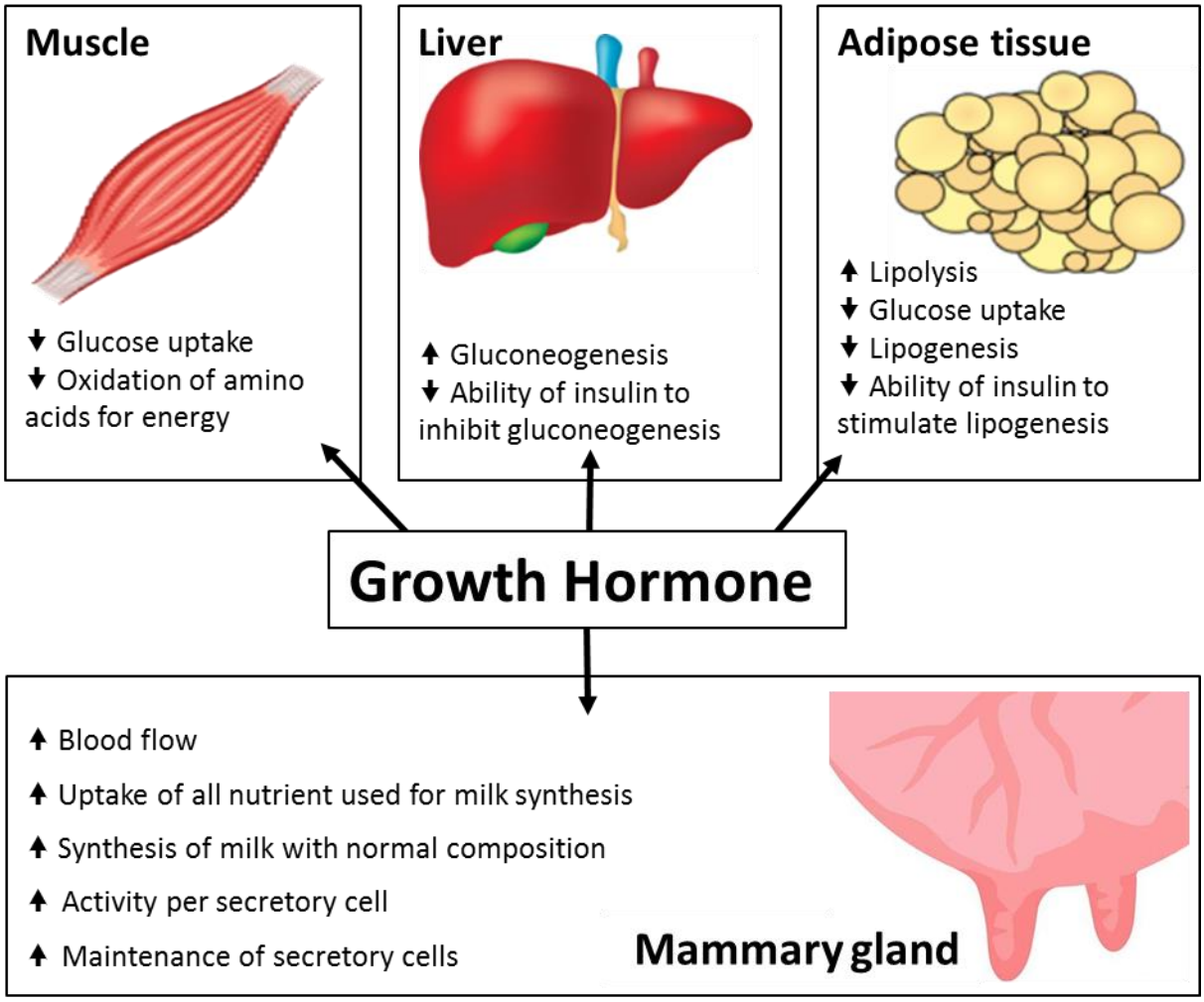

Figure 2.5. The organ-specific effect of growth hormone on nutrient partitioning to support increased milk production in ruminants. Modified from Capuco and Akers (2011)

2.4.6.1 Somatotropic Axis (GH-IGF-1 axis)

The somatotropic axis, primarily consisting of GH, GHR, IGF-1, IGF-1 receptors, and IGF-binding proteins, is one of the major hormonal systems regulating physiological mechanisms involving the hypothalamus, anterior pituitary gland, and liver (Renaville et al., 2002). In brief, IGF-1 is produced primarily by the liver through GH stimulation, which in turn, inhibits GH secretion by the pituitary gland via a negative feedback loop (Burton et al., 1994). During catabolic states (peripartum and/or NEB), there is a reduction in GHR expression in the liver, thereby compromising GH stimulus for IGF-1 synthesis, which relieves the IGF-1 negative

feedback and elevates blood GH, a process commonly referred to as the uncoupling of the somatotropic axis (Burton et al., 1994; Kim, 2014; Wankhade et al., 2017).

The uncoupling of the somatotropic axis increases GH concentration while concentrations of IGF-I and insulin remain low, resulting in a relatively catabolic state (higher GH and lesser IGF-I) which combined with the anti-insulinemic effects of GH, promotes mobilization of body reserves in support of lactation even during periods of undernutrition (Lucy, 2008). The recoupling of the somatotropic axis occurs as nutrient intake improves, and milk yield decreases with the advance of lactation (Burton et al., 1994).

2.4.7 Cortisol

Cortisol is the most potent glucocorticoid hormone synthesized from cholesterol in the adrenal cortex and regulated via the hypothalamic-pituitary-adrenal (HPA) axis (Burdick et al., 2011). **Figure 2.6** illustrates the HPA axis pathway, associated hormones, and target tissues. In general, stressful situations activate the HPA-axis, resulting in increased plasma concentrations of corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), norepinephrine, adrenaline, and cortisol (Lucy, 2008).

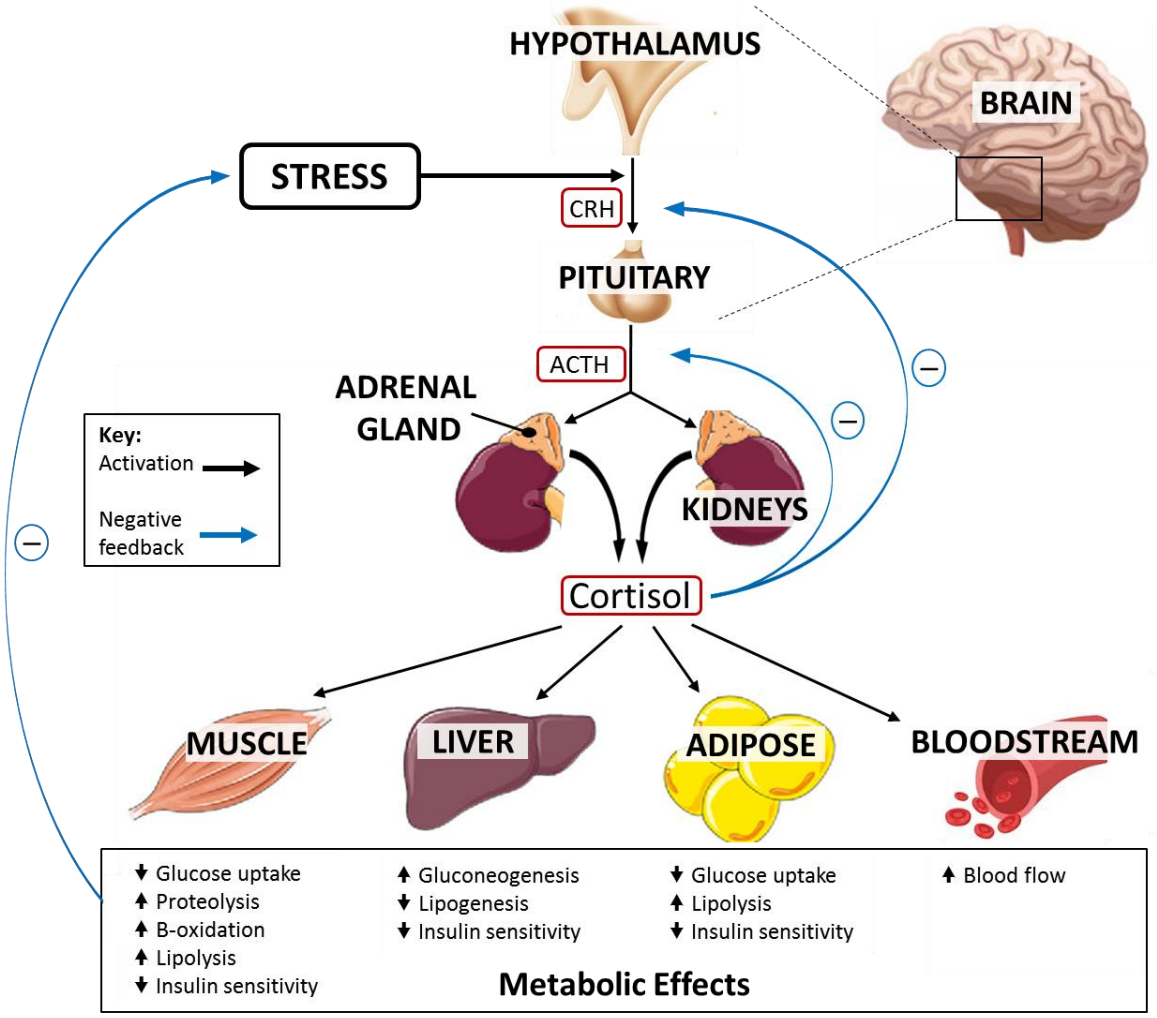

Figure 2.6. Schematic representation of the hypothalamic-pituitary-adrenocortical axis responses to stress, and the cortisol-induced metabolic responses in target tissues. CRH = corticotrophin-releasing hormone, ACTH = adrenocorticotropic hormone

Although the concentrations of all these hormones have been used in the study of stress, cortisol is the primary hormone responsible for stress responses, and the most commonly used for the assessment of animal welfare (Burdick et al., 2011). Several internal or external stressor factors, such as animal handling, transportation, milking, and extreme temperatures, have all been shown to increased cortisol secretion in goats (Komara and Marnet, 2009; Kruger et al., 2016; Bomfim et al., 2018). Fluctuations of plasma cortisol are also influenced by the physiological status of the dam (e.g., estrous cycle, pregnancy, parturition, lactation). Such

fluctuations are demonstrated in goats by Mahmoud and Azab (2014), in which a sharp increase (more than 20-fold) of maternal plasma cortisol was recorded at parturition.

The overall effect of cortisol on metabolism is a temporary increase in energy production and consumption, diverting metabolism from anabolic to catabolic state, at the expense of processes that are not required for immediate survival (Meij and Mol, 2008). According to Binsiya et al. (2017), a tendency to hyperglycemia, increased gluconeogenesis, lipolysis, proteolysis, blood flow, and reduced lipogenesis, glucose uptake, and insulin sensitivity are all related to cortisol stimulation in livestock. In summary, cortisol is an important promoter of milk production because cortisol stimulates the partition of nutrients away from storage and toward mammary utilization (Herdt, 1988; Lérias et al., 2014).

Although corticoids are among the hormones essential for the initiation, and in some cases, maintenance of lactation (Hydbring et al., 1999; Neville et al., 2002), during prolonged stress, a chronic elevation of cortisol levels might suppress immune responses and inflammatory pathways, thereby resulting in greater vulnerability to infectious diseases and prolonged healing times (Meij and Mol, 2008).

2.4.8 Mammogenic, Lactogenic and Galactopoietic Hormones

The so-called reproductive hormones include progesterone, estrogen, oxytocin, PL, and prolactin, have a crucial role in synchronizing maternal adaptations to pregnancy, parturition, and lactation (Hurley and Loor, 2011), but also influence the coordination of nutrient partitioning between fetoplacental unit, mammary and extramammary tissues (Lacasse et al., 2016; Napso et al., 2018).

Estrogen and progesterone are the chief pregnancy hormones. Together they are responsible for both the establishment and maintenance of pregnancy, for the stimulation of maternal behavior, and have a pivotal rule in the hormonal control of mammogenesis and onset of lactation (Napso et al., 2018). Increased levels of circulating estrogen and progesterone are

essential for the proliferation and maturation of mammary epithelial cells while keeping them nonfunctional until parturition (Neville et al., 2002; Akers, 2017). That is because progesterone interferes with prolactin binding to the receptors on the alveolar cells, suppressing prolactin capacity to stimulate the final differentiation of mammary cells for milk production and secretion (Hurley and Loor, 2011), thereby indirectly securing nutrient supply for the conceptus (Bauman, 2000). Moreover, the direct effects of estrogen and progesterone on the regulation of nutrient partitioning during pregnancy have been demonstrated in humans and ruminants (Bell and Bauman, 1997; Napso et al., 2018).

At the end of pregnancy, a gradual fall in progesterone levels triggers secretory activity, while the combination of prolactin, PL, GH, thyroid hormones, and cortisol stimulate copious milk synthesis and initiate lactation (Hydbring et al., 1999; Neville et al., 2002). Prolactin, GH, and PL are members of a family of placental hormones that share similarities in chemical structure and biological actions, as three hormones exhibit both lactogenic and somatotropic properties (Lacasse et al., 2016; Napso et al., 2018).

During pregnancy, prolactin is synthesized and secreted by both the anterior pituitary gland and placenta; however, as pregnancy advances, the placenta gradually becomes the predominant source of prolactin (Napso et al., 2018). Although prolactin is a crucial regulator of mammogenesis and lactogenesis in goats (Lacasse et al., 2016), researchers have reported conflicting results for the effects of the suppression of prolactin secretion on galactopoiesis (milk production in established lactations) in goats (Lérias et al., 2014; Lacasse et al., 2016). There are also indications that, in rodents, prolactin increases pancreatic insulin production but reduces insulin sensitivity (Napso et al., 2018). Also, studies in ruminants have shown that prolactin increases feed intake in dairy cows (Lacasse et al., 2016), and that prolactin concentration is significantly affected by changes in photoperiod and temperature in cows and goats (Lacasse et al., 2016; Pehlivan et al., 2018).

Placental lactogens are secreted from the placenta, and PL exerts its actions by binding (with varying affinity) to GHR and prolactin-receptors (Gertler and Djiane, 2002). In pregnant goats, PL concentration in blood is positively associated with litter size, placental mass, udder volume, colostrum yield, and subsequent milk production (Hurley and Loor, 2011; Lérias et al., 2014), providing indirect evidence for a relevant mammogenic role for PL. Additionally, PL has a clear effect on mammary epithelial cell differentiation, as it can stimulate lactogenesis when prolactin is absent or suppressed in sheep or goats (Lérias et al., 2014). Given that PL is a member of the GH-prolactin family, it mimics some but not all somatogenic and metabolic activities of GH and prolactin in ruminants (Gertler and Djiane, 2002). Such partial mimicking of GH-prolactin action could explain the relationship between PL and udder size, colostrum yield, and degree of insulin resistance (Bell and Ehrhardt, 2000; Hurley and Loor, 2011).

2.5 Factors Affecting Goat Milk Yield and Composition

Milk yield refers to the quantity of milk produced by an animal throughout a determined period (Goetsch et al., 2011). As seen in section 1.1.2., the level of milk production has a significant impact on nutrient demands and energy balance. Consequently, factors affecting milk production are likely to affect endocrine and metabolic adaptations during the transition period. Previous studies have shown that goat milk yield and composition can be significantly affected by several non-genetic factors, such as nutrition, the month of kidding, environmental conditions (e.g., photoperiod, temperature, humidity, rainfall), stage of lactation, parity, age, litter size, production systems (e.g., intensive, semi-intensive, extensive), milk frequency, and individual or herd health (Carnicella et al., 2008; Goetsch et al., 2011; Idowu and Adewumi, 2017).

2.5.1 Environmental Conditions, Stage of Lactation, and Month of Kidding

The seasonal variations in milk yield and composition are well documented in dairy goats. Such seasonality can be largely explained by changes in environmental conditions throughout the year and its simultaneous impact on feed quantity/quality and on animal behavior and physiology (Cannas and Pulina, 2008; Clark and García, 2017; Kljajevic et al., 2018). For instance, the literature shows that, in goats, blood concentrations of GH, prolactin, and thyroid hormones (TH) are markedly influenced by changes in photoperiod and temperature. Long photoperiods stimulate secretion of GH, prolactin, and TH in summer, but elevated temperatures have a negative impact on TH (Hashizume et al., 1999; Todini, 2007; Jin et al., 2012). Therefore, changes in environmental conditions are likely to have an indirect impact on endocrine regulation of nutrient partitioning, thereby influencing overall milk production.

Indeed, multiple studies have reported a positive relationship between photoperiod and milk production in dairy goats (Flores et al., 2011; Russo et al., 2013; Arnal et al., 2018), and a negative correlation between the temperature-humidity index (THI) and contents of fat, protein, and lactose in goat milk (Salama et al., 2014; Kljajevic et al., 2018). Additionally, changes in environmental conditions have a direct influence on the quantity and quality of feedstuffs and grazing crops available but also impact on voluntary feed intake and water consumption (Cannas and Pulina, 2008). Thus, seasonal variations in goat milk yield and composition are even more marked when goats are managed under extensive or semi-extensive systems than when the herd is kept indoors (Garcia-Hernandez et al., 2007; Cannas and Pulina, 2008; Flores et al., 2011).

The stage of lactation is an important factor affecting milk yield and quality in goats. Various studies have shown that in goats, daily milk yield tends to increase continuously from parturition until reaching its peak around 5-8 weeks postpartum, gradually declining after that as lactation progresses (Idowu and Adewumi, 2017). In contrast, most of the major components

in goat's milk (e.g., fat and protein) show an opposite trend, decreasing from the beginning of lactation to a minimum in mid-lactation, increasing again towards the end of the lactation (Idowu and Adewumi, 2017; Park et al., 2017).

According to Safayi et al. (2010), the changes in milk yield over the lactation period are determined by changes in the number and the secretory activity of milk-synthesizing epithelial cells and changes in nutrient supply to the mammary gland. Nevertheless, it is often difficult to distinguish the relative influence of changes in environmental conditions from the effects of stage of lactation on milk yield and composition. That is because changes in environmental conditions might have a significant impact on animal nutrition, behavior, and physiology, which in its turn, might affect metabolic and endocrine regulation of mammary metabolism and secretory capacity (Safayi et al., 2010; Goetsch et al., 2011; Lérias et al., 2014; Idowu and Adewumi, 2017).

Not surprisingly, various authors have demonstrated that the month of kidding can have a significant impact on the shape and scale of the lactation curve in goats (Crepaldi et al., 1999; León et al., 2012; Arnal et al., 2018). These authors observed that although peak yield and total milk produced per lactation is usually higher in goats kidding in warm months, their lactation persistence (ability to maintain production at a higher level after peak yield) is inferior to that of goats kidding during cold months.

2.5.2 Parity Number

Advanced parity is associated with increased milk production in dairy goats (Lérias et al., 2014), typically reaching its maximum between the third and fourth lactations. The increasing milk yield with increasing parity is probably due to the reduction in the percentage of nutrients used for maternal growth and the gradual decline in accumulation of mammary alveoli from the previous lactation as goats get older (Lérias et al., 2014; Idowu and Adewumi, 2017). Parity number is also known to affect milk composition and the shape of the lactation curve in goats.

First-parity goats usually have lower initial and peak yields, higher lactation persistency (Gipson and Grossman, 1990), higher fat and protein percentages, and the lower somatic cell count (SCC) than later-parity goats (Carnicella et al., 2008; Goetsch et al., 2011; Idowu and Adewumi, 2017).

2.5.3 Litter Size

Several authors have reported a positive association between litter size and milk production in goats (Crepaldi et al., 1999; Carnicella et al., 2008; Lérias et al., 2014). As pointed out in section 2.4.8, the placenta synthesizes and secretes a variety of mammogenic hormones (Napso et al., 2018). In goats, increasing litter size is associated with increased placental mass, placental hormones concentration, udder volume, and colostrum and milk yields (Hurley and Loor, 2011; Lérias et al., 2014). Therefore, the higher milk yield found in goats delivering multiple kids is likely to be the result of greater prepartum stimulation of mammary gland development due to higher levels of mammogenic and lactogenic hormones (e.g., progesterone, estrogen, PL, GH, prolactin) compared to goats delivering single kids. However, contrasting the positive effect on milk yield, the literature suggests that litter size is negatively associated with the contents of milk fat and protein in early lactation (Lérias et al., 2014).

2.6 Periparturient Metabolic Diseases

During the transition period, NEB-related metabolic stress coupled with other stressors associated with pregnancy, parturition, and adjustments to lactation can predispose dairy goats to so-called production diseases such as gestational and lactational ketosis, fatty liver, milk fever, and ruminal acidosis (Cannas and Pulina, 2008; Brozos et al., 2011; Matthews, 2016). Some of the main predisposing factors associated with periparturient metabolic diseases in goats are over or under condition, multiple pregnancies, high milk yield, and stress factors (e.g.,

concurrent diseases, dietary changes, severe weather, housing) (Cannas and Pulina, 2008; Matthews, 2016).

Ketosis and hypocalcemia are among the most common diseases affecting periparturient goats and ewes (Rook, 2000; Smith and Sherman, 2009). In goats, ketosis occurs more commonly during the last stage of pregnancy especially in goats carrying multiple fetuses, while hypocalcemia is most common in high-yielding goats in the first few weeks of lactation, when the sudden increase in demand of calcium for milk synthesis cannot be met quickly enough (Matthews, 2016). The high incidence of multiple births, combined with the increased milk yield of modern dairy goats, might increase their risks of developing ketosis and hypocalcemia both in late pregnancy and early lactation (Cannas and Pulina, 2008; Smith and Sherman, 2009). Nevertheless, despite the vast information on etiology and treatment of metabolic diseases in goats (Rook, 2000; Cannas and Pulina, 2008; Smith and Sherman, 2009; Brozos et al., 2011; Matthews, 2016), a perusal of the literature revealed only one large epidemiologic study on gestational ketosis carried out using dairy goats from commercial herds in Canada (Doré et al., 2015), and two studies on the prevalence of ketosis in small herds of dual-purpose goats from Jordan (Ismail et al., 2015) and India (Yadav et al., 2015).

Production diseases can result in significant economic losses in dairy herds by adversely affecting both production and fertility, but also by increasing involuntary culling and treatment costs (Smith and Sherman, 2009; Overton et al., 2017; Wankhade et al., 2017). However, contrasting the abundant information on performance and health of transition dairy cows (Ospina et al., 2013; Overton et al., 2017; Wankhade et al., 2017), little is known about the incidence and economic impacts of metabolic disorder in periparturient dairy goats.

2.7 Conclusions

The transition period is the most critical life stage in dairy goats, during which profound physiological, endocrine, and metabolic adaptations are necessary to support pregnancy and

lactation. Previous studies in dairy cows have shown that an inability to adapt to this challenging situation might have a substantial impact on maternal health and productive efficiency beyond the transition period.

However, in contrast to the extensive information on transition dairy cows, there is little data describing changes in the blood biochemical parameters in periparturient goats. Therefore, studies on metabolic and endocrine responses to support pregnancy and lactation in dairy goats may help to further our understanding of mechanisms of metabolic adaptations and its overall outcomes within this species, thereby contributing to the improvement of guidelines for the management strategies during the transition period.

2.8 References

Akers, R. M. 2017. A 100-year review: Mammary development and lactation. J. Dairy Sci., 100 (12):10332-10352. https://doi.org/10.3168/jds.2017-12983

Albay, M. K., M. Ç. Karakurum, Ş. Şahinduran, K. Sezer, R. Yıldız, and T. Büyükoğlu. 2014. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Vet. Med-Czech, 59 (7):336-342. https://doi.org/10.17221/7620-vetmed

Arnal, M., C. Robert-Granié, and H. Larroque. 2018. Diversity of dairy goat lactation curves in France. J. Dairy Sci., 101 (12):11040-11051. https://doi.org/10.3168/jds.2018-14980

Baldi, A., S. Modina, F. Cheli, F. Gandolfi, L. Pinotti, L. B. Scesi, F. Fantuz, and V. Dell'Orto. 2002. Bovine somatotropin administration to dairy goats in late lactation: effects on mammary gland function, composition, and morphology. J. Dairy Sci., 85 (5):1093-1102. http://dx.doi.org/10.3168/jds.S0022-0302(02)74170-2

Bauman, D. E. 2000. Regulation of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. Pages 311-328 in Ruminant physiology: Digestion, metabolism, growth, and reproduction. P. B. Cronje, ed. CABI. Wallingford, UK. https://doi.org/10.1079/9780851994635.0311

Bauman, D. E., and R. G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Annu. Rev. Nutr., 13 (1):437-461. https://doi.org/10.1146/annurev.nu.13.070193.002253

Baumgard, L. H., R. J. Collier, and D. E. Bauman. 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. J. Dairy Sci., 100 (12):10353-10366. https://doi.org/10.3168/jds.2017-13242

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci., 73 (9):2804-2819. http://dx.doi.org/10.2527/1995.7392804x

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia, 2 (3):265-278. https://doi.org/10.1023/A:1026336505343

Bell, A. W., and R. A. Ehrhardt. 2000. Regulation of macronutrient partitioning between maternal and conceptus tissues in the pregnant ruminant. Pages 275-293 in Ruminant physiology: Digestion, metabolism, growth and reproduction. P. B. Cronje, ed. CABI. Wallingford, UK. https://doi.org/10.1079/9780851994635.0275

Bequette, B. J., C. E. Kyle, L. A. Crompton, V. Buchan, and M. D. Hanigan. 2001. Insulin regulates milk production and mammary gland and hind-leg amino acid fluxes and blood flow in lactating goats. J. Dairy Sci., 84 (1):241-255. https://doi.org/10.3168/jds.S0022-0302(01)74474-8

Bergman, R. N., Y. Z. Ider, C. R. Bowden, and C. Cobelli. 1979. Quantitative estimation of insulin sensitivity. Am. J. Physiol. Gastrointest. Liver Physiol., 236 (6):G667-677. https://doi.org/10.1152/ajpendo.1979.236.6.E667

Binsiya, T. K., V. Sejian, M. Bagath, G. Krishnan, I. Hyder, A. Manimaran, A. M. Lees, J. B. Gaughan, and R. Bhatta. 2017. Significance of hypothalamic-pituitary-adrenal axis to adapt to climate change in livestock. Int. Res. J. Agri. Food Sci., 2 (1):1-20. https://prudentjournals.com/prja97540518/

Bogaert, H., J. De Koster, W. Van Den Broeck, M. Van Eetvelde, and G. Opsomer. 2018. Effects of overconditioning on pancreatic insulin secretory capacity, fat infiltration, and the number and size of islets in dairy cows at the end of the dry period. J. Dairy Sci., 101 (12):11413-11420. https://doi.org/10.3168/jds.2018-14931

Bomfim, G. F., G. K. F. Merighe, S. A. Oliveira, and J. A. Negrao. 2018. Effect of acute stressors, adrenocorticotropic hormone administration, and cortisol release on milk yield, the expression of key genes, proliferation, and apoptosis in goat mammary epithelial cells. J. Dairy Sci., 101 (7):6486-6496. https://doi.org/10.3168/jds.2017-14123

Boston, R. C., D. Stefanovski, P. J. Moate, A. E. Sumner, R. M. Watanabe, and R. N. Bergman. 2003. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol. Ther., 5 (6):1003-1015. https://doi.org/10.1089/152091503322641060

Brozos, C., V. S. Mavrogianni, and G. C. Fthenakis. 2011. Treatment and control of peri-parturient metabolic diseases: pregnancy toxemia, hypocalcemia, hypomagnesemia. Vet. Clin. Food Anim., 27 (1):105-113. https://doi.org/10.1016/j.cvfa.2010.10.004

Brun-Bellut, J. 1997. Urea recycling in the rumen of dairy goats: effects of physiological stage and composition of intake. Small Rum. Res., 23 (2-3):83-90. https://doi.org/10.1016/S0921-4488(96)00910-8

Burdick, N. C., R. D. Randel, J. A. Carroll, and T. H. Welsh. 2011. Interactions between temperament, stress, and immune function in cattle. Int. J. Zool. (373197):1-9. https://doi.org/10.1155/2011/373197

Burton, J. L., B. W. McBride, and E. Block. 1994. A review of bovine growth hormone. Can. J. Anim. Sci., 74:167-201. http://pubs.aic.ca/doi/pdf/10.4141/cjas94-027

Cai, J., F. Q. Zhao, J. Liu, and D. Wang. 2018. Local mammary glucose supply regulates availability and intracellular metabolic pathways of glucose in the mammary gland of lactating dairy goats under malnutrition of energy. Front. Physiol., 9 (1467):1-13. https://doi.org/10.3389/fphys.2018.01467

Cannas, A., and G. Pulina. 2008. Dairy goats feeding and nutrition. CABI International. Bologna, Italy. https://doi.org/10.1079/9781845933487.0000

Capuco, A. V., and R. M. Akers. 2011. Lactation | Galactopoiesis, effect of treatment with bovine somatotropin. Pages 26-31 in Encyclopedia of Dairy Sciences. 2nd Ed. J. W. Fuquay, P. F. Fox and P. L. H. McSweeney, ed. Academic Press. San Diego, USA. https://doi.org/10.1016/B978-0-12-374407-4.00252-1

Carnicella, D., M. Dario, M. C. C. Ayres, V. Laudadio, and C. Dario. 2008. The effect of diet, parity, year and number of kids on milk yield and milk composition in Maltese goat. Small Rum. Res., 77 (1):71-74. https://doi.org/10.1016/j.smallrumres.2008.02.006

Castagnino, D. S., C. J. Härter, A. R. Rivera, L. D. Lima, H. G. O. Silva, B. Biagioli, K. T. Resende, and I. A. M. A. Teixeira. 2015. Changes in maternal body composition and metabolism of dairy goats during pregnancy. R. Bras. Zootec., 44 (3):92-102. https://doi.org/10.1590/S1806-92902015000300003

Celi, P., A. Di Trana, and S. Claps. 2008. Effects of perinatal nutrition on lactational performance, metabolic and hormonal profiles of dairy goats and respective kids. Small Rum. Res., 79 (2-3):129-136. https://doi.org/10.1016/j.smallrumres.2008.07.010

Chadio, S. E., G. Zervas, K. Kiriakou, C. Goulas, and J. Menegatos. 2000. Effects of recombinant bovine somatotropin administration to lactating goats. Small Rum. Res., 35 (3):263-269. https://doi.org/10.1016/S0921-4488(99)00104-2

Chakraborty, D., D. Kumar, P. M. Azhar, S. G. Singh, S. G. Singh, and A. Sudan. 2016. Growth Hormone Receptor (GHR) gene and its applications in livestock - A review. Agric. Rev., 37 (3):250-254. http://dx.doi.org/10.18805/ag.v37i3.3541

Chilliard, Y., A. Ferlay, Y. Faulconnier, M. Bonnet, J. Rouel, and F. Bocquier. 2000. Adipose tissue metabolism and its role in adaptations to undernutrition in ruminants. Proc. Nutr. Soc., 59 (1):127-134. https://doi.org/10.1017/S002966510000015X

Cincović, M. R., R. Đoković, B. Belić, I. Lakić, N. Stojanac, O. Stevančević, and N. Staničkov. 2018. Insulin resistance in cows during the periparturient period. Acta agric. Serb., 23 (46):233-245. https://doi.org/10.5937/AASer1846233C

Clark, S., and M. B. M. García. 2017. A 100-year review: Advances in goat milk research. J. Dairy Sci., 100 (12):10026-10044. https://doi.org/10.3168/jds.2017-13287

Cohick, W. S. 1998. Role of the insulin-like growth factors and their binding proteins in lactation. J. Dairy Sci., 81 (6):1769-1777. https://doi.org/10.3168/jds.S0022-0302(98)75746-7

Crepaldi, P., M. Corti, and M. Cicogna. 1999. Factors affecting milk production and prolificacy of Alpine goats in Lombardy (Italy). Small Rum. Res., 32 (1):83-88. https://doi.org/10.1016/S0921-4488(98)00156-4

De Koster, J., M. Hostens, K. Hermans, W. Van den Broeck, and G. Opsomer. 2016. Validation of different measures of insulin sensitivity of glucose metabolism in dairy cows using the hyperinsulinemic euglycemic clamp test as the gold standard. Domest. Anim. Endocrinol., 57:117-126. http://dx.doi.org/10.1016/j.domaniend.2016.06.004

De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. Food Anim., 29 (2):299-322. http://dx.doi.org/10.1016/j.cvfa.2013.04.002

Debras, E., J. Grizard, E. Aina, S. Tesseraud, C. Champredon, and M. Arnal. 1989. Insulin sensitivity and responsiveness during lactation and dry period in goats. Am. J. Physiol. Endocrinol. Metab., 256 (2):E295-E302. https://doi.org/10.1152/ajpendo.1989.256.2.E295

Disenhausat, C., H. Jammesb, J. Hervieua, F. Ternoisa, and D. Sauvanta. 1995. Effects of recombinant bovine somatotropin on goat milk yield, composition and plasma metabolites. Small Rum. Res. (15):139-148. https://doi.org/10.1016/0921-4488(94)00019-4

Doré, V., J. Dubuc, A. M. Bélanger, and S. Buczinski. 2015. Definition of prepartum hyperketonemia in dairy goats. J. Dairy Sci., 98 (7):4535-4543. http://dx.doi.org/10.3168/jds.2014-9172

Duehlmeier, R., I. Fluegge, B. Schwert, and M. Ganter. 2013. Insulin sensitivity during late gestation in ewes affected by pregnancy toxemia and in ewes with high and low susceptibility to this disorder. J. Vet. Intern. Med., 27 (2):359-366. https://doi.org/10.1111/jvim.12035

Dunshea, F., A. Bell, and T. Trigg. 1990. Body composition changes in goats during early lactation estimated using a two-pool model of tritiated water kinetics. Br. J. Nutr., 64 (1):121-131. https://doi.org/10.1079/BJN19900015

Dunshea, F. R., A. W. Bell, and T. E. Trigg. 1988. Relations between plasma non-esterified fatty acid metabolism and body tissue mobilization during chronic undernutrition in goats. Br. J. Nutr., 60 (3):633-644. https://doi.org/10.1079/BJN19880133

Eknæs, M., Ø. Havrevoll, H. Volden, and K. Hove. 2009. Fat content, fatty acid profile and off-flavours in goats milk-Effects of feed concentrates with different fat sources during the grazing season. Anim. Feed Sci. Technol., 152 (1-2):112-122. https://doi.org/10.1016/j.anifeedsci.2009.04.006

Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nut., 122 (3 Suppl.):850-854. https://doi.org/10.1016/j.smallrumres.2011.09.025

Engelking, L. R. 2015. Chapter 22 - Glucose Trapping. Pages 141-146 in Textbook of Veterinary Physiological Chemistry. 2nd Ed. L. R. Engelking, ed. Academic Press. Boston, USA. https://doi.org/10.1016/B978-0-12-391909-0.50022-0

Etherton, T. D., and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev., 78 (3):745-761. https://doi.org/10.1152/physrev.1998.78.3.745

Flores, M. J., J. A. Flores, J. M. Elizundia, A. Mejía, J. A. Delgadillo, and H. Hernández. 2011. Artificial long-day photoperiod in the subtropics increases milk production in goats giving birth in late autumn. J. Anim. Sci., 89 (3):856-862. https://doi.org/10.2527/jas.2010-3232

Forbes, J. M. 2007. Voluntary food intake and diet selection in farm animals. 2nd Ed. CABI Leeds, UK. https://doi.org/10.1079/9781845932794.0000

Fukao, T., G. A. Mitchell, J. O. Sass, T. Hori, K. Orii, and Y. Aoyama. 2014. Ketone body metabolism and its defects. J. Inherit. Metab. Dis., 37 (4):541-551. https://doi.org/10.1007/s10545-014-9704-9

Garcia-Hernandez, R., G. Newton, S. Horner, and L. C. Nuti. 2007. Effect of photoperiod on milk yield and quality, and reproduction in dairy goats. Livest. Sci., 110 (3):214-220. https://doi.org/10.1016/j.livsci.2006.11.005

Gertler, A., and J. Djiane. 2002. Mechanism of ruminant placental lactogen action: molecular and in vivo studies. Mol. Genet. Metab., 75 (3):189-201. https://doi.org/10.1006/mgme.2002.3303

Gipson, T. A., and M. Grossman. 1990. Lactation curves in dairy goats: a review. Small Rum. Res., 3 (4):383-396. https://doi.org/10.1016/0921-4488(90)90019-3

Goetsch, A. L., S. S. Zeng, and T. A. Gipson. 2011. Factors affecting goat milk production and quality. Small Rum. Res., 101 (1-3):55-63. https://doi.org/10.1016/j.smallrumres.2011.09.025

Guesnet, P. M., M. J. Massoud, and Y. Demarne. 1991. Regulation of adipose tissue metabolism during pregnancy and lactation in the ewe: the role of insulin. J. Anim. Sci., 69 (5):2057-2065. https://doi.org/10.2527/1991.6952057x

Hahn, R. G., S. Ljunggren, F. Larsen, and T. Nyström. 2011. A simple intravenous glucose tolerance test for assessment of insulin sensitivity. Theor. Biol. Medical Model., 8 (1):12. https://doi.org/10.1186/1742-4682-8-12

Harðarson, G. H., and K. L. Ingvartsen. 2005. Energy metabolism in the periparturient dairy cow. Paper presented at the Energy metabolism in the periparturient dairy cow, Faroe Islands, Denmark.

Härter, C. J., J. L. Ellis, J. France, K. T. Resende, and I. A. M. A. Teixeira. 2016. Net energy and protein requirements for pregnancy differ between goats and sheep. J. Anim. Sci., 94 (6):2460-2470. https://doi.org/10.2527/jas2015-9673

Hashizume, T., Y. Takahashi, M. Numata, K. Sasaki, K. Ueno, K. Ohtsuki, M. Kawai, and A. Ishii. 1999. Plasma profiles of growth hormone, prolactin and insulin-like growth factor-I during gestation, lactation and the neonatal period in goats. J. Reprod. Dev., 45 (4):273. https://doi.org/10.1262/jrd.45.273

Herdt, T. H. 1988. Fuel homeostasis in the ruminant. Vet. Clin. Food Anim., 4 (2):213-231. https://doi.org/10.1016/S0749-0720(15)31045-8

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. Vet. Clin. Food Anim., 16 (2):215-230. https://doi.org/10.1016/S0749-0720(15)30102-X

Hurley, W. L., and J. J. Loor. 2011. Mammary gland | Growth, development and involution. Pages 338-345 in Encyclopedia of Dairy Sciences. 2nd Ed. J. W. Fuquay, P. F. Fox and P. L. H. McSweeney, ed. Academic Press. San Diego, USA. https://doi.org/10.1016/B978-0-12-374407-4.00291-0

Hydbring, E., A. Madej, E. MacDonald, G. Drugge-Boholm, B. Berglund, and K. Olsson. 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. J. Endocrinol., 160 (1):75-86. https://doi.org/10.1677/joe.0.1600075

Idamokoro, E. M., V. Muchenje, and P. J. Masika. 2017. Peri-and post-parturient consequences of maternal undernutrition of free ranging does: A review. Livest. Res. Rural Dev., 29:1-18. http://www.lrrd.org/public-lrrd/proofs/lrrd2910/mond29202.html

Idowu, S. T., and O. O. Adewumi. 2017. Genetic and non-genetic factors affecting yield and milk composition in goats. J. Adv. Dairy Res., 5 (175):1-4. https://doi.org/0.4172/2329-888X.1000175

Ismail, Z. B., O. Al-Rawashdeh, A. M. Al-Majali, and F. Amireh. 2015. Prevalence and risk factors for pregnancy toxemia of goats in Jordan. Anim. Biol. Anim. Husb. (ABAH), 7 (1):53-59. http://www.abah.bioflux.com.ro/docs/2015.53-59.pdf

Jibir, M., I. Jibrila, S. Garba, A. M. Isa, and M. L. Sonfada. 2013. Fat deposition and distribution in autochthonous goats of the semi-arid zone of Northwestern Nigeria. J. Anim. Sci. Adv., 3 (9):481-484.

Jin, J. I. N., T. Yaegashi, K. Sawai, and T. Hashizume. 2012. Effects of photoperiod on the secretion of growth hormone in female goats. Anim. Sci. J., 83 (8):610-616. https://doi.org/10.1111/j.1740-0929.2011.00998.x

Kampmann, U., S. Knorr, J. Fuglsang, and P. Ovesen. 2019. Determinants of maternal insulin resistance during pregnancy: an updated overview. J. Diabetes Res., 2019 (5320156):1-9. https://doi.org/10.1155/2019/5320156

Khan, J. R., and R. S. Ludri. 2002. Hormonal profiles during periparturient period in single and twin fetus bearing goats. Asian-Australas. J. Anim. Sci., 15 (3):346-351.

Kim, J. W. 2014. Modulation of the somatotropic axis in periparturient dairy cows. Asian-Australas. J. Anim. Sci., 27 (1):147-154. http://dx.doi.org/10.5713/ajas.2013.13139

Kljajevic, N. V., I. B. Tomasevic, Z. N. Miloradovic, A. Nedeljkovic, J. B. Miocinovic, and S. T. Jovanovic. 2018. Seasonal variations of Saanen goat milk composition and the impact of climatic conditions. J. Food Sci. Technol., 55 (1):299-303. https://doi.org/10.1007/s13197-017-2938-4

Komara, M., and P. G. Marnet. 2009. Endocrine responses and milk emission characteristics in high yielding Alpine dairy goats under once-daily milking management. Small Rum. Res., 87 (1-3):64-69. https://doi.org/10.1016/j.smallrumres.2009.10.003

Kruger, L. P., T. L. Nedambale, M. M. Scholtz, and E. C. Webb. 2016. The effect of environmental factors and husbandry practices on stress in goats. Small Rum. Res., 141:1-4. http://dx.doi.org/10.1016/j.smallrumres.2016.06.004

Lacasse, P., S. Ollier, V. Lollivier, and M. Boutinaud. 2016. New insights into the importance of prolactin in dairy ruminants. J. Dairy Sci., 99 (1):864-874. http://dx.doi.org/10.3168/jds.2015-10035

Larsen, M., and N. B. Kristensen. 2013. Precursors for liver gluconeogenesis in periparturient dairy cows. Animal, 7 (10):1640-1650. https://doi.org/10.1017/S1751731113001171

Lean, I. J., M. L. Bruss, R. L. Baldwin, and H. F. Troutt. 1992. Bovine ketosis: A review. II. Biochemestry and prevention. Vet. Bull., 62 (February):1-14.

Lean, I. J., R. Van Saun, and P. J. DeGaris. 2013. Energy and protein nutrition management of transition dairy cows. Vet. Clin. Food Anim., 29 (2):337-366. http://dx.doi.org/10.1016/j.cvfa.2013.03.005

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev., 56 Suppl:S29-35. https://doi.org/10.1262/jrd.1056s29

Lehninger, A. L. 2017. Principles of biochemistry 7th Ed. Macmillan Learning New York, USA.

León, J. M., N. P. P. Macciotta, L. T. Gama, C. Barba, and J. V. Delgado. 2012. Characterization of the lactation curve in Murciano-Granadina dairy goats. Small Rum. Res., 107 (2-3):76-84. http://dx.doi.org/10.1016/j.smallrumres.2012.05.012

Lérias, J. R., L. E. Hernández-Castellano, A. Suárez-Trujillo, N. Castro, A. Pourlis, and A. M. Almeida. 2014. The mammary gland in small ruminants: major morphological and functional events underlying milk production–a review. J. Dairy Res., 81 (3):304-318. https://doi.org/10.1017/S0022029914000235

Lima, M. S., R. A. Pascoal, and G. T. Stilwell. 2012. Glycaemia as a sign of the viability of the foetuses in the last days of gestation in dairy goats with pregnancy toxaemia. Ir. Vet. J., 65 (1):1-1. https://doi.org/10.1186/2046-0481-65-1

Lucy, M. C. 2008. Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for post-partum nutrition and reproduction. Rep. Dom. Anim., 43 (SUPPL.2):31-39. https://doi.org/10.1111/j.1439-0531.2008.01140.x

Magistrelli, D., and F. Rosi. 2014. Trend analysis of plasma insulin level around parturition in relation to parity in Saanen goats. J. Anim. Sci., 92 (6):2440-2446. http://dx.doi.org/10.2527/jas2013-6993

Mahmoud, S., and M. Azab. 2014. Regulation of glucose level during late pregnancy and onset of lactation in Egyptian female Baladi goats. Small Rum. Res., 121 (2-3):320-324. http://dx.doi.org/10.1016/j.smallrumres.2014.06.009

Mann, S., F. A. L. Yepes, M. Duplessis, J. J. Wakshlag, T. R. Overton, B. P. Cummings, and D. V. Nydam. 2016. Dry period plane of energy: Effects on glucose tolerance in transition dairy cows. J. Dairy Sci., 99 (1):701-717. http://dx.doi.org/10.3168/jds.2015-9908

Marett, L. C., M. J. Auldist, P. J. Moate, W. J. Wales, K. L. Macmillan, F. R. Dunshea, and B. J. Leury. 2015. Response of plasma glucose, insulin, and nonesterified fatty acids to intravenous glucose tolerance tests in dairy cows during a 670-day lactation. J. Dairy Sci., 98 (1):179-189. http://dx.doi.org/10.3168/jds.2014-8205

Marinković, M. D., B. Belić, M. R. Cincović, R. Đoković, I. Lakić, N. Stojanac, O. Stevančević, and G. Devečerski. 2019. Relationship between insulin, glucose, non-esterified fatty acid and indices of insulin resistance in obese cows during the dry period and early lactation. Acta Vet. Brno, 88 (2):143-155. https://doi.org/10.2754/avb201988020143

Marutsova, V., and R. Binev. 2017. Body condition score, nonesterified fatty acids and beta-hydroxybutyrate concentrations in goats with subclinical ketosis. Agri. Sci. Tech., 9 (4):282-285. https://doi.org/10.15547/ast.2017.04.053

Matthews, J. G. 2016. Diseases of the goat. 4th Ed. John Wiley & Sons Ltd. Chelmsford, Essex, UK. https://doi.org/10.1002/9781119073543.ch4

Meij, B. P., and J. A. Mol. 2008. Adrenocortical function. Pages 605-622 in Clinical Biochemistry of Domestic Animals. 6th Ed. J. J. Kaneko, J. W. Harvey and M. L. Bruss, ed. Academic Press. San Diego. https://doi.org/10.1016/B978-0-12-370491-7.00019-2

Napso, T., H. E. J. Yong, J. Lopez-Tello, and A. N. Sferruzzi-Perri. 2018. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. Front. Physiol., 9:1091. http://dx.doi.org/10.3389/fphys.2018.01091

Neville, M. C., T. B. McFadden, and I. A. Forsyth. 2002. Hormonal regulation of mammary differentiation and milk secretion. J. Mammary Gland Biol. Neoplasia, 7 (1):49-66. https://doi.org/10.1023/a:1015770423167

Nielsen, M. O., T. G. Madsen, and A. M. Hedeboe. 2001. Regulation of mammary glucose uptake in goats: role of mammary gland supply, insulin, IGF-1 and synthetic capacity. J. Dairy Res., 68 (3):337-349. https://doi.org/10.1017/s002202990100499x

National Research Council. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Academic Press. Washington, DC.

Ospina, P. A., J. A. McArt, T. R. Overton, T. Stokol, and D. V. Nydam. 2013. Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. Vet. Clin. Food Anim., 29 (2):387-412. http://dx.doi.org/10.1016/j.cvfa.2013.04.003

Overton, T. R., J. A. A. McArt, and D. V. Nydam. 2017. A 100-Year Review: Metabolic health indicators and management of dairy cattle. J. Dairy Sci., 100 (12):10398-10417. https://doi.org/10.3168/jds.2017-13054

Park, Y. W., C. Jeanjulien, and A. Siddique. 2017. Factors affecting sensory quality of goat milk cheeses: a review. J. Adv. Dairy Res., 5 (185):2. https://doi.org/10.4172/2329-888X.1000185

Pehlivan, E., H. Polat, and G. Dellal. 2018. Annual change of prolactin and growth hormone in Angora goats. Biol. Rhythm Res., 49 (6):908-915. https://doi.org/10.1080/09291016.2018.1429551

Petersen, M. C., and G. I. Shulman. 2018. Mechanisms of insulin action and insulin resistance. Physiol. Rev., 98 (4):2133-2223. https://doi.org/10.1152/physrev.00063.2017

Radin, L., M. Šimpraga, S. Vince, A. Kostelić, and S. Milinković-Tur. 2015. Metabolic and oxidative status of Saanen goats of different parity during the peripartum period. J. Dairy Res., 82 (4):426-433. https://doi.org/10.1017/S0022029915000552

Renaville, R., M. Hammadi, and D. Portetelle. 2002. Role of the somatotropic axis in the mammalian metabolism. Domest. Anim. Endocrinol., 23 (1-2):351-360. https://doi.org/10.1016/S0739-7240(02)00170-4

Rhonda, D. K., M. Rio-Moreno, and A. Sarmento-Cabral. 2018. 40 Years of IGF1: Understanding the tissue-specific roles of IGF1/IGF1R in regulating metabolism using the Cre/loxP system. J. Mol. Endocrinol., 61 (1):T187. https://doi.org/10.1530/JME-18-0076

Rook, J. S. 2000. Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. Food Anim., 16 (2):293-317. https://doi.org/10.1016/S0749-0720(15)30107-9

Rui, L. 2011. Energy metabolism in the liver. Compr. physiol., 4 (1):177-197. https://doi.org/0.1002/cphy.c130024

Russo, V. M., A. W. N. Cameron, F. R. Dunshea, A. J. Tilbrook, and B. J. Leury. 2013. Artificially extending photoperiod improves milk yield in dairy goats and is most effective in late lactation. Small Rum. Res., 113 (1):179-186. https://doi.org/10.1016/j.smallrumres.2013.01.002

Safayi, S., P. K. Theil, V. S. Elbrønd, L. Hou, M. Engbaek, J. V. Nørgaard, K. Sejrsen, and M. O. Nielsen. 2010. Mammary remodeling in primiparous and multiparous dairy goats during lactation. J. Dairy Sci., 93 (4):1478-1490. https://doi.org/10.3168/jds.2009-2422

Salama, A. A. K., G. Caja, S. Hamzaoui, B. Badaoui, A. Castro-costa, M. M. Guilhermino, and R. Bozzi. 2014. Different levels of response to heat stress in dairy goats. Small Rum. Res., 121:73-79. https://doi.org/10.1016/j.smallrumres.2013.11.021

Sasaki, S. 2002. Mechanism of insulin action on glucose metabolism in ruminants. Anim. Sci. J., 73 (6):423-433. https://doi.org/10.1046/j.1344-3941.2002.00059.x

Singh, B., and A. Saxena. 2010. Surrogate markers of insulin resistance: A review. World J. Diabetes, 1 (2):36. https://doi.org/10.4239/wjd.v1.i2.36

Smith, M. C., and D. M. Sherman. 2009. Goat Medicine. 2nd Ed. Wiley-Blackwell. Iowa, USA. https://doi.org/10.1002/9780813818825

Sonagra, A. D. 2014. Normal pregnancy - a state of insulin resistance. J. Clin. Diagn. Res.:3-5. https://doi.org/10.7860/JCDR/2014/10068.5081

Souza, L. M., C. L. Mendonça, R. N. Assis, E. F. Oliveira, G. S. L. Soares, R. J. C. Souto, P. C. Soares, and J. A. B. Afonso. 2020. Changes in cardiac biomarkers in goats naturally affected by pregnancy toxemia. Res. Vet. Sci., 130:73-78. https://doi.org/10.1016/j.rvsc. 2020.02.016

Todini, L. 2007. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal (1:7):997-1008. https://doi.org/10.1017/S1751731107000262

Tokarz, V. L., P. E. MacDonald, and A. Klip. 2018. The cell biology of systemic insulin function. J. Cell Biol., 217 (7):2273-2289. https://doi.org/10.1083/jcb.201802095

Van Saun, R. J. 2009. Chapter 40 - Metabolic profiling. Pages 153-162 in Food Animal Practice. 5th Ed. D. E. Anderson and D. M. Rings, ed. Sauders. Saint Louis, USA. https://doi.org/10.1016/B978-141603591-6.10040-5

Wankhade, P. R., A. Manimaran, A. Kumaresan, S. Jeyakumar, K. P. Ramesha, V. Sejian, D. Rajendran, and M. R. Varghese. 2017. Metabolic and immunological changes in transition dairy cows: A review. Vet. World, 10 (11):1367-1377. https://doi.org/ 10.14202/ vetworld.2017

Watanabe, M., H. Hayasaki, T. Tamayama, and M. Shimada. 1998. Histologic distribution of insulin and glucagon receptors. Braz. J. Med. Biol., 31 (2):243-256. https://doi.org/ 10.1590/s0100-879x1998000200008

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. Rev., 26 (2):19-39. https://www.ncbi.nlm.nih.gov/pubmed/16278749

Williams, C. C., K. J. Calmes, J. M. Fernandez, C. C. Stanley, J. C. Lovejoy, H. G. Bateman II, L. R. Gentry, D. T. Gantt, and G. D. Harding. 2004. Glucose metabolism and insulin sensitivity in Gulf Coast Native and Suffolk ewes during late gestation and early lactation. mall Rum. Res., 54 (3):167-171. https://doi.org/10.1016/j.smallrumres. 2003.11.007

Yadav, S. N., D. N. Kalila, A. Phukan, G. Mahala, S. Tamuly, T. C. Dutta, A. Saleque, D. Barman, and P. Thakuria. 2015. Prevalence of sub-clinical ketosis in goats of Guwahati, Assam. J. Anim. Vet. Adv., 14 (13):382-385. http://dx.doi.org/10.36478/javaa. 2015.382.385

Zhao, F.-Q., and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. J. Dairy Sci., 90:E76-E86. https://doi.org/10.3168/jds.2006-470

Chapter 3 - Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia

F. Zamuner*[1], K. DiGiacomo*, A.W.N. Cameron†, B.J. Leury*

* Faculty of Veterinary and Agricultural Sciences - The University of Melbourne, Parkville, Victoria 3010, Australia.

† Meredith Dairy Pty Ltd, 106 Cameron Rd, Meredith, Victoria 3333, Australia.

3.1 Manuscript 1

Chapter 3 investigated the impacts of month of kidding, parity, and litter size on milk yield and composition within a herd of Saanen dairy goats at Meredith Dairy commercial farm.

This work has been published in the Journal of Dairy Science;

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia. J. Dairy Sci., 103 (1):954-964. https://doi.org/10.3168/jds.2019-17051

Therefore, the paper has been included in this chapter as a manuscript.

3.2 Abstract

The aim of this observational study was to identify the influence of key nongenetic factors such as month of kidding, parity, and litter size on milk yield and composition of Australian dairy goats throughout lactation. The study was conducted over 4 consecutive kidding seasons from June 2016 to March 2017. Data from 940 lactations of Saanen goats from a commercial herd were used to observe the effects of month of kidding, parity number, and litter size on total milk yield (L/goat) in early lactation (kidding to 90 d in milk; DIM), mid-lactation (91–180 DIM), and late lactation (181–270 DIM), cumulative milk yield (from kidding to 270 DIM; CMY), average lactation length, proportion (%) of goats reaching their target lactation length (270 DIM), somatic cell count (SCC), and percentages of milk fat and protein in early lactation. The mean herd responses throughout the entire study were as follows: CMY = 519 L/goat; lactation length = 233 d, with 70% of goats reaching 270 DIM; milk fat = 4.2%; milk protein = 2.9%; and SCC = 6.2×10^5 cells/mL. Average milk production peaked in February and was lowest in June (2.4 vs. 1.8 L/goat per day, respectively). Milk yield was affected by month of kidding, parity number, and litter size in all phases of lactation. November kidders had the greatest CMY, and March kidders had the lowest CMY. March kidders had the shortest lactation length and the lowest proportion of goats reaching 270 DIM. June kidders had the longest lactation length, whereas September kidders had the highest proportion of goats reaching 270 DIM. Maximum milk yield was attained in third parity. Goats in fourth or greater parity had the shortest lactation length, the lowest proportion of goats reaching 270 DIM, and the highest SCC. Goats delivering single kids had lower CMY, lower SCC, and higher percentages of fat and protein than goats delivering multiple kids. Our findings indicate that milk yield was primarily influenced by month of kidding, and the effects of month of kidding on milk yield were accentuated during mid-lactation. However, the effects of month of kidding on milk yield varied significantly among parities.

Key words: dairy goat, kidding season, lactation phase, lactation curve

3.3 Introduction

The Australian dairy goat industry has experienced a phase of dramatic growth in recent years. The Australian dairy goat sector is estimated to be growing at around 20%/year (Cameron, 2014), with the number of milking goats and production volume increasing more than 62% in the past 7 years (Zalcman and Cowled, 2018). This expansion has been stimulated predominantly by the increased popularity of fresh goat dairy products among Australian consumers (Stubbs and Abud, 2009). Today, around 60% of national goat milk production goes into fresh cheese making, and this requires a constant supply of milk year-round. However, Australian goat milk production remains seasonal, with production surpluses in spring-summer and shortfalls in winter (Cameron, 2003; Celi and White, 2012).

As a consequence of this productive and reproductive seasonality, significant farm management changes are necessary to achieve uniform milk production consistent with market needs (Stubbs and Abud, 2009). Accurate information about performance traits such as conception rates, total milk yield, and lactation persistency based on goat category (e.g., time of kidding, parity, litter size) is crucial for the optimization of herd management plans to achieve a more uniform distribution of milk production per year (Cameron, 2003; Carnicella et al., 2008). However, information on the productive performance of dairy goats raised under Australian conditions is still limited. Therefore, dairy goat farmers often extrapolate information either from overseas data or gathered from the cattle dairy sector (Celi and White, 2012).

Europe has the most organized market for goat milk and the largest source of information on dairy goat systems (Haenlein, 2001; Miller and Lu, 2019). However, European systems are mostly seasonal, with 1 to 2 kidding seasons per year (Pulina et al., 2018). Consequently, even though out-of-season breeding, and management of dairy systems based on multiple kidding

seasons per year are matters of considerable interest to Australian farmers, the literature on these topics is still scarce. Additionally, economic, climatic, and market characteristics of dairy goat farming in Australia are distinct from those of European dairy goat systems (Stubbs and Abud, 2009). For instance, the Australian dairy goat industry is characterized by relatively large farms (~450 milking goats/farm) and increased intensification of husbandry systems. Australian farmers are among the least subsidized in the world (OCDE, 2018; Zalcman and Cowled, 2018). On the other hand, European dairy goat herds are relatively small (~100 milking goats/farm), predominantly managed in extensive and semi extensive systems, and a direct subsidy is paid on milk production (Escareño et al., 2013; Pulina et al., 2018). Therefore, this study aimed to characterize and evaluate the effects of main nongenetic factors, such as month of kidding, parity number, and litter size, on milk yield and composition, as well as their possible interaction effects on lactation curves of Australian dairy goats.

3.4 Materials & Methods

All experimental procedures were approved by the Faculty of Veterinary and Agricultural Sciences Animal Ethics and Welfare Committee of The University of Melbourne, Australia (no. 1613846.1.).

3.4.1 Animals and Management

This study was conducted at Meredith Dairy commercial farm (Meredith, Australia; 37°50′S 144°04′E) over 4 consecutive kidding seasons from June 2016 to March 2017. The climate in the area is defined as "mild temperate" by the Australian Bureau of Meteorology, with summer beginning in December and winter beginning in June. The farm has 2,200 does and 116 bucks and follows a reproductive calendar based on 4 mating periods per year (males introduced for 30 d in October, January, April, and June), aiming for 600 goats kidding in each of the 4 kidding periods: March (MAR), June (JUN), September (SEP), and November (NOV).

The herd distribution according to parity is 35, 30, 20, and 15% for goats in first, second, third, and fourth (or greater) parity, respectively. In total, 1,000 (~250 per kidding season) clinically healthy pregnant goats (≤ 7 years old) were selected from the herd to be monitored throughout lactation. To get a representative subset of the population, goats were selected based on their parity number, aiming for parity distribution similar to that observed in the main herd.

Pregnancy and litter size were determined at 60 to 80 d post-breeding using transabdominal ultrasound (Jones and Reed, 2017). Pregnant goats were dried off 60 d before their expected kidding date. Enrolled goats were kept with the main herd throughout the study and were housed in a 1-sided shed (naturally ventilated; north-south oriented with the open side facing east) with a stocking density of 1.5 m^2 of floor space per head and 0.3 linear meters of feeder space per head. Fresh straw bedding was applied daily. Goats were fed a TMR once daily at around 0700 h. Diets were formulated as described by McDonald et al. (2002), aiming for 3.5 kg of DMI/goat. Approximate proportions (DM basis) of the components in the diet were wheat/barley (20%), fava beans (12%), legume hay (6%), lucerne silage (28%), ryegrass silage (27%), and commercial pellets (7%; 25% CP, 14 MJ of ME/kg, 29% NDF, 65 g of Ca/kg, 16 g of P/kg). The nutrient composition (per kg of DM) of the diet was 28% NDF, 16% CP, 10 MJ of ME, 18 g of Ca, and 9.0 g of P. Weekly adjustments were made to the amount of TMR offered to maintain a minimum 5% refusal rate, ensuring ad libitum feeding.

3.4.2 Measurements

Goat BW and BCS were measured within 1 wk after delivery (before feeding and after the morning milking). The BCS was scored by the same person, adopting a 6-point scale method (Villaquirán et al., 2004).

Does were weaned from their kids within 1 d of parturition, and the kids were reared as a separate animal group and fed milk replacer via automatic feeders. Does were milked twice daily in a 36-sided herringbone system fitted with automatic cup removers and in-line

electronic milk meters (MidiLine SG Parlor, DeLaval Inc., Westmeadows, VIC, Australia), and individual milk volume was recorded at 0600 and 1600 h. Although data from the general herd were used to estimate average milk yield (**Figure 3.1 c**) and bulk-tank milk composition (**Figure 3.1 b**), only data from experimental animals were included in the statistical analysis. The lactation period was up to 270 DIM and was assessed in 3 phases: early lactation (kidding to 90 DIM; EMY), mid-lactation (91–180 DIM; MMY), and late lactation (181–270 DIM; LMY).

The bulk-tank milk samples were collected daily, preserved with bronopol (Broad Spectrum Microtabs II, D & F Control Systems Inc., Norwood, MA), stored at 4°C, and shipped to the laboratory (DTS Food Assurance, North Melbourne, VIC, Australia) at weekly intervals for analysis of fat, protein, lactose, and TS using a CombiFoss FT+ milk analyzer (Foss, Hillerød, Denmark). Herd test was performed once in early lactation, and individual milk samples were collected from all goats on the same day (48 ± 13.0 DIM; mean ± SD). Individual milk samples were preserved with bronopol and transported on ice (4°C) to the laboratory at Herd Improvement Co-Operative (Maffra, VIC, Australia) on the same day of sample collection. Samples were heated to 40°C before the determination of milk fat, protein, and SCC using a NexGen milk analyzer (Bentley Instruments, Chaska, MN).

The environmental conditions on each day throughout the study were obtained from Sheoaks meteorological station database (BOM, 2017; 16 km from the farm), and monthly variations in daylight length (hours of light per day) and maximum and minimum temperatures (°C) are presented in **Figure 3.1 a.**

3.4.3 Dry-Off Threshold

Goats that had an average milk yield of <3.0 L/wk for 2 consecutive weeks were dried off irrespective of whether they had met their lactation target (270 DIM). Goats that maintained

production above 3.0 L/wk for the entire lactation were dried off 60 d before their expected kidding date.

3.4.4 Statistical Analyses

The effects of month of kidding, parity number, and litter size and all possible interactions on milk yield and composition were determined using restricted maximum likelihood (GenStat, 18th ed.; VSN International Ltd., Hemel Hampstead, UK). Before statistical analysis, SCC values were transformed into somatic cell score (SCS) to achieve normality using the equation SCS = log2 (SCC/100) + 3, where SCC is in cells per microliter (Weigel, and Shook, 2018). Due to reduced animal numbers, goats of parity 4, 5, and 6 were grouped as parity 4+, and goats carrying 2 or 3 fetuses were grouped as litter size 2+. The month of kidding × litter size, parity number × litter size, and month of kidding × parity number × litter size interactions were not significant for all variables analyzed, and only the main effects for LS were retained in the model

$$Y_{ijkl} = \mu + MK_i + PN_j + (MK_i \times PN_j) + LS_k + R_l + E_{ijkl},$$

where Y_{ijkl} = calculated variable; μ = overall mean; MK_i = month of kidding (i = MAR, JUN, SEP, NOV); PN_j = parity number (j = 1, 2, 3, 4+); LS_k = litter size (k = 1 or 2+); R_l = random effect of goat (l = 1, . . ., n); and E_{ijkl} = residual error assumed to be normally and independently distributed. The Bonferroni method with a 95 % confidence interval was used for pairwise comparisons. Statistical significance was declared at $P < 0.05$. Data are presented as means ± standard error unless declared otherwise. The time to dry-off was analyzed using the Wilcoxon (Peto-Prentice) nonparametric survival analysis test (Collett, 2015). The analysis was performed using the Kaplan-Meier and RSTEST (95% confidence interval) procedures in GenStat (18th ed.).

3.5 Results

Out of the 1,000 animals, 60 were excluded from the study due to abortions, death, or kidding delays (total n = 940). As the study progressed, goats were dried off in line with commercial practice, as previously described, and the proportion of goats still milking along the 270-d lactation is described in **Figure 3.2**. The number of goats commencing each lactational phase being dried off and proportion of goats reaching the end of each lactational phase are presented in **Table 3.1**. Descriptive statistics of all variables analyzed are reported in **Table 3.2**. The means (± SD) of bulk-tank milk content during the 18mo of study were as follows: fat, 3.6 ± 0.54%; protein, 3.1 ± 0.39%; lactose, 4.8 ± 0.13%; TS, 12.1 ± 1.02%; and ash, 0.61 ± 0.23%.

3.5.1 Month of Kidding

The main effects of month of kidding on milk yield, milk composition in early lactation, lactation length, and SCC are presented in **Table 3.3**. Monthly variations in daylight length, temperature, milk yield (general herd), bulk-tank milk composition, and lactation curves of goats that kidded in MAR, JUN, SEP, and NOV are depicted in **Figure 3.1**. Month of kidding affected ($P < 0.001$) milk yield in all phases of lactation. Goats that kidded in NOV had the highest cumulative milk yield (**CMY**), and goats that kidded in MAR had the lowest CMY (578 ± 18.4 vs. 455 ± 17.5 L, respectively; $P < 0.001$).

Goats that kidded in NOV averaged greater EMY (+15%; $P < 0.001$) compared with goats that kidded in MAR, JUN, and SEP. Goats kidding in SEP and NOV (spring) averaged greater EMY (+10%; $P < 0.01$) and greater MMY (+28%; $P < 0.001$) than goats that kidded in MAR and JUN (autumn-winter). Goats kidding in NOV reached the highest peak milk yield ($P < 0.001$), and goats kidding in MAR had the lowest peak milk yield (2.8 ± 0.05 vs. 2.4 ± 0.05 L/d, respectively; **Figure 3.1 c**).

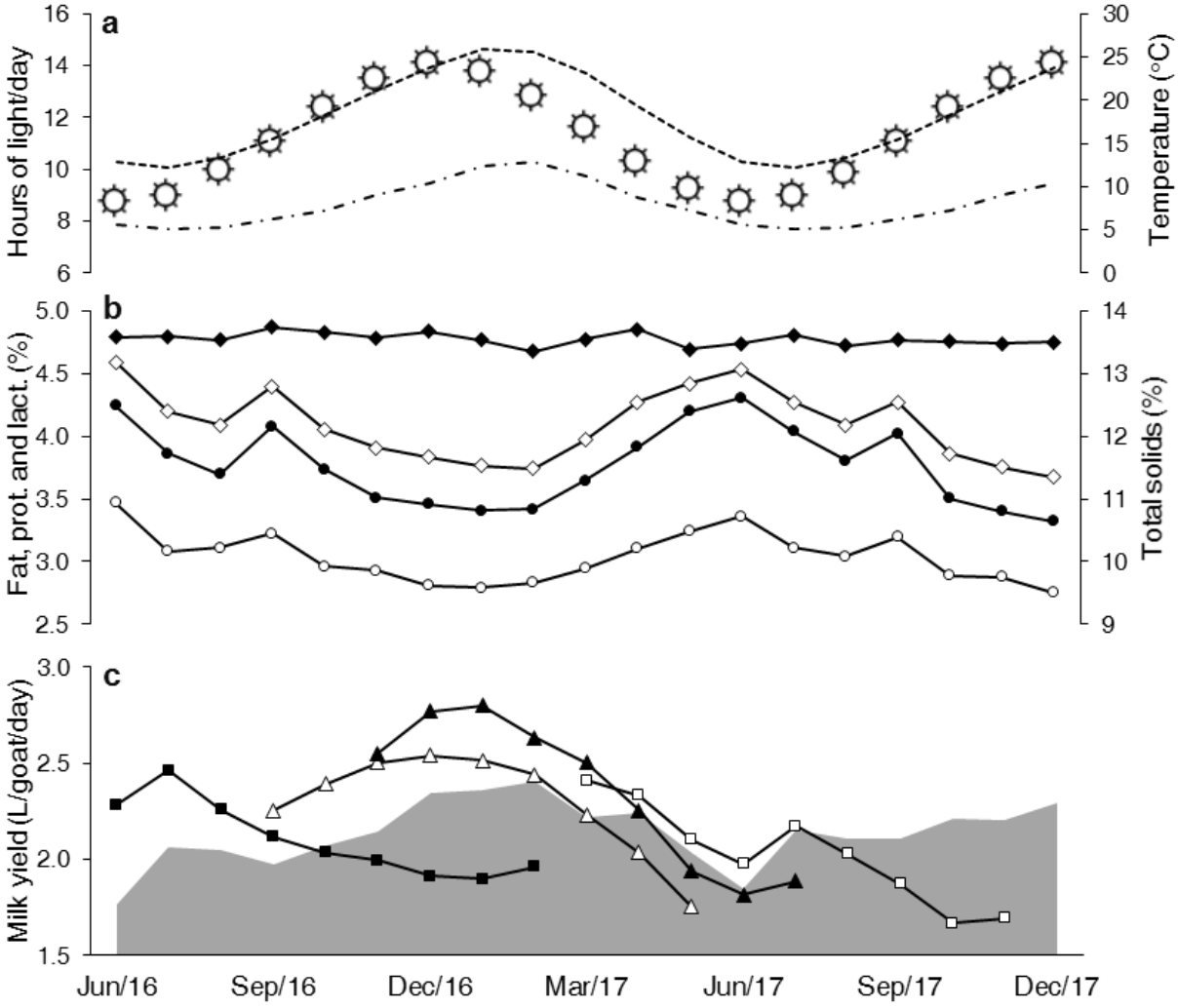

Figure 3.1. (a) Monthly variations in daylight length (hours of light/d; sun symbol) and mean maximum (-----) and minimum (— · —) ambient temperature (°C). (b) Changes in the contents (%) of fat (●), protein (prot.; ○), lactose (lact.; ◆), and TS (◇) in bulk milk samples. (c) Lactation curves of milk yield (L/d) of goats that kidded in June (■; n = 242), September (△; n = 223), November (▲; n = 239), and March (□; n = 237) and the herd milk yield (gray area; the number of goats in milk, n ~ 1,500) at Meredith Dairy (Meredith, VIC, Australia) from June 2016 to December 2017

Goats that kidded in MAR had the shortest ($P < 0.001$) lactation length and lowest ($P = 0.001$) proportion of goats reaching 270 DIM (**Table 3.1**). Goats that kidded in JUN had the longest ($P < 0.001$) lactation length, whereas goats that kidded SEP had the highest ($P = 0.001$)

proportion of goats reaching 270 DIM (**Table 3.1**). Goats that kidded in NOV (late spring) had the highest SCC, and goats that kidded in JUN (winter) had the lowest SCC (7.7 vs. 6.0 × 10^5 cells/ mL, respectively; $P < 0.001$).

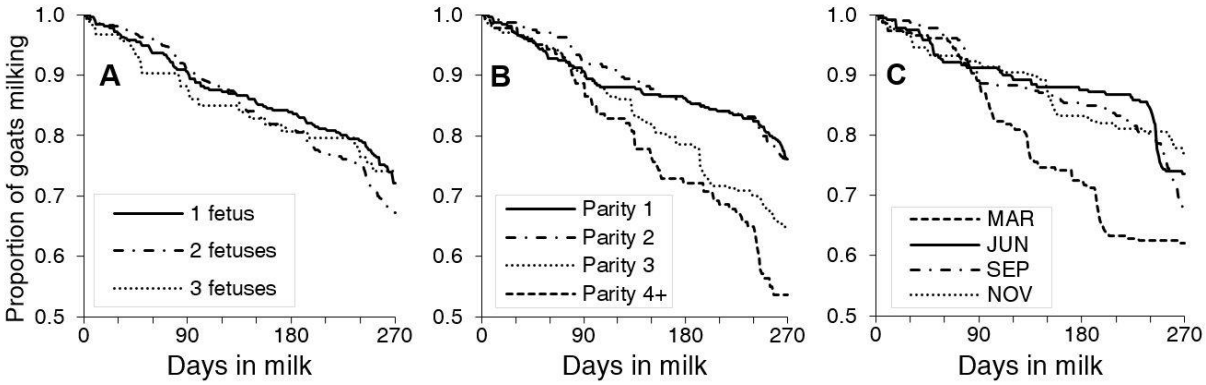

Figure 3.2. Kaplan-Meier survival curves for the proportion of does (n = 940) still milking during a 270-d lactation at Meredith Dairy (Meredith, VIC, Australia) analyzed by (a) litter size, (b) parity number, and (c) month of kidding (MAR = March, JUN = June, SEP = September, NOV = November). Days in milk were measured from the kidding date for each doe, and data were censored at 270 DIM.

3.5.2 Parity Number

The main effects of parity number on milk yield, milk composition, lactation length, and SCC are presented in **Table 3.3**. Goats in first parity had lower CMY (-26%; $P < 0.001$) than goats in third parity. However, the difference between first and third parity gradually decreased as goats advanced into lactation [-33% for EMY ($P < 0.001$), -27% for MMY ($P < 0.001$), and -21% for LMY ($P < 0.001$)]. There were no differences among second and third parity for EMY, MMY, LMY, and CMY. The differences between goats in fourth or greater parity and third parity gradually increased as goats advanced into lactation [-6% for EMY ($P = 0.153$), -11% for MMY ($P = 0.002$), and -12% for LMY ($P = 0.007$)]. Goats in fourth or greater parity had the shortest ($P < 0.01$) lactation length, the lowest ($P < 0.001$) proportion of goats reaching

270 DIM (**Table 3.1**), and the highest ($P < 0.001$) SCC. Parity number did not affect contents (%) of milk fat and protein in early lactation.

3.5.3 Combined Effects of Month of Kidding and Parity Number

The month of kidding did not affect MMY and LMY of first-parity goats (**Table 3.4**). Goats in fourth or greater parity kidding in SEP and NOV (spring) had considerably higher MMY (+51%; $P < 0.001$) and LMY (+33%; $P < 0.001$) than goats in fourth or greater parity kidding in MAR and JUN (autumn-winter). Except for goats kidding in MAR, third-parity goats had the highest ($P < 0.05$) milk yield in all lactation phases regardless of month of kidding. Third-parity goats kidding in NOV (late spring) had the highest ($P < 0.05$) EMY (287 ± 9.3 L/goat) and MMY (258 ± 7.3 L/goat), whereas third-parity goats kidding in SEP (early spring) had the highest ($P < 0.05$) LMY (198 ± 11.1 L/goat).

3.5.4 Litter Size

Percentages of single, twin, and triplet kids were 35.2, 54.8, and 9.9%, respectively. Goats delivering multiple kids had higher EMY (+6%; $P < 0.01$), MMY (+9%; $P < 0.001$), LMY (+8%; $P < 0.001$), and CMY (+7%; $P < 0.05$) than goats delivering single kids (**Table 3.3**). Goats delivering single kids had higher percentages of milk fat (4.29 vs. 4.15%; $P < 0.05$), higher percentages of milk protein (2.95 vs. 2.87%; $P < 0.01$), and lower SCC (5.6 vs. 7.5 × 10^5 cells/mL; $P < 0.01$) than goats delivering multiple kids (**Table 3.3**). There was no significant effect of litter size on lactation length (**Table 3.3**) on the proportion of goats reaching 270 DIM (**Table 3.1**).

Table 3.1. Number of goats commencing and dried-off in each phase of lactation1, and survival probabilities (Kaplan-Meier method) censured at 90, 180 and 270 days in milk (DIM), grouped by litter size (LS), parity number (PN), and month of kidding (MK)

	Total	LS		PN				MK				P – value[2]		
Period of censoring		1	2+	1	2	3	4+	MAR	JUN	SEP	NOV	LS	PN	MK
Milking at 1 DIM	940	330	612	319	245	237	140	237	242	223	239			
Dried off ≤ 90 DIM	89	31	58	30	19	24	16	26	21	17	25			
Still milking at 90 DIM[3](%)	0.91	0.91	0.91	0.91	0.92	0.90	0.89	0.89	0.91	0.92	0.90	NS	NS	NS
Milking at 91 DIM	851	300	553	290	225	213	125	212	223	207	215			
Dried off (91-180 DIM)	79	22	57	14	15	27	23	40	9	20	10			
Still milking at 180 DIM[3](%)	0.82	0.84	0.82	0.72	0.67	0.74	0.72	0.72	0.88	0.83	0.85	NS	**	***
Milking at 181 DIM	772	279	500	277	211	187	103	172	214	186	206			
Dried off (180-270 DIM)	117	39	78	32	26	33	26	24	34	14	45			
Still milking at 270 DIM[3] (%)	0.70	0.72	0.68	0.76	0.75	0.65	0.54	0.62	0.74	0.77	0.67	NS	***	***

[1]Early lactation = kidding to 90 DIM; mid lactation = 91–180 DIM; late lactation = 181–270 DIM

[2]Wilcoxon (Peto-Prentice) nonparametric survival analysis test.

[3]Kaplan-Meier survival probabilities measured from kidding date for each doe and data censored at 90, 180 and 270 DIM.

**P < 0.01; ***P < 0.001; NS = not significant.

3.6 Discussion

To our knowledge, this is the first study comparing the effects of month of kidding, parity, and litter size on productive traits of commercial dairy goats raised in intensive feeding systems and managed in multiple kidding seasons per year. The large field data set available has enabled a more in-depth analysis of how different factors affect the evolution of milk yield, milk quality, lactation length, and proportion of goats still milking during a 270-d lactation. The major finding from this study was that the month of kidding was the primary factor influencing differences in productivity of multiparous goats, particularly during mid-lactation.

Table 3.2. Descriptive statistics for BCS, BW, and total milk yield in early, mid, and late lactation,[1] cumulative milk yield from kidding to 270 DIM (CMY, L/goat), lactation length, SCS, SCC, and percentage of milk fat and protein of a commercial dairy goat herd in Australia

Item	N	Mean	SE	SD	CV (%)
BCS	940	2.6	0.01	0.24	9.3
BW (kg)	940	68	0.5	15.7	23.0
Total milk yield (L/goat)					
Early lactation	940	211	2.5	75.3	35.7
Mid lactation	851	194	2.4	70.9	36.5
Late lactation	772	161	2.2	59.7	37.1
CMY (L/goat)	940	519	7.3	224.6	43.3
Lactation length (d)	940	233	2.4	72.2	31.0
Fat[2] (%)	878	4.2	0.03	0.93	22.1
Protein[2] (%)	878	2.9	0.01	0.36	12.6
SCS2[2]	878	5.6	0.1	1.8	31.9
SCC[3] (x10^5/ml)		6.2	-	0.3	-

[1]Early lactation = kidding to 90 DIM; mid lactation = 91–180 DIM; late lactation = 181–270 DIM.

[2]Corresponds to individual milk samples collected once in early lactation. Milk samples were collected from all goats on the same day (48 ± 13.0 DIM; mean ± SD).

[3]Back-transformed values.

SE = standard error

SD = standard deviation

3.6.1 Milk Yield

The mean milk production of the present study (519 ± 7.3 kg for CMY and 233 ± 2.4 d for lactation length; **Table 3.2**) was inferior to the national average of French goat herds (949 kg/goat; IDELE, 2017) and to those published by other observations in dairy goats of a similar breed composition (Zoa-Mboé et al., 1997; Solis-Ramirez, 2014), showing considerable potential for improvement of Australian dairy goat productivity.

Overall, superior milk production (+17%) was observed in goats that kidded in SEP and NOV (spring) compared with goats that kidded in MAR and JUN (autumn-winter). The higher milk yield in spring kidders was likely driven by the longer photoperiod during mid-lactation, as September kidding would result in peak production (mid-lactation) in December when the photoperiod is maximal, whereas March kidding would result in peak production in June when the photoperiod is minimum. Goats that kidded in JUN (winter) reached peak lactation at around 40 DIM and slowly reduced daily yield in the subsequent months. Conversely, goats that kidded in SEP (early spring) reached peak lactation much later, at around 90 DIM, and sharply decreased after 150 DIM (**Figure 3.1 c**). Furthermore, the herd's average milk yield peaked in February 2017 (summer) and was lowest in June 2016 (winter; 2.4 ± 0.19 vs. 1.8 ± 0.10 L/d, respectively; **Figure 3.1 c**), a clear indication of substantial effects of photoperiod on goat productivity.

Arnal et al. (2018) demonstrated that the shape of the goat lactation curve is tied to the duration of the photoperiod, and that month of kidding is strongly linked to mid-lactation production. Moreover, multiple studies have reported the influence of month of kidding (season of the year, more specifically) on peak yield, lactation persistence (ability to maintain production at a higher level after peak yield), and CMY of dairy goats (Montaldo et al., 1997; León et al., 2012). The findings presented here agree with previous studies that have shown

that although goats kidding in winter have lower production at the beginning of lactation, they maintain peak production for longer than goats kidding during the spring.

Several studies have shown that seasonal variations of environmental factors such as feed quality, air temperature, relative humidity, rainfall, and solar radiation have a significant effect on milk yield and physicochemical milk composition (Nardone et al., 2010; Salari et al., 2016; Clark and García, 2017). In this study, the flock was housed in 1-sided sheds and fed a TMR year-round. Therefore, the influence of changes in feed quality on variations in milk yield was likely to be minimal due to the lack of grazing and goats being fed to match their nutrient requirements. Thus, higher herd milk yield in summer was more likely driven by the longer photoperiod.

Abundant literature demonstrates that photoperiod is a primary environmental variable influencing milk yield and feed intake of dairy goats (Neville et al., 2002; Garcia-Hernandez et al., 2007; Flores et al., 2011). Although feed intake was not measured in this study, increased feed intake in the longer days was presumably the primary source of variation in milk yield. In a previous study on the same farm, Russo et al. (2013) stated that goats exposed to 16 h of artificial light had higher milk yield and higher live weight and that analysis of their metabolic profile indicated better energy balance than a control group exposed to natural light (9.5–10 h of light/d).

Table 3.3. Main effects of litter size (SL), parity number (PN) and month of kidding (MK) on total milk yield (L/goat) early, mid, and late-lactation1, cumulative milk yield at 270 DIM (CMY), lactation length (total DIM), milk fat, milk protein, SCS, and SCC of Australian dairy goats (n = 940)2

	LS		PN				MK				SED[3]			P-value		
Item	1	2+	1	2	3	4+	MAR	JUN	SEP	NOV	LS	PN	MK	LS	PN	MK
Total milk yield (L/goat)																
Early lactation	210^{b}	223^{a}	163^{b}	230^{a}	244^{a}	229^{a}	206^{b}	206^{b}	210^{b}	244^{a}	4.6	6.4	6.4	**	***	***
Mid lactation	188^{b}	204^{a}	161^{c}	209^{ab}	220^{a}	195^{b}	165^{b}	179^{b}	218^{a}	223^{a}	4.6	6.4	6.5	***	***	***
Late lactation	155^{b}	168^{a}	140^{c}	172^{ab}	178^{a}	157^{b}	139^{c}	161^{b}	182^{a}	164^{b}	4.3	6.1	6.1	**	***	***
CMY[4]	508^{b}	543^{a}	431^{c}	570^{ab}	587^{a}	514^{b}	455^{c}	513^{b}	555^{ab}	578^{a}	14.8	20.6	20.6	*	***	***
Lactation Length	232	231	241^{a}	242^{a}	230^{ab}	217^{b}	216^{b}	241^{a}	234^{a}	236^{a}	5.0	7.0	6.9	NS	**	***
Fat[5] (%)	4.29^{a}	4.15^{b}	4.13	4.27	4.21	4.26	4.33^{a}	4.20^{a}	3.95^{b}	4.41^{a}	0.100	0.093	0.093	*	NS	***
Protein[5] (%)	2.95^{a}	2.87^{b}	2.92	2.89	2.88	2.93	3.10^{a}	3.02^{a}	2.68^{c}	2.83^{b}	0.030	0.33	0.033	**	NS	***
SCS[5]	5.5^{b}	5.9^{a}	5.3^{b}	5.5^{b}	5.7^{ab}	6.2^{a}	5.6^{ab}	5.4^{b}	5.8^{ab}	5.9^{a}	0.13	0.17	0.17	**	***	***
SCC[6] (x10^{5}/ml)	(5.6)	(7.5)	(5.1)	(5.7)	(6.6)	(9.2)	(6.0)	(5.4)	(7.1)	(7.7)						
Animals sampled[7] (no.)	305	573	294	230	221	133	217	228	227	206						

$^{a-c}$Means within a row with different lowercase superscripts differ (P < 0.05).

[1]Early lactation = kidding to 90 DIM; mid lactation = 91–180 DIM; late lactation = 181–270 DIM.

[2]Values are the restricted maximum likelihood predicted means.

[3]Pooled standard error of differences.

[4]Number of goats in each category is presented in Table 3.1.

[5]Corresponds to individual milk samples collected once in early lactation. Milk samples were collected from all goats on the same day (48 ± 13.0 DIM; mean ± SD).

[6]Back-transformed means.

[7]Number of animals sampled in each category for the determination of milk fat, protein, and SCC.

*P < 0.05; **P < 0.01; ***P < 0.001; NS = P > 0.05.

Recent studies suggested that the prepartum photoperiod affects mammary development and subsequent milk production of dairy cows, ewes, and does (Mabjeesh et al., 2007; Mikolayunas et al., 2008; Lacasse et al., 2014). Interestingly, all studies reported that animals exposed to a short-day photoperiod during the last trimester prepartum produced more milk in the subsequent lactation than animals exposed to a long-day photoperiod. It is reasonable to conclude that variations in prepartum photoperiod have influenced our results to some extent. However, the present study was not designed to measure such effects. Thus, the characterization of potential prepartum photoperiod effects on milk yield and composition of Australian dairy goats is a matter for further research.

In this study, month of kidding affected EMY, MMY, LMY, and CMY of goats in all parities. However, month of kidding affected the yield of primiparous goats less than that of multiparous goats. Further, differences in milk yield between goats of equal parity that kidded in different months were accentuated during mid-lactation (**Table 3.4**). The effect of parity on milk yield showed an almost steady increasing trend from first to third parity in all phases of lactation. First-parity goats had the lowest milk yields, and third-parity goats had the greatest milk yields (431 vs. 587 L, respectively; $P < 0.001$), with goats in fourth or greater parity performing intermediately (**Table 3.3**). This increase and decrease according to parity number agrees with several published studies in dairy goats (Gipson and Grossman, 1990; Goetsch et al., 2011; Arnal et al., 2018). According to Lérias et al. (2014), the greater milk production in multiparous goats can be explained by older goats tending to have a higher proportion of alveoli developed in the previous lactation added to those developed in subsequent lactations, increasing secretory parenchyma and udder volume compared with primiparous goats. Furthermore, as animals get older there is a reduction in the use of energy for growth, favoring partitioning of nutrients toward milk production (Lérias et al., 2014).

In the present study, differences in productivity varied as goats advanced into lactation, gradually decreasing between goats in first and third parity and increasing between goats in third and fourth or greater parity. Our results are supported by others who reported increased initial and peak milk yields with increasing parity (until about the third or fourth parity), later peak with greater persistence in first-parity goats, and decreased persistence with increasing parity (Gipson and Grossman, 1990; León et al., 2012; Arnal et al., 2018).

Figure 3.2 shows the proportion of goats being removed from the milking herd (dried off or culled) as lactation progresses. The low milk yield recorded for goats kidding in MAR may explain the greater removal in this group, whereas the gradual increase in the removal rate with increasing parity is more likely linked to the natural decline of resilience against illness and disease as animals get older. However, information obtained from farm records were not detailed enough, and we could not test for associations between nongenetic factors and the proportion of goats removed for involuntary (e.g., disease, injury, death) or economic (e.g., low yield, milk surplus) reasons.

Moreover, despite goats being weaned from their kids within 1 d of parturition and the kids being reared artificially, larger litter size positively affected EMY (+6%), MMY (+9%), and LMY (+8%). As a result, goats delivering multiple kids had greater CMY (+35 L) than goats delivering single kids (**Table 3.3**). Our findings agree with those of several authors who found a higher milk production in multiple deliveries in commercially milked goats (Browning et al., 1995; Crepaldi et al., 1999; Brito et al., 2011). Litter size is positively associated with antepartum levels of mammogenic hormones such as placental lactogen, progesterone, and prolactin (Lérias et al., 2014). Thus, it is plausible to assume that the greater milk yield observed in goats delivering multiple kids was a consequence of intensified stimulation of the mammary gland development during gestation.

Table 3.4. Combined effects of parity number (PN) and month of kidding (MK) on total milk yield (L/goat) in early, mid, and late lactation[1], and cumulative milk yield at 270 DIM (CMY) of Australian dairy goats (n = 940)[2]

Milk yield (L/goat)	PN	MK				SED[3]	P-Value
		MAR	JUN	SEP	NOV	PN × MK	PN × MK
Early lactation	1	170^{abB}	154^{abB}	141^{bB}	187^{aB}	12.6	***
	2	229^{A}	215^{A}	229^{A}	248^{A}		
	3	204^{bA}	241^{abA}	243^{abA}	287^{aA}		
	4+	220^{A}	215^{A}	226^{A}	255^{A}		
Mid lactation	1	148	153^{B}	168^{B}	176^{B}	12.8	***
	2	192^{ab}	186^{bAB}	231^{abA}	226^{aA}		
	3	174^{c}	213^{bA}	234^{abA}	258^{aA}		
	4+	148^{b}	162^{bB}	238^{aA}	231^{aA}		
Late lactation	1	139	147^{B}	135^{B}	139^{B}	12.1	***
	2	157	182^{A}	183^{A}	165^{AB}		
	3	134^{b}	174^{aAB}	211^{aA}	191^{aA}		
	4+	128^{b}	142^{bB}	198^{aA}	160^{abAB}		
CMY[4]	1	411	418^{B}	415^{B}	479^{B}	40.8	**
	2	529	544^{A}	623^{A}	586^{AB}		
	3	445^{b}	600^{aA}	621^{abA}	681^{aA}		
	4+	434	491^{B}	563^{AB}	568^{AB}		

$^{a-c}$Means within a row with different lowercase superscripts differ ($P < 0.05$).

^{A,B}Means within a column with different uppercase superscripts differ ($P < 0.05$).

[1]Early lactation = kidding to 90 DIM; mid lactation = 91–180 DIM; late lactation = 181–270 DIM.

[2]Values are the restricted maximum likelihood predicted means.

[3] SED = Pooled standard error of differences.

[4]Number of goats in each category is presented in Table 3.1.

**P < 0.01; ***P < 0.001.

3.6.2 Milk Composition

The basic composition of bulk-tank milk showed marked seasonal variation during the study (**Figure 3.1 b**). Overall, bulk-tank milk collected during winter months had higher contents of fat (+16%), protein (+14%), and TS (+9%) than milk collected during summer, whereas lactose content remained relatively constant throughout the year. In this study, the herd was divided

into 4 kidding seasons per year, managed indoors, and fed a constant ration for the entire lactation. Therefore, variation in climatic conditions was presumably a primary factor affecting bulk milk composition. The effects of both climatic conditions and stage of lactation change over time and the precise effect of one factor is often confounded by the effects of the other (Salama et al., 2014). The distinction between the effects of these 2 factors is even less evident in dairy systems where goats kid once yearly in spring. In such systems, mid-lactation, when milk fat (%) is expected to reach its lowest point during lactation (Goetsch et al., 2011), coincides with summer, when the increasing air temperature, temperature−humidity index, and solar radiation are known to negatively affect the main physicochemical characteristics of goat milk (Salama et al., 2014; Kljajevic et al., 2018). Our results are consistent with previous observations that have demonstrated that the effects of climatic conditions on milk composition vary according to the stage of lactation (Salama et al., 2014) but also indicate that the influence of the former surpasses the effects of the latter.

Despite the considerable variation in bulk milk composition, results from individual milk samples collected in early lactation (**Table 3.3**) show a much smaller degree of variation. Milk from JUN kidders (sampled in mid-July) had a higher content of fat (+6.7%) but lower protein (-4.8%) than milk from NOV kidders (sampled in mid-January). Additionally, milk yield and milk solids (%) varied in an opposite manner throughout the year (**Figure 3.1**), suggesting that the decline in total milk solids (%) during summer was, to some extent, a reflection of the increased milk yield with increasing photoperiod (the dilution effect).

Our results also show that parity number did not affect the percentages of milk fat or protein (**Table 3.3**). However, results regarding the effects of parity on milk composition in dairy goats are conflicting. For instance, authors have reported that increased parity increases fat (Šlyžius et al., 2017), increases protein content with no effect on fat (Mioč et al., 2008), or increases both fat and protein percentages (Carnicella et al., 2008). Further, we observed a higher content

of fat (+3.4%) and protein (+2.8%) in milk from goats delivering 1 kid (**Table 3.3**). Our results contrast with those of Carnicella et al. (2008), who reported that litter size had no significant effect on milk composition of Maltese goats.

Although there is no mention in the Australian legislation regarding a standard limit value for SCC in goat milk, the herd-average SCC ($6.2 \times 10^5 \pm 0.3$ cells/ mL) was below the threshold of 7.0×10^5 cells/mL that indicates changes in the immune status of the goat mammary gland (Clark and García, 2017). Milk SCC is normally higher in goats than in cows because the apocrine secretory system of goats naturally results in a greater number of skin cells and cell fragments being present in the milk compared with milk from healthy cows (Clark and García, 2017). In the present study, lactations initiated in warmer months, increased parity, and multiple pregnancies all increased SCC. After an extensive review of factors affecting SCC in dairy goats, Granado et al. (2014) suggested that more extended exposure to pathogens might be the reason for higher SCC in older animals and that heat is directly responsible for the higher SCC in warmer months by favoring cell growth. However, the explanation for the influence of litter size on SCC is still insufficient because the authors have found that goats delivering multiple kids showed higher SCC regardless of whether the goats were suckled by their kids or machine milked (Granado et al., 2014).

3.6.3 Industry Implications

Australian dairy goat farmers are aiming to continue increasing milk production over the next 5 years with the goal of achieving production levels comparable with their European counterparts (Zalcman and Cowled, 2018). Our results showed that month of kidding had a considerable effect on lactation curves of Australian dairy goats, indicating that light manipulation, a cost-effective and straightforward method (GarciaHernandez et al., 2007; Russo et al., 2013; Cameron, 2014), could accelerate increments in the national herd productivity. Moreover, this study has identified significant interactions between month of

kidding and parity number, suggesting that the effects of such factors on milk traits are not independent of each other and that interactive effects should be considered when analyzing individual performance.

From a higher-level perspective, these results are consistent with previous findings observed in European studies. However, this study not only gives additional information on how nongenetic and environmental factors can affect milk production of commercial dairy goats but, most importantly, also has produced new knowledge regarding productive traits of dairy goats raised in intensive feeding systems and managed in multiple kidding seasons per year.

3.7 Conclusions

In this study, month of kidding had the greatest effect on CMY and lactation curves of multiparous goats. Goats kidding in spring produced more milk than goats kidding in the autumn and winter. Additionally, the effects of month of kidding on milk yield increased with increasing parity and were accentuated during mid-lactation. Maximum milk production was attained in third parity, and goats delivering multiple kids had slightly superior milk yield in detriment of milk fat and protein.

3.8 Acknowledgments

Fernanda Zamuner received a Ph.D. scholarship financed by the Australian Government Research Training Program (Melbourne, VIC, Australia). The authors gratefully acknowledge Meredith Dairy Ltd. (Meredith, VIC, Australia) personnel for their assistance throughout this study. The authors declare there was no conflict of interest during the entire process of this study.

3.9 References

Arnal, M., C. Robert-Granié, and H. Larroque. 2018. Diversity of dairy goat lactation curves in France. J. Dairy Sci., 101 (12):11040-11051. https://doi.org/10.3168/jds.2018-14980

BOM (Australian Bureau of Meteorology) 2017. Climate statistics for Australian locations. Accessed Oct. 02, 2018. http://www.bom.gov.au/climate/averages/tables/cw_087168_All.shtml

Brito, L. F., F. G. Silva, A. L. P. Melo, G. C. Caetano, R. A. Torres, M. T. Rodrigues, and G. R. O. Menezes. 2011. Genetic and environmental factors that influence production and quality of milk of Alpine and Saanen goats. Genet. Mol. Res., 10 (4):3794-3802. http://dx.doi.org/10.4238/2011

Browning Jr, R., M. L. Leite-Browning, and T. Sahlu. 1995. Factors affecting standardized milk and fat yields in Alpine goats. Small Rum. Res., 18 (2):173-178. https://doi.org/10.1016/0921-4488(95)00719-2

Cameron, A. W. N. 2003. Increase in autumn and winter milk production in dairy goats. Rural Industries Research and Development Corporation (RIRDC). Australia. Accessed on 13/04/2020. https://www.agrifutures.com.au/wp-content/uploads/publications/03-105.pdf

Cameron, A. W. N. 2014. Optimising genetics, reproduction and nutrition of dairy sheep and goats. Rural Industries Research and Development Corporation (RIRDC). Australia. Accessed on Accessed Sep. 20, 2018. Retrieved from https://www.agrifutures.com.au/wp-content/uploads/publications/14-083.pdf

Carnicella, D., M. Dario, M. C. C. Ayres, V. Laudadio, and C. Dario. 2008. The effect of diet, parity, year and number of kids on milk yield and milk composition in Maltese goat. Small Rum. Res., 77 (1):71-74. https://doi.org/10.1016/j.smallrumres.2008.02.006

Celi, P., and P. White. 2012. Dairy goat farming in Australia: current challenges and future developments. Paper presented at the First Asia Dairy Goat Conference, Kuala Lumpur, Malaysia. http://www.fao.org/docrep/017/i2891e/i2891e00.pdf

Clark, S., and M. B. M. García. 2017. A 100-year review: Advances in goat milk research. J. Dairy Sci., 100 (12):10026-10044. https://doi.org/10.3168/jds.2017-13287

Collett, D. 2015. Modelling survival data in medical research. Chapman & Hall. London, UK.

Crepaldi, P., M. Corti, and M. Cicogna. 1999. Factors affecting milk production and prolificacy of Alpine goats in Lombardy (Italy). Small Rum. Res., 32 (1):83-88. https://doi.org/10.1016/S0921-4488(98)00156-4

Escareño, L., H. Salinas-Gonzalez, M. Wurzinger, L. Iñiguez, J. Sölkner, and C. Meza-Herrera. 2012. Dairy goat production systems: status quo, perspectives and challenges. Trop. Anim. Health Prod., 45 (1):17-34. https://doi.org/10.1007/s11250-012-0246-6

Flores, M. J., J. A. Flores, J. M. Elizundia, A. Mejía, J. A. Delgadillo, and H. Hernández. 2011. Artificial long-day photoperiod in the subtropics increases milk production in goats giving birth in late autumn. J. Anim. Sci., 89 (3):856-862. https://doi.org/10.2527/jas.2010-3232

Garcia-Hernandez, R., G. Newton, S. Horner, and L. C. Nuti. 2007. Effect of photoperiod on milk yield and quality, and reproduction in dairy goats. Livestock Science, 110 (3):214-220.

Garcia-Hernandez, R., G. Newton, S. Horner, and L. C. Nuti. 2007. Effect of photoperiod on milk yield and quality, and reproduction in dairy goats. Livest. Sci., 110 (3):214-220. https://doi.org/10.1016/j.livsci.2006.11.005

Gipson, T. A., and M. Grossman. 1990. Lactation curves in dairy goats: a review. Small Rum. Res., 3 (4):383-396. https://doi.org/10.1016/0921-4488(90)90019-3

Goetsch, A. L., S. S. Zeng, and T. A. Gipson. 2011. Factors affecting goat milk production and quality. Small Rum. Res., 101 (1-3):55-63. https://doi.org/10.1016/j.smallrumres.2011.09.025

Granado, R. J., M. S. Rodríguez, C. Arce, and V. R. Estévez. 2014. Factors affecting somatic cell count in dairy goats: a review. Span. J. Agric. Res. (1):133-150. http://dx.doi.org/10.5424/sjar/2014121-3803

Haenlein, G. F. W. 2001. Past, present, and future perspectives of small ruminant dairy research. J. Dairy Sci., 84 (9):2097-2115. https://doi.org/10.3168/jds.S0022-0302(01)74655-3

IDELE (Institut de l'Élevage et de la CNE) 2017. Caprins 2017: Productions lait et viande (Goats 2017: Milk and meat products). Accessed Sep. 16, 2018. http://idele.fr/?eID=cmis_download&oID=workspace://SpacesStore/dc364a28-06b7-4fb5-891a-ae7254d8c252

Jones, A. K., and S. A. Reed. 2017. Benefits of ultrasound scanning during gestation in the small ruminant. Small Rum. Res., 149:163-171. http://dx.doi.org/10.1016/j.smallrumres. 2017.02.008

Kljajevic, N. V., I. B. Tomasevic, Z. N. Miloradovic, A. Nedeljkovic, J. B. Miocinovic, and S. T. Jovanovic. 2018. Seasonal variations of Saanen goat milk composition and the impact of climatic conditions. J. Food Sci. Technol., 55 (1):299-303. https://doi.org/10.1007/s13197-017-2938-4

Lacasse, P., C. M. Vinet, and D. Petitclerc. 2014. Effect of prepartum photoperiod and melatonin feeding on milk production and prolactin concentration in dairy heifers and cows. J. Dairy Sci. (97):3589-3598.

León, J. M., N. P. P. Macciotta, L. T. Gama, C. Barba, and J. V. Delgado. 2012. Characterization of the lactation curve in Murciano-Granadina dairy goats. Small Rum. Res., 107 (2-3):76-84. http://dx.doi.org/10.1016/j.smallrumres.2012.05.012

Lérias, J. R., L. E. Hernández-Castellano, A. Suárez-Trujillo, N. Castro, A. Pourlis, and A. M. Almeida. 2014. The mammary gland in small ruminants: major morphological and functional events underlying milk production–a review. J. Dairy Res., 81 (3):304-318. https://doi.org/10.1017/S0022029914000235

Mabjeesh, S. J., O. Gal-Garber, and A. Shamay. 2007. Effect of photoperiod in the third trimester of gestation on milk production and circulating hormones in dairy goat. J. Dairy Sci., 90:699-705. https://doi.org/10.3168/jds.S0022-0302(07)71553-9

McDonald, P., R. A. Edwards, J. F. D. Greenhalgh, and C. A. Morgan. 2002. Animal nutrition. 6th ed. Pearson Education Ltd. Essex, England.

Mikolayunas, C. M., D. L. Thomas, G. E. Dahl, T. F. Gressley, and Y. M. Berger. 2008. Effect of prepartum photoperiod on milk production and prolactin concentration of dairy ewes. J. Dairy Sci. (91):85-90. https://doi.org/10.3168/jds.S0022-0302(07)71553-9

Miller, B. A., and C. D. Lu. 2019. Current status of global dairy goat production: an overview. Asian-Australas. J. Anim. Sci., 32 (8):1219. https://doi.org/10.5713/ajas.19.0253

Mioč, B., Z. Prpić, I. Vnučec, Z. Barać, V. Sušić, D. Samaržija, and V. Pavić. 2008. Factors affecting goat milk yield and composition. Mljekarstvo Dairy, 58 (4).

Montaldo, H., A. Almanza, and A. Juárez. 1997. Genetic group, age and season effects on lactation curve shape in goats. Small Rum. Res., 24 (3):195-202. https://doi.org/10.1016/S0921-4488(96)00946-7

Nardone, A., B. Ronchi, N. Lacetera, M. S. Ranieri, and U. Bernabucci. 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livest. Sci., 130 (1):57-69. https://doi.org/10.1016/j.livsci.2010.02.011

Neville, M. C., T. B. McFadden, and I. A. Forsyth. 2002. Hormonal regulation of mammary differentiation and milk secretion. J. Mammary Gland Biol., 7 (1):49-66. https://doi.org/10.1023/a:1015770423167

OCDE. 2018. Agricultural Policy Monitoring and Evaluation. Organisation for Economic Co-Operation and Development. OECD Publishing, Paris, France. Accessed Sep. 16, 2018. http://dx.doi.org/10.1787/agr_pol-2018-en

Pulina, G., M. J. Milán, M. P. Lavín, A. Theodoridis, E. Morin, J. Capote, D. L. Thomas, A. H. D. Francesconi, and G. Caja. 2018. Invited review: Current production trends, farm structures, and economics of the dairy sheep and goat sectors. J. Dairy Sci., 101 (8):6715-6729. https://doi.org/10.3168/jds.2017-14015

Rabasco, A., J. M. Serradilla, J. A. Padilla, and A. Serrano. 1993. Genetic and non-genetic sources of variation in yield and composition of milk in Verata goats. Small Rum. Res., 11 (2):151-161. https://doi.org/10.1016/0921-4488(93)90148-B

Russo, V. M., A. W. N. Cameron, F. R. Dunshea, A. J. Tilbrook, and B. J. Leury. 2013. Artificially extending photoperiod improves milk yield in dairy goats and is most effective in late lactation. Small Rum. Res., 113 (1):179-186. https://doi.org/10.1016/j.smallrumres.2013.01.002

Salama, A. A. K., G. Caja, S. Hamzaoui, B. Badaoui, A. Castro-costa, M. M. Guilhermino, and R. Bozzi. 2014. Different levels of response to heat stress in dairy goats. 121:73-79. https://doi.org/10.1016/j.smallrumres.2013.11.021

Salari, F., I. Altomonte, N. L. Ribeiro, M. N. Ribeiro, R. Bozzi, and M. Martini. 2016. Effects of season on the quality of Garfagnina goat milk. Ital. J. Anim. Sci., 15 (4):568-575. http://dx.doi.org/10.1080/1828051X.2016.1247658

Šlyžius, E., B. Šlyžienė, and V. Lindžiūtė. 2017. Factors affecting goat milk fat yield. Žemės ūkio mokslai, 24 (3). https://doi.org/10.6001/zemesukiomokslai.v24i3.3555

Solis-Ramirez, J. 2014. A genetic improvement program for New Zealand dairy goats. PhD thesis. Massey University, Palmerston North, New Zealand,

Stubbs, A. K., and G. L. Abud. 2009. Farming and Marketing Goat and Sheep Milk Products. Rural Industries Research and Development Corporation (RIRDC). Barton, ACT, Australia. Accessed Mar. 18, 2016. https://www.agrifutures.com.au/wp-content/uploads/publications/08-207.pdf

Villaquiran, M., T. A. Gipson, R. C. Merkel, A. L. Goetsch, and T. Sahlu. 2004. Body condition scores in goats. American Institute for Goat Research. Langston University, OK, USA. Accessed on Accessed Mar. 18, 2016. http://www.luresext.edu/sites/default/files/BCS_factsheet.pdf

Weigel, K. A., and G. E. Shook. 2018. Genetic Selection for Mastitis Resistance. Vet. Clin. North Am. Food Anim. Pract., 34 (3):457-472. https://doi.org/10.1016/j.cvfa.2018.07.001

Zalcman, E., and B. Cowled. 2018. Farmer survey to assess the size of the Australian dairy goat industry. Aust. Vet. J., 96 (9):341-345. https://doi.org/10.1111/avj.12734

Zoa-Mboé, A., C. Michaux, J. C. Detilleux, C. Kebers, F. P. Farnir, and P. L. Leroy. 1997. Effects of parity, breed, herd-year, age, and month of kidding on the milk yield and composition of dairy goats in Belgium. J. Anim. Breed. Genet., 114:201-213. https://doi.org/10.1111/j.1439-0388.1997.tb00506.x

Chapter 4 - Endocrine and metabolic status of commercial dairy goats during the transition period

F. Zamuner*1, K. DiGiacomo*, A.W.N. Cameron†, B.J. Leury*

* Faculty of Veterinary and Agricultural Sciences - The University of Melbourne, Parkville, Victoria 3010, Australia.

† Meredith Dairy Pty Ltd, 106 Cameron Rd, Meredith, Victoria 3333, Australia.

4.1 Manuscript 2

Chapter 4 investigated the temporal variations in concentrations of selected hormones and metabolites involved in the regulation of energy metabolism and examined how parity number, litter size, and the level of milk production in early lactation, affect the metabolic profile of commercial dairy goats during the transition period.

This work has been published in the Journal of Dairy Science;

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Endocrine and metabolic status of commercial dairy goats during the transition period. J. Dairy Sci., 103 (6):5616-5628. https://doi.org/10.3168/jds.2019-18040

Therefore, the paper is included in this chapter as a manuscript.

4.2 Abstract

The aim of this study was to evaluate temporal variations in circulating levels of selected hormones and metabolites in commercial dairy goats during the transition period. Blood samples from 940 goats were collected weekly, from -3 to 3 wk relative to delivery, to measure the effects of level of milk production, parity number, and litter size on concentrations of glucose, β-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), and plasma urea nitrogen (PUN). A subset of 80 goats [40 low-yielding (LY, < 1.8 L/d) and 40 high-yielding goats (HY, > 3.7 L/d)] were selected from the study population to measure the effects of level of milk production on plasma concentration of insulin, prolactin, and growth hormone. Average (± SD) milk yield (from 3 to 30 d in milk), body weight, and body condition score for the study population were 2.4 ± 0.78 L/d, 70 ± 16.0 kg, and 2.5 ± 0.28 units, respectively. Milk yield was moderately correlated with parity number (r = 0.49) but had a weak correlation with litter size (r = 0.14). In multiparous but not in primiparous goats, antepartum concentrations of NEFA and BHB increased with increasing litter size. Concentrations of NEFA, BHB, and PUN were consistently lower in primiparous goats compared with those in second or greater parity. Postpartum HY goats had higher ratios of glucose, NEFA, and BHB to insulin than did LY goats, which might explain the greater mobilization of body tissues and enhanced milk production observed in this group. Collectively, our results indicate that increased milk yield has the most significant influence on the magnitude of body tissue mobilization. Our results also show that goats of higher parity display higher levels of lipid mobilization, and that both pregnancy and lactation are less able to elicit lipomobilization in primiparous compared with multiparous goats.

Keywords: milk yield, parity, litter size, peripartum

4.3 Introduction

The Australian dairy goat industry has experienced unprecedented growth in recent years. Stimulated by increasing demand for healthy and niche dairy goat products, national goat milk production has more than doubled, from 8 million L in 2012 to 16.9 million L in 2016, and the enterprise structure is rapidly moving from small family-owned businesses to large-scale production and intensive husbandry systems (Zalcman and Cowled, 2018). However, the adoption of intensive production systems and genetic selection for increased milk yield is likely to increase the incidence of peripartum disorders as a consequence of intensified metabolic burden in high-yielding goats (Celi et al., 2008).

The risk of metabolic and infectious diseases is particularly high during the transition period (3 weeks before to 3 weeks after parturition) because around this time a substantial increase in energy requirements to support pregnancy and lactation coincides with a period of depressed feed intake, often resulting in maternal negative energy balance (NEB; Sundrum, 2015; Matthews, 2016). Research in dairy cows has shown that the greater the duration and severity of NEB, the higher the risk of disease development and milk loss (Overton et al., 2017). Similarly, in dairy goats, multiple pregnancies and high milk yield are considered predisposing factors for several metabolic diseases, because both potentially exacerbate NEB (Albay et al., 2014; Radin et al., 2015). Given the high prolificacy and improved milk production of modern dairy goats, commercial goats might be at risk of developing metabolic complications, both ante- and postpartum (Stelletta et al., 2008; Matthews, 2016).

Dairy ruminants cope with NEB through an intricate mechanism of hormonal and metabolic adaptations that regulate interactions among nutrients and nutrient-using organs and tissues (Herdt, 2000). The success or failure of such adaptations will determine animal performance in the ensuing lactation (Stelletta et al., 2008; Matthews, 2016). Therefore, correct understanding and characterization of both endocrine and metabolic changes during the

transition period are of utmost importance for accurate prediction and prevention of NEB-related metabolic disorders (Matthews, 2016; Overton et al., 2017).

The metabolic health of transitioning dairy cows has been the subject of numerous reviews (Sundrum, 2015; Overton et al., 2017; Wankhade et al., 2017). However, only limited information is available on transitioning dairy goats. Relatively few studies have examined periparturient metabolic changes in dairy goats with respect to parity (Magistrelli and Rosi, 2014; Radin et al., 2015) and litter size (Castagnino et al., 2015; Cappai et al., 2019), and these have had limited numbers of animals in each group (n = 4 to 22). Moreover, to the best of our knowledge, no studies have described changes in hormonal and metabolic profiles of transition dairy goats raised on large-scale commercial farms.

Thus, the aims of this study were (1) to evaluate temporal variations in circulating levels of selected hormones and metabolites involved in the regulation of energy metabolism during the transition period and (2) to investigate the effects of level of milk production, parity number, and litter size on the metabolic profile of periparturient dairy goats from a commercial herd in Australia.

4.4 Materials & Methods

All experimental procedures were approved by the Faculty of Veterinary and Agricultural Sciences Animal Ethics and Welfare Committee of The University of Melbourne, Australia (No 1613846.1.).

4.4.1 Animals and Management

This experiment was conducted at Meredith Dairy commercial farm (Meredith, Australia, 37°50′S, 144°04′E). The farm follows a reproductive calendar based on 4 kidding seasons per year, aiming for 600 goats kidding in each of the 4 kidding periods: March, June, September, and November. In total, 1,000 Saanen goats (~250 different goats per season) were selected

from the herd over 4 consecutive kidding seasons (June 2016 to March 2017) to be monitored in this study. Further details on animals, management, and measurements of milk yield and milk composition are available in Zamuner et al. (2020). In brief, approximately 1 month before kidding, primiparous and multiparous goats were brought together and moved to the kidding shed and began to be fed the fresh-goat TMR. Enrolled goats were kept with their contemporary group (~600 goats) in a collective pen. Goats were fed a TMR ad libitum once daily at around 0700 h; nutrient composition of the diet (per kg of DM) was 28% NDF, 16% CP, 10 MJ of ME, 18 g of Ca, and 9.0 g of P. Milk composition was measured at wk 6, and goat BW and BCS were measured at wk -6, 0, and 6 relative to delivery (always before feeding and after the morning milking). The BCS was scored by the same person, adopting a 6-point scale method (Villaquiran et al., 2004).

4.4.2 Blood Sampling: All Does

Blood samples were collected from all goats on the same day (~250 each kidding season), starting at around 1100 h, always after the morning milking. Feed was withheld until after blood collection. Goats were sampled in random order, and the interval between the first and last animals sampled was 166 ± 30 min (mean ± SD). Blood samples were taken at weekly intervals from wk -3 to 3 relative to the expected kidding date. The actual day of sampling (relative to delivery) was determined by subtracting the date of kidding from the date of sampling, and data were segmented into intervals of 1 wk (i.e., -21 ± 3d = wk -3, -14 ± 3d = wk -2, and so forth). Blood was collected via jugular venipuncture using vacuum tubes (10 mL) coated with lithium-heparin (BD Vacutainer, Plymouth, UK). Blood samples were immediately placed on ice and centrifuged (1,250 × *g*, 4°C) for 12 min within 1 to 3 h after collection. Isolated plasma was stored at -20°C until analysis.

4.4.3 Blood Sampling: Subset Group (High vs. Low Milk Yield)

After the end of the last kidding season (Mar. 2017), a total of 80 goats in second or third lactation were selected (retrospectively) from the study population based on their average milk yield from 3 to 30 DIM. The 10 highest and 10 lowest-yielding goats of each month of kidding were selected for analysis of plasma insulin, prolactin, and growth hormone (GH) concentrations at wk -3, -1, 1, and 3, relative to delivery.

4.4.4 Metabolite Analysis

The whole-blood concentration of BHB was measured using a hand-held meter (FreeStyle Optium Precision Neo, Abbott Diabetes Care Ltd., Oxfordshire, UK) before plasma separation. Plasma nonesterified fatty acid (NEFA), glucose, and urea concentrations were determined spectrophotometrically using commercial kits [respectively, NEFA-C ACS-ACOD method, Wako Pure Chemical Industries, Ltd., Osaka, Japan (modified as per the methods of Johnson and Peters (1993); Infinity Glucose Oxidase Liquid, Thermo Fisher Scientific, Waltham, MA; and Infinity Urea Liquid Stable Reagent, Thermo Fisher Scientific]. The inter-, and intra-assay coefficients of variation (CV) were < 6.0 and < 3.4% for glucose, < 8.5 and < 3.5% for NEFA, and < 6.3 and < 3.4% for plasma urea nitrogen (PUN).

4.4.5 Hormone Analysis (High- vs. Low-Yield Subset Groups)

Plasma prolactin and GH concentrations were measured in duplicate using the method described by Thomas et al. (1990). The sensitivity of the assay was 0.2 ng/mL for prolactin and 0.3 ng/mL for GH. The intra-assay CV were < 10% between 0.8 and 27.3 ng/mL for prolactin and < 10% between 1.2 and 51.9 ng/mL for GH. Plasma insulin concentration was measured in duplicate using RIA kits (Porcine Insulin Cat. # PI- 12K, Millipore Corporation, Billerica, MA). The assay sensitivity range was 0.4 to 0.7 μU/mL, the intra-assay CV was < 10% between 1.5 and 212.1 μU/mL, and inter-assay CV was 5.3% at 16.0 μU/mL and 5.9% at 49.5 μU/mL.

4.4.6 Calculations and Statistical Analyses

To account for confounding effects of labor-induced changes in the concentrations of hormones and metabolites, antepartum calculations included observations from d -24 to -3, and postpartum calculations included observations from d 3 to 24 relative to delivery. The level of milk production was determined retrospectively, based on the average milk yield (L/d) from 3 to 30 DIM. The ratios of glucose, NEFA, and BHB to insulin were calculated as 1 unit of glucose, NEFA, or BHB (mmol/L) per 1 unit of insulin (μU/mL).

Due to the potential effects of animal handling on milk production, individual milk produced the day before blood sampling (Milk-S) was used to calculate associations between simultaneous observations of blood parameters and milk yield. Primiparous goats produced approximately 40% less milk (3 to 30 DIM) than did multiparous goats. To avoid bias due to this considerably lower milk yield, only data from multiparous goats were used to calculate (1) the effects of the level of milk production on metabolite concentrations and (2) the effects of elevated NEFA and BHB on Milk-S.

For the purposes of data analysis, the following intervals were used as cut points for BHB concentrations: < 0.8, 0.8 to 1.6, and > 1.6 mmol/L (Albay et al., 2014; Marutsova and Binev, 2017; Caré et al., 2018). The cut point for elevated NEFA in postpartum dairy cows (≥ 0.7 mmol/L; Overton et al., 2017) was adapted to the following intervals: < 0.8, 0.8 to 1.6, and > 1.6 mmol/L. Blood samples were dichotomized into 4 yes or no groups, according to NEFA and BHB intervals. The total numbers of positive events (0, 1, 2, or 3 times) antepartum (d -24 to -1) and postpartum (d 0 to 24) were calculated for each goat separately. The percentage of goats with at least 1 positive event above the cut point was calculated as the number of positive events ÷ number of goats tested × 100. Goats were dichotomized based on the dry-off date (≤ 30 DIM = yes or no).

Data were categorized into the week of blood sampling (-3, -2, -1, 0, 1, 2, and 3), period (ante- and postpartum), month of kidding (Mar., Jun., Sep., and Nov.), parity number (1, 2, 3, and 4+), litter size (LS: 1, 2, and 3 fetuses), and level of milk production [low yield (LY), < 2.4 L/d; medium yield (MY), 2.4 to 3.1 L/d; and high yield (HY), > 3.1 L/d]. Statistical analyses were performed using Minitab software (version 18.1; Minitab Inc., State College, PA). All outcome variables were screened for normality by calculation of kurtosis and skewness and by visual assessment of standardized residuals distribution. Data from repeated measurements were analyzed using the Mixed Effects Model (restricted maximum likelihood, 2-sided 95% CI), and data from non-repeated-measurements were analyzed using the General Linear Model (2-sided 95% CI) of Minitab. For all models, the random effect of goat was nested with month of kidding to account for variations between month of kidding. The effects of parity number, LS, BW, BCS, and time of blood sampling, as well as suspected 2-way interactions, were tested in all models and retained when significant ($P < 0.05$). The Bonferroni method with 95% CI was used for pairwise comparisons. The Spearman rho correlation was used to examine relationships between variables of interest (using simultaneous observations for each goat at each sampling event). Binary logistic regression was used to evaluate associations between the number of events at or above NEFA and BHB cut points (continuous variable) and removal from the milking herd ≤ 30 DIM (binary response).

4.4.7 Study Population

Values for glucose, NEFA, BHB, PUN, and NEFA-to-BHB ratio were log-transformed to achieve normality. The interaction week of blood sampling × parity number × LS was not significant, but preliminary analysis showed significant differences between primiparous and multiparous goats of equal LS. Hence, the effects LS on ante- and postpartum concentrations of metabolites were analyzed in primiparous and multiparous goats separately. Data for NEFA

and BHB in multiparous goats were grouped as < 0.8, 0.8 to 1.6, and > 1.6 mmol/L to estimate Milk-S of each group.

4.4.8 High Versus Low-Yield Subset Groups

Values for NEFA, BHB, insulin, GH, prolactin, and ratios of glucose, NEFA, and BHB to insulin were log-transformed to achieve normality. Values for ratios of glucose, NEFA, and BHB to insulin were analyzed for wk 1 and 3 separately.

4.4.9 Data Presentation

Results for log-transformed variables were reported after back-transformation. Data are presented as means ± SE unless declared otherwise.

4.5 Results

Out of 1,000 animals, 60 were excluded from the experiment due to abortions, death, or kidding delays. Thus, 940 goats were included in the final analysis. The final number of enrolled goats is listed in **Table 4.1**. The percentages of goats delivering 1, 2, or 3 kids were 35, 55, and 10%, respectively, and the average litter size was 1.75 kids per goat.

Production data according to level of milk production, parity number, and litter size are reported in **Table 4.2**. The mean (± SD) milk yield, BW, and BCS for the study population were, respectively, 2.4 ± 0.78 L/d, 70 ± 16.0 kg, and 2.5 ± 0.28 units. Milk yield was moderately correlated with BW (r = 0.52; $P < 0.001$) but not with BCS (r = 0.01; $P = 0.919$). Litter size was weakly correlated with BCS (r = -0.25; $P < 0.001$).

Table 4.1. Counts of participant goats listed by factor combination

Item		Weeks from delivery							Parity				Litter			LMP[1]			
		-3	-2	-1	0	1	2	3	1	2	3	4+	1	2	3	LY	MY	HY	Total[2]
Month of kidding	Mar.	214	230	209	191	183	183	170	84	31	88	34	73	139	25	51	58	44	237
	Jun.	209	233	236	225	227	210	204	84	71	53	34	84	135	23	59	61	39	242
	Sep.	212	222	220	209	196	193	189	74	64	48	36	67	112	43	45	69	33	222
	Nov.	176	232	220	193	203	194	192	77	78	48	36	108	129	2	40	59	64	239
LMP[1]	LY	154	179	175	165	166	162	160	-	93	60	42	57	118	20				195
	MY	224	241	228	219	215	210	200	-	96	98	53	78	140	29				247
	HY	177	187	179	162	160	150	149	-	56	79	45	40	119	21				180
Litter size	1	276	322	307	280	278	271	263	158	69	67	38							332
	2	451	504	489	452	449	426	412	138	154	140	83							515
	3	84	91	89	86	82	83	80	23	21	30	19							93
Parity number	1	258	311	303	272	269	258	246											319
	2	214	237	230	218	216	205	200											244
	3	208	232	218	204	201	194	191											237
	4+	131	137	134	124	123	123	118											140
Total[3]		811	917	885	818	809	780	755											940

[1]LMP = Level of milk production based on average milk yield from 3 to 30 DIM. LY = low-yield (≤ 2.4 L/d), MY = medium-yield (2.4-3.1 L/d), and HY = high-yield (≥3.1 L/d).

[2]Total = number of goats commencing the experiment.

[3]Number of goats tested in each week.

Table 4.2. Effect of level of milk production (LMP), parity number (PN) and litter size (LS) on mean body weight (BW; kg), body condition score (BCS), and average daily milk yield (L/d) of commercial dairy goats during the first month of lactation (n = 940)[1]

Item	BW[2]	BCS[2]	Milk[3]
LMP			
LY	76^{B}	2.5^{A}	2.0^{C}
MY	78^{B}	2.5^{AB}	2.7^{B}
HY	80^{A}	2.4^{B}	3.5^{A}
SED	1.0	0.03	0.03
P-value	0.049	0.043	<0.001
PN			
1	54^{D}	2.4^{B}	1.9^{C}
2	72^{C}	2.5^{A}	2.6^{B}
3	76^{B}	2.4^{B}	2.8^{A}
4+	82^{A}	2.5^{AB}	2.7^{AB}
SED	1.1	0.03	0.06
P-value	<0.001	<0.001	<0.001
LS			
1	72^{A}	2.6^{A}	2.4^{B}
2	70^{B}	2.4^{B}	2.5^{A}
3	70^{AB}	2.4^{B}	2.6^{A}
SED	1.1	0.03	0.07
P-value	0.035	<0.001	0.002

$^{A-C}$Means within a column with different uppercase superscripts differ (P < 0.05).
[1]The number of goats (by factor combination) is presented in Table 4.1.
[2]Measured within 1 wk relative to parturition.
[3]Average milk yield (L/d) from 3 to 30 DIM (excluding data for primiparous goats). LY = low yield (≤ 2.4 L/d), MY = medium yield (2.4 to 3.1 L/d), and HY = high yield (≥ 3.1 L/d), SED = Pooled standard error of differences.

4.5.1 Metabolite Concentrations During the Transition Period

For all metabolites we discovered a significant (P < 0.001) difference between ante- and postpartum concentrations [glucose: 3.3 vs. 3.1, standard error of differences (SED) = 0.02; NEFA: 0.42 vs. 0.81, SED = 0.016; BHB: 0.24 vs. 0.43, SED = 0.011; and PUN: 6.9 vs. 8.4,

SED = 0.08, ante- vs. postpartum, respectively]. Weekly variations in plasma concentration of glucose, NEFA, BHB, and PUN are reported in **Table 4.3**. Concentrations of glucose, BHB, and PUN were relatively constant until wk -1, sharply increasing (except for PUN) at wk 0, the week of parturition. Plasma glucose reached a peak of 3.6 mmol/L at wk 0, rapidly decreasing after birth. Plasma NEFA concentration increased continuously ($P < 0.001$) from wk -1 to its highest concentration (0.89 mmol/L) at wk 3, and BHB concentration rose sharply from wk -1 to a peak of 0.44 mmol/L at wk 1 and remaining above 0.4 mmol/L until the end of the study. The concentration of PUN gradually increased after birth, reaching its highest concentration (8.6 mmol/L) at wk 3. Milk yield was moderately (positively) correlated with NEFA and BHB (**Table 4.4**) but weakly correlated with NEFA-to-BHB ratio (r = 0.15; $P < 0.001$).

Table 4.3. Weekly concentrations (mmol/L)[1] of β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose and plasma urea nitrogen (PUN) in commercial dairy goats during the transition period (n = 940)[1]

	Weeks relative to delivery								
Item	-3	-2	-1	0	1	2	3	SED[2]	P-value
Milk-S[3]					2.25^{c}	2.48^{b}	2.54^{a}	0.028	< 0.001
Glucose	3.29^{b}	3.23^{bc}	3.18^{bcd}	3.63^{a}	3.15^{bdc}	3.08^{d}	3.10^{cd}	0.061	< 0.001
NEFA	0.36^{e}	0.43^{d}	0.44d	0.55^{c}	0.67^{b}	0.83^{a}	0.89^{a}	0.034	< 0.001
BHB	0.24^{c}	0.25^{c}	0.26^{c}	0.37^{b}	0.44^{a}	0.42^{a}	0.41^{a}	0.016	< 0.001
PUN	6.91^{c}	6.97^{c}	7.26^{c}	7.02^{c}	8.01^{b}	8.43^{a}	8.63^{a}	0.169	< 0.001

$^{a-d}$Means within a row with different lowercase superscripts differ (P < 0.05).

[1]Values are back-transformed means. The number of goats by factor combination is presented in Table 4.1.

[2]SED = standard error of differences

[3]Milk yield (L/d) 1 d before the blood test

Table 4.4. Spearman rho correlations between parity number (PN), litter size (LS), milk yield 1 d before blood test (Milk-S, L/d), and concentrations (mmol/L) of β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose and plasma urea nitrogen (PUN) in commercial dairy goats during the transition period (n = 940)[1]

	Antepartum					Postpartum					
Item	PN	LS	Gluc.	NEFA	BHB	PN	LS	Gluc.	NEFA	BHB	PUN
Milk-S	-	-	-	-	-	0.49***	0.14***	-0.28***	0.39***	0.38***	0.26***
Glucose	-0.10***	-0.17***				-0.16***	-0.04†				
NEFA	0.12***	0.28***	-0.45***			0.16***	0.06**	-0.51***			
BHB	0.17***	0.25***	-0.23***	0.40***		0.29***	0.12***	-0.29***	0.58***		
PUN	0.11***	-0.04*	-0.02ns	-0.04*	-0.06**	0.13***	-0.06**	-0.06**	0.07**	0.06**	-

[1]Number of goats (by factor combination) is presented in Table 4.1. Antepartum calculations between plasma metabolites included 2745 simultaneous observations, from d -24 to -3, and postpartum calculations between plasma metabolites and milk yield included 2390 simultaneous observations from d 3 to 24.

*** P < 0.001; ** P < 0.01; * P < 0.05; † P < 0.1; ns P > 0.1

4.5.2 Effects of Litter Size

Increasing litter size increased ($P < 0.05$) milk yield and decreased ($P < 0.05$) BW and BCS at parturition. Primiparous goats were less prolific than multiparous (1.6 vs. 1.8 kid/goat; $P < 0.001$). The effects of litter size on ante- and postpartum concentrations of glucose, NEFA, BHB, and PUN in primiparous and multiparous goats are presented in Error! Reference source not found.. For all metabolites, we found no significant difference in ante- or postpartum concentration between primiparous goats of different litter size. Multiparous goats had consistently higher ($P < 0.05$) NEFA and BHB than primiparous goats of equal litter size. Before parturition, multiparous goats carrying triplets had lower glucose (-0.20 mmol/L; $P < 0.05$) and higher NEFA (+0.20 mmol/L; $P < 0.05$) and BHB (+0.11 mmol/L; $P < 0.05$) levels

than goats carrying singles. The effects of litter size disappeared after birth in both primiparous and multiparous goats.

Table 4.5. Effects of litter size (LS) on antepartum (AP) and postpartum (PP) concentrations (mmol/L) of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and plasma urea nitrogen (PUN) in primiparous (PRIM) and multiparous (MULT) goats[1]

		AP		PP		SED[2]		P-value[2]	
Item	LS	PRIM	MULT	PRIM	MULT	AP	PP	AP	PP
Glucose	1	3.5	3.3^{AB}	3.2	3.1	0.01	0.11	0.33	0.98
	2	3.4^{a}	3.2^{BCb}	3.2	3.1				
	3	3.5^{a}	3.1^{Cb}	3.2	3.0				
NEFA	1	0.29^{b}	0.37^{Ba}	0.57^{b}	0.84^{a}	0.03	0.039	0.09	0.36
	2	0.34^{b}	0.47^{ABa}	0.64^{b}	0.83^{a}				
	3	0.35^{b}	0.57^{Aa}	0.61^{b}	0.84^{a}				
BHB	1	0.18^{b}	0.22^{Ca}	0.30^{b}	0.44^{a}	0.03	0.036	0.01	0.56
	2	0.19^{b}	0.25^{Ba}	0.30^{b}	0.45^{a}				
	3	0.20^{b}	0.33^{Aa}	0.29^{b}	0.48^{a}				
PUN	1	6.9	7.1	7.8^{b}	8.7^{a}	0.02	0.02	0.98	0.80
	2	6.8	6.9	7.6^{b}	8.6^{a}				
	3	6.9	7.1	7.2^{b}	8.1^{a}				

[a,b]Means antepartum or postpartum, within a row, with different lowercase superscripts differ ($P < 0.05$).

[A-C]Means within a column with different uppercase superscripts differs ($P < 0.05$).

[1]Values are back-transformed means. Antepartum calculations included samples from d -24 to -3 relative to parturition. Postpartum calculations included samples from d 3 to 24. The number of goats tested each week is presented in Table 4.1.

[2]Joint effects of parity × litter size. SED = standard error of differences.

4.5.3 Effects of Parity Number

Despite constant BW increase with increasing parity, the greatest milk yields were attained in third parity (**Table 4.2**). Parity number was positively correlated with levels of Milk-S, NEFA, BHB, and PUN, and negatively correlated with glucose levels (**Table 4.4**). Milk yield was positively correlated with BW in both multiparous and primiparous goats (r = 0.13, P = 0.002; and r = 0.40, $P < 0.001$, respectively). Milk-S correlated ($P < 0.001$) with NEFA and BHB levels in both multiparous and primiparous goats, but values varied slightly between groups (r = 0.27 vs. 0.32, and r = 0.26 vs. 0.21, for NEFA and BHB in primiparous vs. multiparous goats, respectively). The average weekly concentrations of NEFA, BHB, and PUN were consistently lower in first-parity goats than in goats of greater parities (**Figure 4.1**). Despite the numerical increase in NEFA and BHB concentrations with increasing parity, no significant differences were detected for these parameters between parities 2, 3, and 4+ (**Figure 4.1**).

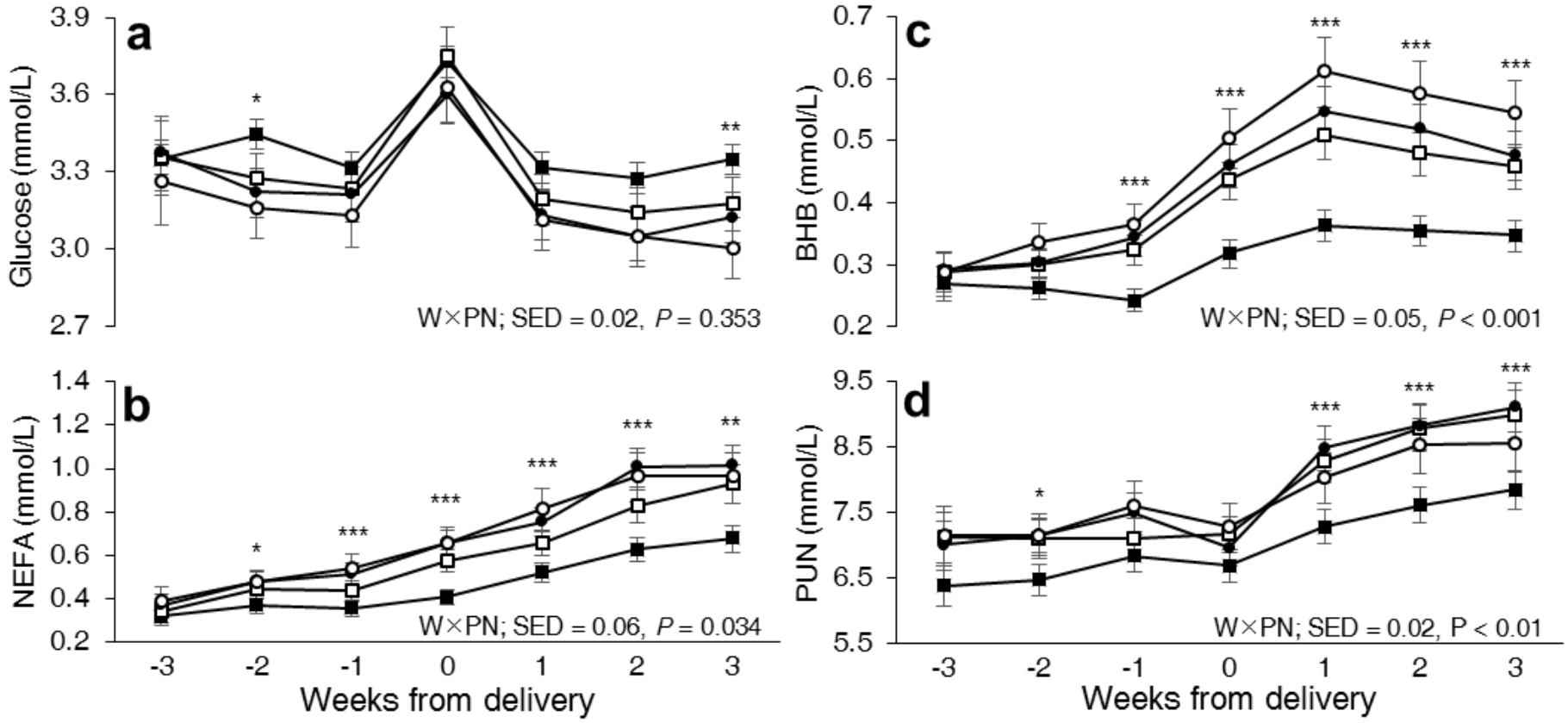

Figure 4.1. Effects of parity number (PN; 1 (■), 2(□), 3 (●), and 4 or greater parity (○)) on weekly (W) variations in concentrations of (a) glucose, (b) non-esterified fatty acids (NEFA), (c) β-hydroxybutyrate (BHB), and (d) plasma urea nitrogen (PUN) in periparturient dairy goats. Values are back-transformed means; error bars represent 95% CI. SED = standard error of differences.Weekly comparisons between groups; *** P < 0.001; ** P < 0.01; * P < 0.05. The number of goats tested each week is presented in Table 4.1

4.5.4 Effects of Level of Milk Production

No significant interaction occurred between the level of milk production and parity (2, 3, or 4+) on any of the variables analyzed (BW, BCS, milk yield). At parturition, LY goats were lighter (-4.0 kg; $P < 0.01$) than HY goats (**Table 4.2**). However, HY and MY goats lost considerably more weight than LY goats from parturition to wk 6 (-92, -85, and -27 g/d for HY, MY, and LY respectively; SED = 18.3; $P < 0.002$). The effects of the level of milk production on weekly concentrations of glucose, NEFA, BHB, and PUN are presented in **Figure 4.2**. Concentrations of NEFA, BHB, and PUN were greater ($P < 0.01$) post- than antepartum in all groups. Glucose concentration was lower ($P < 0.001$) post- than antepartum in both MY (-7%) and HY goats (-10%), with no difference in LY goats. Postpartum NEFA, BHB, and PUN increased with increasing level of milk production, and HY goats had greater concentrations of NEFA than LY (+73%) and MY goats (+16%; $P < 0.001$), greater BHB concentrations than LY (+49%) and MY goats (+16%; $P < 0.001$), and greater PUN concentrations than LY (+12%) and MY goats (+8%; $P < 0.01$). The NEFA-to-BHB ratio was greater post- than antepartum in HY (1.9 vs. 1.8; SED = 0.01, $P = 0.023$) and MY goats (1.9 vs. 1.7; SED = 0.01, $P < 0.001$), but we found no change in LY goats. The NEFA-to-BHB ratio was greater in HY and MY goats compared with LY (1.9, 1.8, and 1.6; SED = 0.07, $P < 0.001$) throughout the transition period.

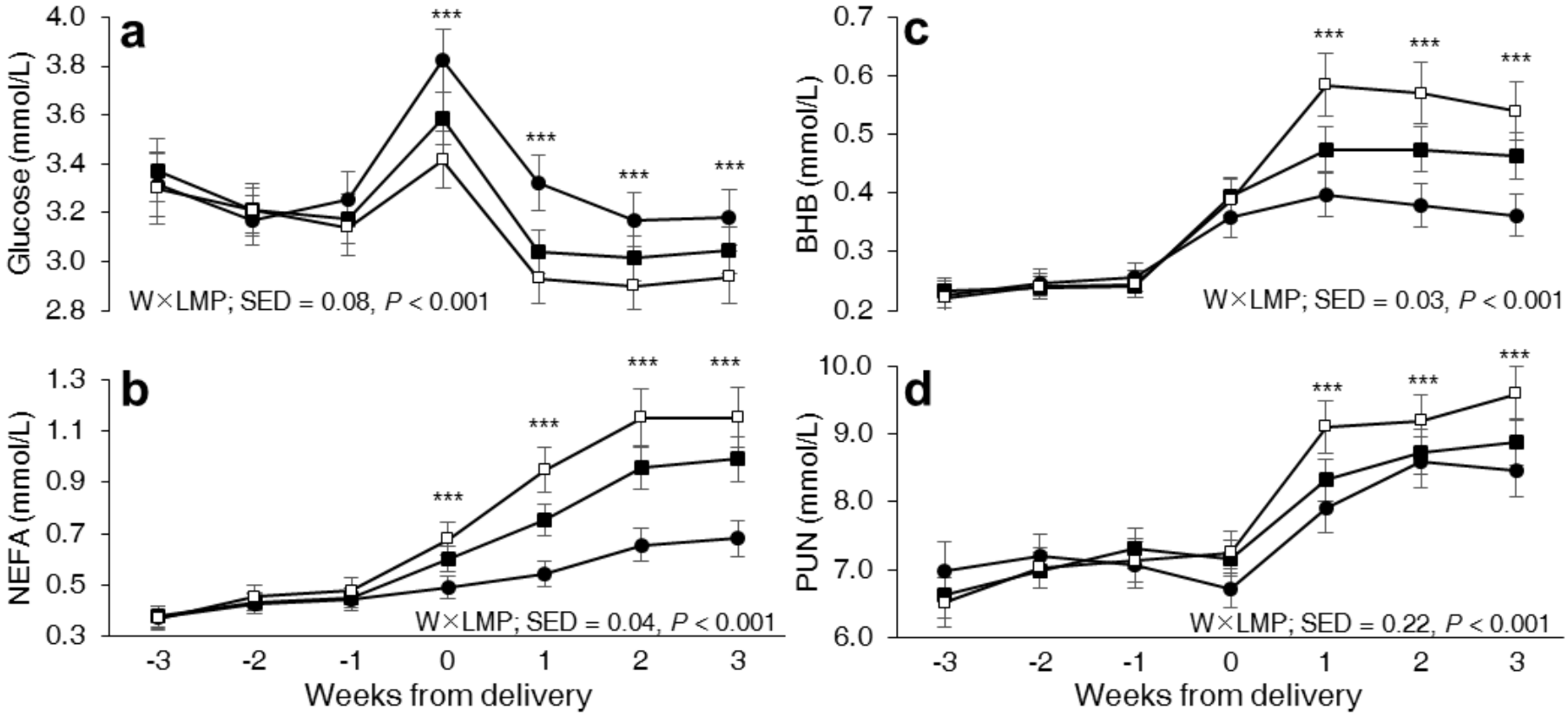

Figure 4.2. Effects of level of milk production (LMP) on weekly (W) variations in concentrations of (a) glucose, (b) non-esterified fatty acids (NEFA), (c) β-hydroxybutyrate (BHB), and (d) plasma urea nitrogen (PUN) in low- (LY (●); < 2.4 L/d), mid- (MY (■); 2.4 to 3.1 L/d) and high-yielding (HY (□); > 3.1 L/d) multiparous dairy goats. Data for primiparous were not included in these calculations. Values are back-transformed means; error bars represent 95% CI. SED = standard error of differences. Weekly comparisons between groups; *** P < 0.001; * P < 0.05. The number of goats tested each week is presented in Table 4.1.

4.5.5 High- Versus Low-Yield Subset Groups

Mean (± SD) milk yield of HY and LY were 3.7 ± 0.31 and 1.8 ± 0.32 L/d, respectively. The HY and LY goats were homogenous for litter size (1.9 ± 0.10 kid/goat) and BCS at parturition (2.5 ± 0.04), but HY goats were heavier and older than LY goats (respectively, 78 vs. 71 kg, SED = 0.07, $P = 0.014$; and 34 vs. 30 moths, SED = 1.4, $P = 0.003$). The effects of

milk production level on concentrations of hormones and metabolites are presented in Figure 4.3. We found no effect of milk production level on concentrations of hormones or metabolites in late pregnancy. Prolactin and insulin concentrations were lower ($P < 0.01$) post- than antepartum in both groups. Insulin was considerably lower in HY than in LY goats at wk 1 (2.0 vs. 6.8 μU/mL; $P < 0.001$). We also observed a tendency for higher GH in HY than in LY goats at wk 3 (2.5 vs. 1.6 ng/mL; $P = 0.075$). After parturition, HY goats had lower glucose (-11%; $P = 0.001$), higher NEFA (+52%; $P < 0.001$), and higher BHB concentrations (+36%; $P < 0.001$) than LY goats. Postpartum insulin was negatively correlated with Milk-S (r = -0.31; $P < 0.001$), NEFA (r = -0.67; $P < 0.001$), and BHB (r = -0.42; $P < 0.001$) only in LY goats, and postpartum ratios of glucose, NEFA, and BHB to insulin were significantly higher in HY than in LY goats **(**Table 4.6).

Table 4.6. Ratios of glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentrations (mmol/L) to insulin (μU/ml) in high-yielding (HY; 3.7 L/d, n = 40) and low-yielding (LY; 1.8 L/d, n = 40) commercial dairy goats at 1 and 3 weeks postpartum[1]

Week	Item	Glucose/Insulin	NEFA/Insulin	BHB/Insulin
1	HY	1.2	0.4	0.2
	LY	0.5	0.1	0.1
	SED	0.12	0.17	0.15
	P-value	0.003	<0.001	<0.001
3	HY	1.5	0.6	0.2
	LY	0.8	0.2	0.1
	SED	0.14	0.14	0.07
	P-value	<0.001	<0.001	<0.001

[1]Values are back-transformed means. SED = standard error of differences.

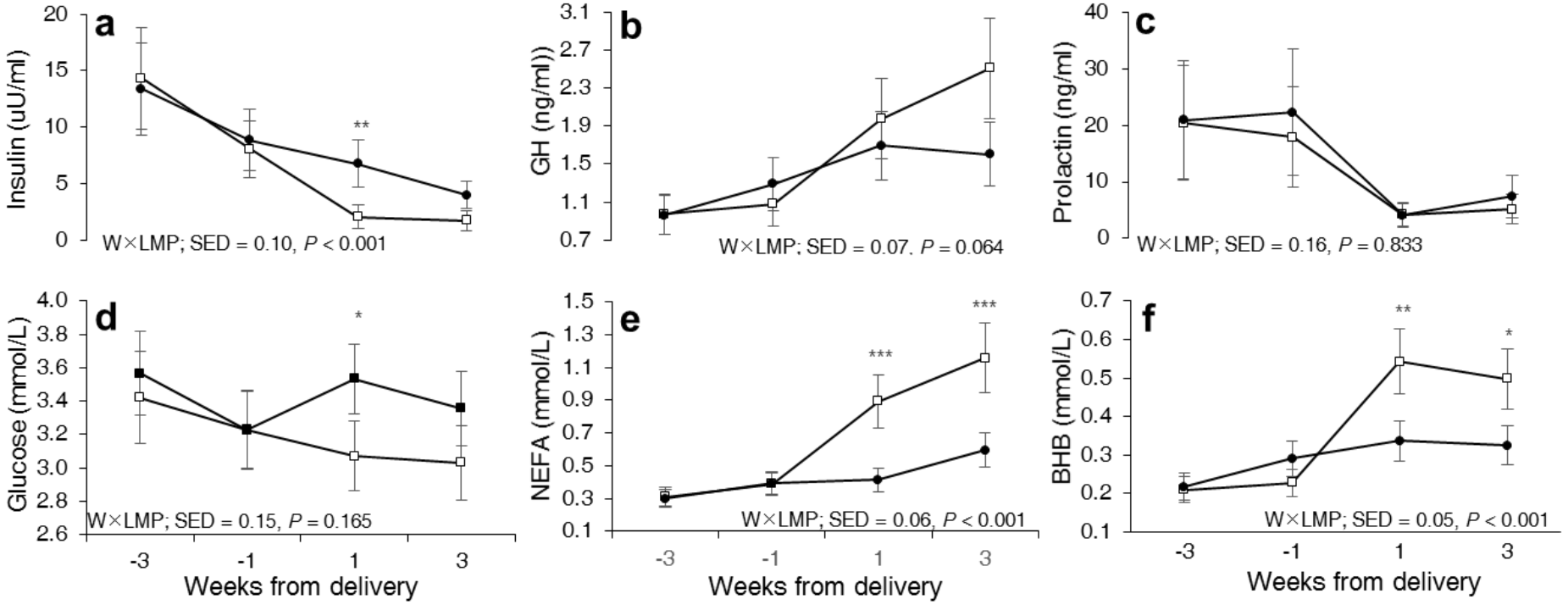

Figure 4.3. Effects of level of milk production (LMP) on weekly (W) variations in concentrations of (a) insulin, (b) growth hormone (GH), (c) prolactin, (d) glucose, (e) non-esterified fatty acids (NEFA), and (f) β-hydroxybutyrate (BHB), in high-yielding (HY(□); 3.7 L/d, n = 40) and low-yielding (LY (●); 1.8 L/d, n = 40) periparturient dairy goats. Values are back-transformed means; error bars represent 95% CI. SED = standard error of differences. Weekly comparisons between groups; *** P < 0.001; ** P < 0.01; * P < 0.05

4.5.6 Effects of Elevated NEFA and BHB on Milk Yield and Culling Rates up to 30 DIM

Effects of Elevated NEFA and BHB on Milk Yield and Culling Rates up to 30 DIM The odds ratio reported in **Table 4.7** indicates the increase in the likelihood of being removed from the milking herd before 30 DIM for every increase in the number of events of BHB and NEFA above cut points. Each of the following positive events significantly increased the chances of early removal: antepartum BHB between 0.8 and 1.6 = 3 times; antepartum BHB > 1.6 = 14.4 times; antepartum NEFA > 1.6 = 3.6 times; and postpartum BHB > 1.6 = 3.4 times.

Table 4.7. Association between the number of events of β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA; mmol/L) at or above cutpoints and the odds of removal from the milking herd ≤ 30 DIM (n = 940)[1]

Cut point	Goats[2] (%)	Coefficient	SE	Odds ratio	95% CI	P-value
Antepartum[3]						
BHB 0.8-1.6	5	1.1	0.33	3.0	1.6-5.7	0.001
BHB > 1.6	2	2.7	0.51	14.4	5.3-39.0	< 0.001
NEFA 0.8-1.6	26	0.3	0.33	1.4	0.9-2.1	0.090
NEFA > 1.6	4	1.3	0.36	3.6	1.8-7.4	< 0.001
Postpartum[4]						
BHB 0.8-1.6	29	-0.2	0.39	0.8	0.4-1.7	0.572
BHB > 1.6	5	1.2	0.36	3.4	1.7-6.9	0.001
NEFA 0.8-1.6	70	-0.3	0.29	0.7	0.4-1.3	0.306
NEFA > 1.6	34	-0.4	0.41	0.7	0.3-1.6	0.394

[1]Number of goats (by factor combination) is presented in Table 4.1.
[2]Percentage of goats with at least 1 positive event above cutpoint.
[3]From d -24 to -1 relative to delivery.
[4]From d 0 to 24 relative to delivery.

Milk yield increased ($P < 0.001$) with increasing NEFA and was greater ($P = 0.008$) in goats with BHB between 0.8 and 1.6 than in goats with BHB below 0.8 mmol/L (**Figure 4.4**). Correlations between Milk-S and NEFA concentration in goats with NEFA < 0.8, 0.8 to 1.6,

and > 1.6 mmol/L were, respectively, r = 0.27, $P < 0.001$; r = 0.11, $P = 0.001$; and r = 0.16, $P = 0.004$. Correlations between Milk-S and BHB concentration in goats with BHB < 0.8, 0.8 to 1.6, and > 1.6 mmol/L were, respectively, r = 0.32, $P < 0.001$; r = 0.07, $P = 0.208$; and r = -0.37, $P = 0.013$.

4.6 Discussion

To the best of our knowledge, this is the first study exploring changes in endocrine and metabolic profiles of transition dairy goats from a large-scale farm where the herd is managed under intensive conditions. Importantly, this study used a considerably larger number of animals than any previous study that has investigated biomarkers of NEB in periparturient dairy goats. The large data set has enabled us to examine interrelationships between hormones and metabolite concentrations over time, to study their relationship with milk yield in early lactation, and to determine how these factors differed according to goats' parity and litter size.

4.6.1 Metabolite Concentrations During the Transition Period

The observed gradual decrease of glucose concentration as the pregnancy progresses is typical for goats and ewes, for which late pregnancy is characterized by exponential fetal growth and progressive reduction in DMI due to physical compression of the rumen (Castagnino et al., 2015). The sudden rise in glucose concentration in the week of parturition (wk 0) is a normal response to parturition-induced endocrine changes that stimulate gluconeogenesis and lipolysis (Bell and Bauman, 1997; Hashizume et al., 1999).

In this experiment, NEFA concentration consistently increased from 1 wk before until 3 wk after parturition. Our results are consistent with reports in high-yielding cows (Wathes et al., 2007; McCarthy et al., 2015) but contrasting with recent studies in postpartum Saanen goats of slightly higher milk production (~3L/d), which reported a decrease (21 to 66%) in NEFA concentration from wk 0 to 2 (Magistrelli and Rosi, 2014; Radin et al., 2015). The reason for

differences between studies is unclear, but it should be noted that, in contrast with the Magistrelli and Rosi (2014) study, where goats were kept in groups of 5 goat/pen, goats of the present experiment were managed (housed, fed, and milked) in a group of ~600 goats. Large group size might enhance food competition, potentially affecting individual DMI (Goetsch et al., 2010) but could also result in underfeeding of higher-yielding goats, thus aggravating NEB in this group (Sundrum, 2015). For example, because all goats were fed the same diet, to meet nutrient requirements, a 70-kg goat producing up to 4.8 L/d would need to consume 1.1 kg of DM/d (+61%) more than a goat of equal BW producing 1.6 L/d (National Research Council, 2007).

Despite the constant increase in NEFA concentrations, BHB reached a plateau in the first week postpartum, remaining below hyperketonemic levels (BHB $\geq$ 0.8 mmol/L; Marutsova and Binev, 2017) throughout the transition period. Although we did not measure changes in BHB production or utilization, it is known that BHB is more readily available for tissue uptake than NEFA (Lean et al., 1992) and that BHB clearance is increased after parturition in dairy cows (Zarrin et al., 2017). Thus, the greater NEFA-to-BHB ratio observed postpartum might be related to an enhanced BHB uptake capacity compared with NEFA.

Moreover, postpartum BHB was lower than reported in dairy cows of similar postpartum NEFA concentration (McCarthy et al., 2015; Caré et al., 2018), which corroborates the findings of Toral et al. (2015), who reported higher BHB concentrations in cows than goats, even though NEFA, glucose, and insulin concentrations were comparable between species. Additionally, dairy goats are reportedly less prone to develop pregnancy toxemia and lactational ketosis than are dairy ewes and cows, respectively (Pezzanite et al., 2009; Marutsova and Binev, 2017). The changes in plasma BHB concentration, in the present and other studies, suggest differences in ketone body metabolism between goats and cows, which in turn could be related to inherent

interspecies differences in the intermediary metabolism of lipids (Delavaud et al., 2019) and mammary lipogenesis (Chilliard et al., 2014).

Furthermore, we observed a significant increase in PUN concentrations after parturition, which agrees with previous reports in dairy goats (Radin et al., 2015; Soares et al., 2018). In the current study, increases in milk yield were paralleled by increases in PUN concentration, and changes in PUN were positively associated with changes in NEFA and BHB. Hence, changes in PUN concentration were most likely a reflection of enhanced catabolism of amino acids to support milk production during NEB. However, such changes in PUN concentration could also be related, at least in part, to changes in DMI (Van Saun, 2009) as well as to physiological and hormonal changes to nitrogen utilization efficiency as goats transition from pregnancy to lactation (Brun-Bellut, 1997).

4.6.2 Litter Size

The metabolic profiles of primiparous goats were not affected by litter size, which, together with the fact that multiparous goats had greater NEFA and BHB concentrations than did primiparous goats of equal litter size, suggests that primiparous goats were partitioning nutrients toward body growth, most likely to the detriment of fetal development. Although we did not measure litter weight, this supposition is supported by the findings of González-García et al. (2014), who reported that, although the metabolic profiles of primiparous ewes were indifferent to litter size, their offspring were lighter and smaller than those born to multiparous dams.

In multiparous goats, increasing litter size increased lipid mobilization, as evidenced by higher NEFA and BHB concentrations and lower BCS in goats delivering 2 or 3 kids compared with goats delivering 1 kid. Moreover, in dairy goats, multiple pregnancies is associated with reduced ingestion capacity (Soares et al., 2018) and higher colostrum yield (Lérias et al., 2014).

Therefore, greater nutrient demands for colostrogenesis might have increased the metabolic burden on goats carrying 2 or 3 kids.

4.6.3 Parity

In multi- but not primiparous goats, NEFA and BHB levels started to rise before the onset of lactation. In goats, the phase of exponential mammary growth starts around 4 wk before delivery, and total udder volume at parturition (parenchyma + colostrum) increases with increasing parity number (Lérias et al., 2014). Thus, the higher antepartum concentrations of NEFA and BHB in multiparous goats might reflect a higher nutrient requirement for colostrogenesis and mammary growth compared with primiparous goats.

Despite considerable variation in milk yield, BW, and BCS among multiparous goats, no significant difference in metabolic profile existed between goats in second or greater parity. On the other hand, primiparous goats had lower concentrations of NEFA, BHB, and PUN, and lower milk yield than multiparous goats. Similar results have been reported in goats, sheep, and cows (Wathes et al., 2007; Magistrelli and Rosi, 2014; González-García et al., 2015). Collectively, previous and present results suggest that nutrient supply for body growth is prioritized over milk production in young ruminants. Additionally, we observed a stronger correlation between BW and milk yield for primiparous goats than for multiparous goats (r = 0.40 and 0.13, respectively), suggesting that in dairy goats, as in dairy cows (Wathes et al., 2007), initial body reserves are not only a key component of improved milk production in early lactation but might also be essential for optimum performance in primiparous goats.

4.6.4 Level of Production

Our results show no endocrine or metabolic differences between HY and LY goats in the antepartum period. On the other hand, similar to what has been reported in high-yielding cows (Bell and Bauman, 1997; Lucy et al., 2009), postpartum HY goats had lower insulin levels and

tended to have greater GH concentration than LY goats. Additionally, the ratios of glucose, NEFA, and BHB to insulin were considerably higher in HY compared with LY goats, indicating a homeorhetic shift in metabolism toward catabolic pathways, which might explain the increased mobilization of body tissues in support of enhanced milk production among this group.

Growth hormone favors nutrient supply to the mammary gland by stimulating gluconeogenesis, lipolysis, and blood flow, but also by antagonizing insulin action in several tissues (Bell and Bauman, 1997). However, postpartum GH was not significantly associated with either Milk-S, hormones, or metabolites in our study. The secretion of GH is pulsatile in goats, and both the amplitude and frequency of GH pulses may vary from goat to goat and according to environmental and physiological conditions (Jin et al., 2012). Therefore, because we used single GH measurements, our results might not be an accurate representation of individual and group-average GH baseline levels.

4.6.5 Effects of Elevated NEFA and BHB on Milk Yield and Culling Rates up to 30 DIM

In goats, plasma BHB ≥ 0.8 mmol/L is associated with increased risk of subclinical ketosis, and BHB > 1.6 mmol/L is associated with the clinical presentation of ketosis (Albay et al., 2014; Doré et al., 2015; Marutsova and Binev, 2017). In our study, almost 30% of goats had at least 1 event of BHB ≥ 0.8 mmol/L postpartum, and the mean concentration of NEFA was consistently above cattle thresholds (≥ 0.7 mmol/L), but only 21 goats (2%) were removed from the milking herd before 30 DIM. Because culling reasons were, in large part, related to animal health (e.g., low milk yield, illness, injury, or death), we anticipated a link between culling rates and elevated NEFA and BHB. This hypothesis was further supported by the increased risk of culling in pregnant goats with BHB ≥ 0.8 mmol/L or NEFA > 1.6 mmol/L, and in postpartum goats with NEFA > 1.6 mmol/L. Interestingly, though, goats with NEFA >

1.6 mmol/L had the highest milk yield, and milk yield decreased only slightly in goats with BHB > 1.6 mmol/L.

The positive association between postpartum NEFA and milk yield appears contrary to previous research in dairy cows (Overton et al., 2017) and suggests that goats might be more resilient to elevated NEFA levels than cows are. However, it is important to note that metabolic profiles may vary from farm to farm, depending on species, genotype, average milk yield, diet composition, and farm management system, as well as other factors (Van Saun, 2009; Overton et al., 2017).

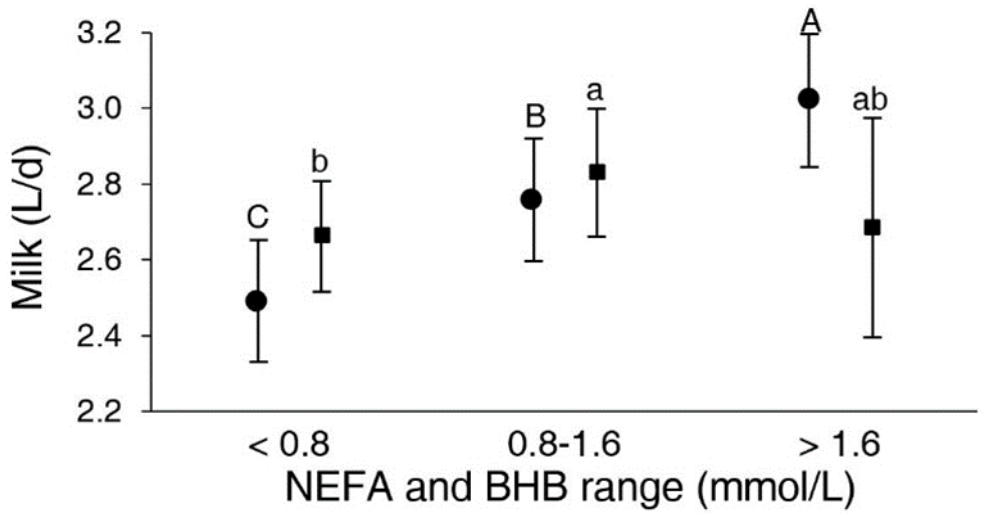

Figure 4.4. Average milk yield (L/d) according to nonesterified fatty acids (NEFA, ●) and BHB (■) level (mmol/L). Data from 622 multiparous goats tested weekly from 3 to 30 DIM. Data for primiparous were not included in these calculations. The number of simultaneous observations (milk + plasma sample) for each plasma level (< 0.8, 0.8 to 1.6, and > 1.6 mmol/L) were 1195, 835 and 387 for NEFA ((standard error of differences, SED = 0.05; P < 0.001), and 2064, 308 and 45 for BHB (SED = 0.11; P = 0.008). Error bars represent 95% CI. ^{a,b}BHB means with different lowercase superscripts, and $^{A-C}$NEFA means with different uppercase superscripts differ (P < 0.05).

4.6.6 Industry Implications

A more thorough understanding of endocrine and metabolic regulation of body tissue mobilization would contribute to the development of feeding and management practices for improved milk production in dairy goats. Because this study was carried out on a commercial farm, it provides a more realistic estimation of the metabolic burden experienced by periparturient dairy goats managed under intensive conditions. Based on the present results, it is plausible to conclude that the nutritional deficit grew with, in order of importance, increasing milk yield, parity, and litter size.

Manipulating the energy density of the diet could help to narrow the gap between energy demand and supply, thereby reducing lipid mobilization in the transition period (Hayirli and Grummer, 2004; Celi et al., 2008). Recent studies have demonstrated that grouping cows based on simultaneous nutritional needs can considerably improve the profitability of dairy farms by increasing milk yield, decreasing risks of metabolic diseases, and reducing nutrient waste and feed costs (Kalantari et al., 2016). It is only logical to suppose that such practices would yield similar results if adopted by goat dairy farmers. Nonetheless, nutritional grouping may not be practical in many goat dairy systems, because managing multiple TMR groups requires greater management complexity, which, in turn, increases labor costs (Kalantari et al., 2016; Goetsch, 2019). In such cases, varying the amount of concentrate fed in the milking parlor could be an effective way to improve the energy balance and overall performance of dairy goat herds. Moreover, recent advances in the feeding practices and nutrition of dairy goats have shown a considerable opportunity for improving the metabolic health of fresh goats and for minimizing production costs through dietary inclusion of alternative feedstuffs (e.g., fats and oils, by-products, former foodstuffs; Goetsch, 2019).

4.7 Conclusions

The presented data indicate that increased milk yield has the most significant influence on the magnitude of body tissue mobilization and suggests that the differing endocrine background of high-yielding goats may favor the partitioning of nutrients into milk. Furthermore, pregnancy and lactation were less able to elicit lipid mobilization in primiparous compared with multiparous goats. On the other hand, associations between milk, NEFA, and BW indicate that BW at birth is of greater importance for improved milk production in primiparous than in multiparous goats. Moreover, goats in late pregnancy were more susceptible to metabolic complications due to elevated NEFA and BHB than goats in early lactation. Nevertheless, NEFA thresholds established for dairy cows were not predictive of dairy goat performance. Further investigation into differences in insulin resistance between high- and low-yielding goats in early lactation, the economic effects of nutritional grouping, and the determination of critical NEFA and BHB thresholds specific to dairy goats is warranted.

4.8 Acknowledgments

Fernanda Zamuner received a Ph.D. scholarship financed by the Australian Government Research Training Program (Melbourne, VIC, Australia). The authors gratefully acknowledge Meredith Dairy Ltd. (Meredith, VIC, Australia) personnel for their assistance throughout this study. The authors declare there was no conflict of interest during the entire process of this study.

4.9 References

Albay, M. K., M. Ç. Karakurum, Ş. Şahinduran, K. Sezer, R. Yıldız, and T. Büyükoğlu. 2014. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Vet. Med-Czech, 59 (7):336-342. https://doi.org/10.17221/7620-vetmed

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol., 2 (3):265-278. https://doi.org/10.1023/A:1026336505343

Brun-Bellut, J. 1997. Urea recycling in the rumen of dairy goats: effects of physiological stage and composition of intake. Small Rumin. Res., 23 (2-3):83-90. https://doi.org/10.1016/S0921-4488(96)00910-8

Cappai, M. G., A. Liesegang, C. Dimauro, F. Mossa, and W. Pinna. 2019. Circulating electrolytes in the bloodstream of transition Sarda goats make the difference in body fluid distribution between single vs. twin gestation. Res. Vet. Sci., 123:84-90. https://doi.org/10.1016/j.rvsc.2018.12.016

Caré, S., E. Trevisi, A. Minuti, A. Ferrari, J. J. Loor, and L. Calamari. 2018. Plasma fructosamine during the transition period and its relationship with energy metabolism and inflammation biomarkers in dairy cows. Livest. Sci., 216:138-147. https://doi.org/10.1016/j.livsci.2018.08.003

Castagnino, D. S., C. J. Härter, A. R. Rivera, L. D. Lima, H. G. O. Silva, B. Biagioli, K. T. Resende, and I. A. M. A. Teixeira. 2015. Changes in maternal body composition and metabolism of dairy goats during pregnancy. R. Bras. Zootec., 44 (3):92-102. https://doi.org/10.1590/S1806-92902015000300003

Celi, P., A. Di Trana, and S. Claps. 2008. Effects of perinatal nutrition on lactational performance, metabolic and hormonal profiles of dairy goats and respective kids. Small Rumin. Res., 79 (2-3):129-136. https://doi.org/10.1016/j.smallrumres.2008.07.010

Chilliard, Y., P. G. Toral, K. Shingfield, J. Rouel, C. Leroux, and L. Bernard. 2014. Effects of diet and physiological factors on milk fat synthesis, milk fat composition and lipolysis in the goat: A short review. Small Rumin. Res., 122 (1-3):31-37. https://doi.org/10.1016/j.smallrumres.2014.07.014

Delavaud, C., H. Fougère, Y. Chilliard, and L. Bernard. 2019. The dietary addition of fish oil or sunflower oil plus starch differently modulates the lipid classes in plasma of lactating cows and goats. Eur. J. Lipid Sci. Technol., 121 (10):1900075. https://doi.org/10.1002/ejlt.201900075

Doré, V., J. Dubuc, A. M. Bélanger, and S. Buczinski. 2015. Definition of prepartum hyperketonemia in dairy goats. J. Dairy Sci., 98 (7):4535-4543. http://dx.doi.org/10.3168/ jds.2014-9172

Goetsch, A. 2019. Recent research of feeding practices and the nutrition of lactating dairy goats. J. Appl. Anim. Res., 47 (1):103-114. https://doi.org/10.1080/09712119.2019.1580585

Goetsch, A. L., T. A. Gipson, A. R. Askar, and R. Puchala. 2010. Invited review: Feeding behavior of goats. J. Anim. Sci., 88 (1):361-373. https://doi.org/10.2527/jas.2009-2332

González-García, E., V. G. De Figuereido, D. Foulquie, E. Jousserand, P. Autran, S. Camous, A. Tesnière, F. Bocquier, and M. Jouven. 2014. Circannual body reserve dynamics and metabolic profile changes in Romane ewes grazing on rangelands. Domest. Anim. Endocrinol., 46:37-48. https://doi.org/10.1016/j.domaniend.2013.10.002

González-García, E., A. Tesnière, S. Camous, F. Bocquier, F. Barillet, and P. Hassoun. 2015. The effects of parity, litter size, physiological state, and milking frequency on the metabolic profile of Lacaune dairy ewes. Domest. Anim. Endocrin., 50:32-44. https://doi.org/10. 1016/j.domaniend.2014.07.001

Hashizume, T., Y. Takahashi, M. Numata, K. Sasaki, K. Ueno, K. Ohtsuki, M. Kawai, and A. Ishii. 1999. Plasma Profiles of Growth Hormone, Prolactin and Insulin-like Growth Factor-I during Gestation, Lactation and the Neonatal Period in Goats. J. Reprod. Develop., 45 (4):273. https://doi.org/10.1262/jrd.45.273

Hayirli, A., and R. R. Grummer. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Can. J. Anim. Sci., 84 (3):337-347. https://doi.org/10.4141/A03-122

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance: Influences on the etiology of ketosis and fatty liver. Vet. Clin. N. Am. Food A., 16 (2):215-230. https://doi.org/10.1016/S0749-0720(15)30102-X

Jin, J. I. N., T. Yaegashi, K. Sawai, and T. Hashizume. 2012. Effects of photoperiod on the secretion of growth hormone in female goats. Anim. Sci. J., 83 (8):610-616. https://doi.org/10.1111/j.1740-0929.2011.00998.x

Johnson, M. M., and J. P. Peters. 1993. An improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci., 71 (3):753-756. https://doi.org/10.2527/1993.713753x

Kalantari, A. S., L. E. Armentano, R. D. Shaver, and V. E. Cabrera. 2016. Economic impact of nutritional grouping in dairy herds. J. Dairy Sci., 99 (2):1672-1692. https://doi.org/10.3168/jds.2015-9810

Lean, I. J., M. L. Bruss, R. L. Baldwin, and H. F. Troutt. 1992. Bovine ketosis: A review. II. Biochemestry and prevention. Vet. Bull., 62 (February):1-14.

Lérias, J. R., L. E. Hernández-Castellano, A. Suárez-Trujillo, N. Castro, A. Pourlis, and A. M. Almeida. 2014. The mammary gland in small ruminants: major morphological and functional events underlying milk production–a review. J. Dairy Res., 81 (3):304-318. https://doi.org/10.1017/S0022029914000235

Lucy, M. C., G. A. Verkerk, B. E. Whyte, K. A. Macdonald, L. Burton, R. T. Cursons, J. R. Roche, and C. W. Holmes. 2009. Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. J. Dairy Sci., 92 (2):526-539. https://doi.org/10.3168/jds.2008-1421

Magistrelli, D., and F. Rosi. 2014. Trend analysis of plasma insulin level around parturition in relation to parity in Saanen goats. J. Anim. Sci., 92 (6):2440-2446. http://dx.doi.org/10.2527/jas2013-6993

Marutsova, V., and R. Binev. 2017. Body condition score, nonesterified fatty acids and beta-hydroxybutyrate concentrations in goats with subclinical ketosis. Agri. Sci. Tech., 9 (4):282-285. https://doi.org/10.15547/ast.2017.04.053

Matthews, J. G. 2016. The Periparturient Goat. Pages 41-60 In Diseases of the goat. 4 ed. John Wiley & Sons Ltd. Chichester, UK.

McCarthy, M. M., S. Mann, D. V. Nydam, T. R. Overton, and J. A. A. McArt. 2015. Concentrations of nonesterified fatty acids and β-hydroxybutyrate in dairy cows are not well correlated during the transition period. J. Dairy Sci., 98 (9):6284-6290. http://dx.doi.org/10.3168/jds.2015-9446

National Research Council. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Academic Press. Washington, DC.

Overton, T. R., J. A. A. McArt, and D. V. Nydam. 2017. A 100-Year Review: Metabolic health indicators and management of dairy cattle. J. Dairy Sci., 100 (12):10398-10417. https://doi.org/10.3168/jds.2017-13054

Pezzanite, L., M. Neary, T. Hutchens, and P. Scharko. 2009. Common diseases and health problems in sheep and goats. Knowledge to Go. Purdue Extension. Kentucky, USA. Accessed on Accessed Jun. 16, 2019. https://www.extension.purdue.edu/extmedia/as/as-595-commondiseases.pdf

Radin, L., M. Šimpraga, S. Vince, A. Kostelić, and S. Milinković-Tur. 2015. Metabolic and oxidative status of Saanen goats of different parity during the peripartum period. J. Dairy Res., 82 (4):426-433. https://doi.org/10.1017/S0022029915000552

Soares, G. S. L., R. J. C. Souto, J. F. P. Cajueiro, J. A. B. Afonso, R. O. Rego, A. T. M. Macêdo, P. C. Soares, and C. L. Mendonça. 2018. Adaptive changes in blood biochemical profile of dairy goats during the period of transition. Revue Méd. Vét., 169 (1-3):65-75.

Stelletta, C., M. Gianesella, and M. Morgante. 2008. Metabolic and nutritional diseases. Pages Pages 263-288 In Dairy goats feeding and nutrition. A. Cannas and G. Pulina, ed. CABI International. Bologna, Italy.

Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals, 5 (4):978-1020. https://doi.org/10.3390/ani5040395

Thomas, G. B., J. E. Mercer, T. Karalis, A. Rao, J. T. Cummins, and I. J. Clarke. 1990. Effect of restricted feeding on the concentrations of growth hormone (GH), gonadotropins, and prolactin (PRL) in plasma, and on the amounts of messenger ribonucleic acid for GH, gonadotropin subunits, and PRL in the pituitary glands of adult ovariectomized ewes. Endocrinology, 126 (3):1361-1367. https://doi.org/10.1210/endo-126-3-1361

Toral, P. G., Y. Chilliard, J. Rouel, H. Leskinen, K. J. Shingfield, and L. Bernard. 2015. Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats. J. Dairy Sci., 98 (10):7277-7297. https://doi.org/10.3168/jds.2015-9649

Van Saun, R. J. 2009. Metabolic Profiling. Pages 153-162 In Vet. Clin. N. Am. Food A. 5 D. E. Anderson and D. M. Rings, ed. W. B. Saunders. Edinburgh, UK.

Villaquiran, M., T. A. Gipson, R. C. Merkel, A. L. Goetsch, and T. Sahlu. 2004. Body condition scores in goats. American Institute for Goat Research. Langston University, OK, USA. Accessed on Accessed Mar. 18, 2016. http://www.luresext.edu/sites/default/files/BCS_factsheet.pdf

Wankhade, P. R., A. Manimaran, A. Kumaresan, S. Jeyakumar, K. P. Ramesha, V. Sejian, D. Rajendran, and M. R. Varghese. 2017. Metabolic and immunological changes in transition dairy cows: A review. Vet. World, 10 (11):1367-1377. https://doi.org/10.14202/vetworld .2017

Wathes, D. C., Z. Cheng, N. Bourne, V. J. Taylor, M. P. Coffey, and S. Brotherstone. 2007. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domest. Anim. Endocrinol., 33 (2):203-225. https://doi.org/10.1016/j.domaniend.2006.05.004

Zalcman, E., and B. Cowled. 2018. Farmer survey to assess the size of the Australian dairy goat industry. Aust. Vet. J., 96 (9):341-345. https://doi.org/10.1111/avj.12734

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia. J. Dairy Sci., 103 (1):954-964. https://doi.org/10.3168/jds.2019-17051

Zarrin, M., L. Grossen-Rösti, R. M. Bruckmaier, and J. J. Gross. 2017. Elevation of blood β-hydroxybutyrate concentration affects glucose metabolism in dairy cows before and after parturition. J. Dairy Sci., 100 (3):2323-2333. https://doi.org/10.3168/jds.2016-11714

Chapter 5 - Associations between non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB) and glucose in periparturient dairy goats

F. Zamuner[*1], K. DiGiacomo[*], A.W.N. Cameron[†], B.J. Leury[*]

[*] Faculty of Veterinary and Agricultural Sciences - The University of Melbourne, Parkville, Victoria 3010, Australia.

[†] Meredith Dairy Pty Ltd, 106 Cameron Rd, Meredith, Victoria 3333, Australia.

5.1 Manuscript 3

Chapter 5 investigated temporal relationships between NEFA, BHB, and glucose concentrations in periparturient dairy goats. We used samples collected on the same days as the experiments in Chapter 4 to estimate the ante- and postpartum prevalence of goats above BHB thresholds and/or with elevated NEFA, as well as to estimate the day of maximal BHB and NEFA concentrations.

This work has been published in the Journal of Dairy Science;

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Associations between nonesterified fatty acids, β-hydroxybutyrate, and glucose in periparturient dairy goats. J. Dairy Sci., 103 (7): 6672-6678 https://doi.org/10.3168/jds.2019-17163

Therefore, the paper is included in this chapter as a manuscript.

5.2 Abstract

The objective of the present study was to use longitudinal data to examine the relationships between blood concentrations of nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and glucose during the transition period in dairy goats. Weekly blood samples were collected from Saanen goats from a commercial herd in Australia [1–7 years; body weight 70 ± 16.0 kg; body condition score 2.5 ± 0.3; and daily milk yield 2.4 ± 0.73 L/d; all mean ± standard deviation (SD)]. The weekly prevalence of goats above hyperketonemic levels (BHB ≥ 0.8 mmol/L) was approximately 6 times greater postpartum than antepartum. As well, of the 935 goats sampled antepartum, 50 (5%) had at least 1 hyperketonemic event, and 823 (88%) had at least 1 event of NEFA above the threshold (≥ 0.3 mmol/L). Of 847 goats tested postpartum, 258 (30%) had at least 1 hyperketonemic event, and 690 goats (81%) had at least 1 event of NEFA above the threshold (≥ 0.7 mmol/L). Substantial variation was found when analyzing the mean days of maximum NEFA and maximum BHB concentrations antepartum (-11 ± 6.6 and -14 ± 7.2 d, respectively, mean ± SD) and postpartum (14 ± 6.6 and 9 ± 6.8 d, respectively, mean ± SD). We observed moderate to strong relationships between NEFA and BHB concentrations (r = 0.66) and between NEFA and glucose concentrations (r = -0.46) throughout the transition period. Our results suggested that 3 to 16 d in milk is the best sampling window for monitoring hyperketonemia in dairy goats, and that results from simultaneous BHB and glucose tests provide an improved indication of the fat mobilization and energy status of the herd when measured close to this timeframe.

Key Words: transition period, energy metabolites, correlation, prevalence

5.3 Introduction

The period from 3 weeks before to 3 weeks after parturition, defined as the transition period in dairy animals (Grummer, 1995), is characterized by depressed feed intake and increased nutrient requirements (for fetal development and milk production), often resulting in negative energy balance (NEB; Wankhade et al., 2017). Intensified mobilization of body fat reserves and elevation of circulating nonesterified fatty acids (NEFA) and BHB is an adaptive response to NEB to supply peripheral tissues with alternative energy sources, sparing glucose for fetal development and milk synthesis (Baumgard et al., 2017). However, a considerable amount of work has linked elevated blood levels of NEFA and BHB to increased incidence of NEB-related diseases in transitioning dairy cows (McArt et al., 2013; Overton et al., 2017).

Over the last 20 years, NEFA and BHB have become the accepted biomarkers of excessive NEB or maladaptation of energy metabolism to lactation in cows (Overton et al., 2017), and antepartum (NEFA ≥ 0.3–0.5 and BHB ≥ 0.6–0.8 mmol/L) and postpartum (NEFA ≥ 0.7–1.0 and BHB ≥ 1.0–1.4 mmol/L) thresholds have been established to predict the risk of NEB-related diseases and milk loss in transition dairy cows (Overton et al., 2017; Wankhade et al., 2017). Similarly, studies in dairy goats have demonstrated that plasma BHB ≥ 0.8 mmol/L is associated with subclinical ketosis, and BHB > 1.6 mmol/L is associated with clinical ketosis (Albay et al., 2014; Doré et al., 2015; Marutsova and Binev, 2017). However, specific NEFA reference values have not been established for goats (Radin et al., 2015).

Although NEFA has been proposed as a better predictor of NEB status and disease risk than BHB and glucose (McArt et al., 2013), testing for NEFA is expensive and practically challenging. In contrast, several point-of-care meters have been validated for measurement of blood BHB and glucose concentrations in cows, ewes, and goats (Pichler et al., 2014; Overton et al., 2017; Panousis et al., 2018). This inexpensive method provides producers, practitioners, and investigators with an important diagnostic and screening tool. However, the determination

of BHB and glucose cut-points associated with negative outcomes in dairy goats would require large epidemiological studies in different herds. A perusal of the literature revealed only 1 cohort study on optimal BHB thresholds for predicting the risk of prepartum hyperketonemia in dairy goats (Doré et al., 2015), and no similar studies have been reported in lactating goats.

The aims of this study were (1) to use longitudinal data to estimate the ante- and postpartum prevalence of goats with BHB levels above thresholds and the prevalence of goats with elevated NEFA (based on dairy cattle thresholds); and (2) to examine the temporal relationships between circulating concentrations of NEFA, BHB, and glucose in periparturient dairy goats from a commercial herd in Australia.

5.4 Material & Methods

All experimental procedures were approved by the Faculty of Veterinary and Agricultural Sciences Animal Ethics and Welfare Committee of the University of Melbourne, Australia (No. 1613846.1).

This experiment was conducted at Meredith Dairy commercial farm (Meredith, Australia, 37°50'S; 144°04'E) over 4 consecutive kidding seasons, from June 2016 to March 2017, in parallel with a production trial using 1,000 Saanen goats [~250 different goats each kidding season; 1–7 years; 69 ± 14.0 kg BW; mean ± standard deviation (SD)]. Further details on animals and management are available in Zamuner et al. (2020). In brief, the enrolled goats were kept with their contemporary group (~600 goats) in a collective pen and began to receive the fresh-goat TMR 1 months before the expected kidding date. Goats were fed ad libitum once per day at approximately 0700 h. The approximate nutrient composition (DM basis) of the diet was as follows: 32% NDF, 16% CP, 10 MJ of ME, 18 g of Ca, and 9.0 g of P/kg of DM.

Blood samples were collected from all goats (~250 each kidding season) on the same day at approximately 1000 h, always after the morning milking. On the day of blood sampling, feed was withheld until after blood collection. Goats were sampled in random order, and the average

interval between the first and last animal sampled was 166 ± 30 min (± SD). Blood collection was carried out once weekly for 7 consecutive weeks, starting at wk -3 relative to the expected kidding date of each kidding season. The actual day of sampling (in relation to delivery) was determined after birth by sampling date - kidding date, and data were segmented in weekly intervals (i.e., -21 ± 3 d = wk -3; -14 ± 3 d = wk -2; and so forth).

Blood was harvested via jugular venipuncture into 10-mL lithium heparin (170 IU) evacuated tubes (BD Vacutainer, Plymouth, UK) and immediately placed on ice until plasma separation. The whole-blood concentration of BHB was measured using a handheld meter (FreeStyle Optium Precision Neo; Abbott Diabetes Care Ltd., Witney, UK) before centrifugation (1,250 × g, 12 min, 4°C). Isolated plasma was stored at −20°C until analysis. Plasma NEFA and glucose concentrations were determined spectrophotometrically using commercial kits: NEFA-C ACS-ACOD Method (Wako Pure Chemical Industries Ltd., Osaka, Japan), modified as per the methods of Johnson and Peters (1993); and Infinity Glucose Oxidase Liquid (Thermo-Scientific, Waltham, MA).

Statistical computations were performed using Minitab software (version 18.1; Minitab Inc., State College, PA). Plasma concentrations of NEFA, BHB, and glucose were screened for normality by calculating kurtosis and skewness, and by visual assessment of standardized residuals distribution. Concentrations of NEFA and BHB were log-transformed to achieve normality. Data for logged NEFA, logged BHB, and glucose concentrations from d -24 to 24 were subjected to repeated-measures ANOVA using a mixed-effects model (REML and 2-sided 95% CI) in Minitab. The fixed effect was the day of sampling, and the random effect was goat nested within kidding season to account for variations between kidding seasons. Fixed effects of parity number and litter size, as well as the covariant effects of time of bleeding, BW, and BCS, were tested in all models and retained when significant ($P < 0.05$). Results for logged NEFA and logged BHB were back-transformed for graphic presentation.

Spearman rho correlations were used to examine the relationships between weekly concentrations of NEFA, BHB, and glucose. A multiple linear regression (2-sided, 95% CI) was used to evaluate the confidence in estimating logged NEFA using logged BHB and glucose concentrations as continuous predictors.

Days of maximum NEFA and BHB concentrations were determined for each goat in the data set before log-transformation and statistical analysis. We used the following cutoffs: ≥ 0.8 mmol/L and > 1.6 mmol/L for elevated BHB; and ≥ 0.3 mmol/L antepartum and ≥ 0.7 mmol/L postpartum for elevated NEFA (McArt et al., 2013). The antepartum calculations included data observations from d -24 to -1, and the postpartum calculations included data observations from d 0 to 24. Statistical significance was declared at $P < 0.05$. Data are presented as mean (95% CI) unless declared otherwise.

5.5 Results & Discussion

Of the 1,000 animals enrolled, 60 were excluded from the experiment due to abortion, death, or kidding delay lay, for a total population of 940. The final number of goats commencing the experiment were subdivided as follows: by season of kidding (March = 237, June = 242, September = 222, and November = 239); by parity number (1 = 319, 2 = 244, 3 = 237, 4 = 85, 5 = 38, and 6 = 17); and by litter size (single = 330, twins = 516, and triplets = 94). The average number of samples collected at any given day between d -24 to 24 was 118 (range 68 to 156). The total number of goats tested each week is presented in **Table 5.1**. The average daily milk yield in the first month was 2.4 ± 0.73 L/d (mean ± SD).

To our knowledge, this is the first report using longitudinal data from a commercial herd to estimate the weekly prevalence of goats above critical levels of NEFA and BHB during the transition period. **Figure 5.1** describes daily variations of NEFA, BHB, and glucose from d 24 prepartum to d 24 postpartum. Previous studies in periparturient dairy goats have reported a similar increase in NEFA and BHB concentrations postpartum (Magistrelli and Rosi, 2014;

Radin et al., 2015). However, these studies did not report the proportion of goats showing elevated plasma NEFA and BHB concentrations.

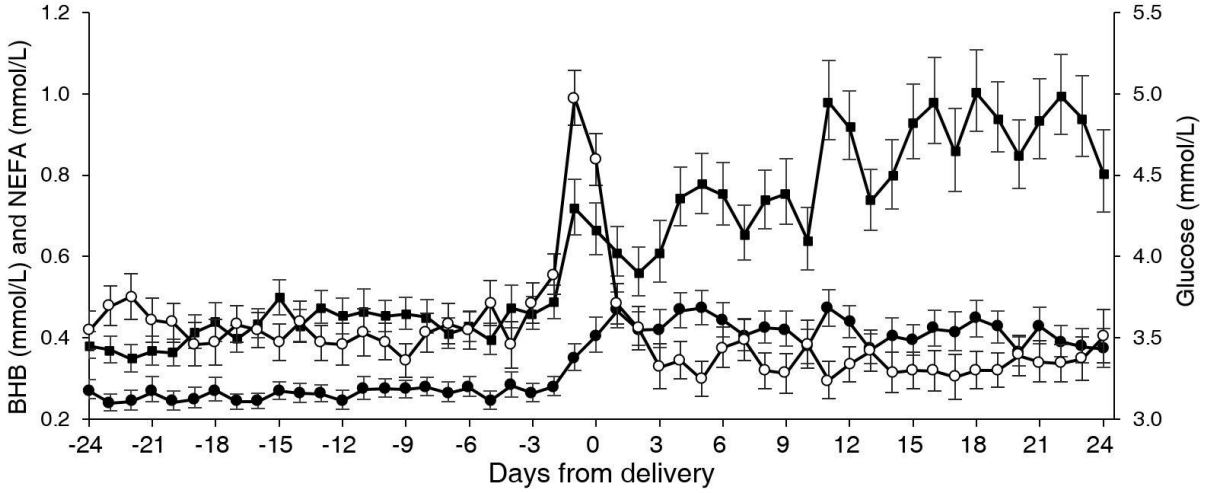

Figure 5.1. Daily plasma concentrations (mmol/L) of β-hydroxybutyrate (BHB; ●), non-esterified fatty acids (NEFA; ■), and glucose (○) in periparturient dairy goats sampled from -24 to 24 days from delivery; error bars represent 95% confidence intervals. Goats were sampled weekly and grouped by 'days from delivery' based on their kidding dates [average number of samples collected at any given day between d -24 to 24 was 118 (range, 68 to 156)]

The present data showed a greater proportion of goats having BHB and NEFA concentrations above critical levels postpartum compared with antepartum (**Table 5.1**). Of the 935 goats tested antepartum (3,011 sampling events), the number of goats with at least 1 event was as follows: BHB ≥ 0.8 mmol/L = 50 (5%); NEFA ≥ 0.3 mmol/L = 823 (88%). From the 847 goats tested postpartum (2,767 sampling events), the number of goats with at least 1 event was as follows: BHB ≥ 0.8 mmol/L = 258 (30%); NEFA ≥ 0.7 mmol/L = 690 (81%).

Ospina et al. (2010) studied more than 2,700 cows in 100 free-stall dairies in the United States and reported that 34% of cows had NEFA ≥ 0.3 mmol/L (d -14 to -3) and 32% of cows had NEFA ≥ 0.7 mmol/L (3 to 14 DIM). Ospina et al. (2010) also reported that cows with values above such NEFA thresholds were twice as likely to be culled within 30 DIM.

Comparatively, in the present study, the prevalence of goats with elevated NEFA was 77% antepartum and 64% postpartum (for the same thresholds and sampling period), whereas only 2% of the herd was culled within 30 DIM, suggesting that the NEFA threshold established for cows might not be predictive of performance in dairy goats.

Ketosis is one of the most common diseases affecting does and ewes regardless of farming purpose (i.e., dairy, meat, or wool/hair production; Rook, 2000). Contrary to the extensive information available on lactational ketosis in dairy cattle (McArt et al., 2012; Suthar et al., 2013), data on hyperketonemia in dairy goats are focused mainly on pregnancy ketosis (Albay et al., 2014; Doré et al., 2015; Vasava et al., 2016) and, to a lesser extent, on lactational ketosis (Yadav et al., 2015; Marutsova and Binev, 2017). Nevertheless, dairy goats with high genetic merit for milk production are prone to intense and prolonged NEB in early lactation (Smith and Sherman, 2009), emphasizing the importance of monitoring the energy status of dairy herds both ante- and postpartum.

In the present study, the weekly prevalence of goats above hyperketonemic levels (BHB $\geq$ 0.8 mmol/L) was approximately 6 times greater postpartum than antepartum (**Table 5.1**). Several authors have suggested that the best sampling window for early detection of hyperketonemia in dairy cows is between 2 to 3 and 14 to 16 DIM (McArt et al., 2012; Ospina et al., 2013; Suthar et al., 2013). In the present study, 258 goats (30%) had at least 1 hyperketonemic episode from 0 to 24 DIM. Of those, 130 goats (50%) had their first hyperketonemic episode between 3 and 16 DIM, indicating that the same sampling window might also be suitable for the observation of hyperketonemia in dairy goats

Table 5.1. Weekly prevalence of goats above critical levels for non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB), and weekly correlations between plasma concentrations (mmol/L) of NEFA, BHB, and glucose in Australian commercial dairy goats.

	Weeks relative to delivery[1]						
Item	-3	-2	-1	0	1	2	3
No. of goats sampled	785	914	883	811	794	776	752
No. (%) of goats at or above cutoffs							
BHB ≥ 0.8 mmol/L	10 (1.3)	16 (1.8)	24 (2.7)	80 (9.9)	121 (15.2)	117 (15.1)	106 (14.1)
BHB > 1.6 mmol/L	1 (0.1)	4 (0.4)	6 (0.7)	13 (1.6)	12 (1.5)	18 (2.3)	13 (1.7)
NEFA[2]	470 (59)	610 (67)	571 (65)	285 (35)	414 (51)	489 (63)	468 (62)
Spearman rho correlation							
NEFA-BHB	0.24***	0.45***	0.43***	0.68***	0.64***	0.58***	0.53***
NEFA-Glucose	-0.37***	-0.50***	-0.47***	-0.25***	-0.54***	-0.52***	-0.47***
BHB-Glucose	-0.14***	-0.27***	-0.25***	-0.23***	-0.36***	-0.26***	-0.23***

[1]Wk -3; d -21 ± 3, ... wk 3; d 21 ± 3.

[2]Cutpoints for elevated NEFA concentration in dairy cows; NEFA ≥ 0.3 mmol/L antepartum (wk -3 to -1) and ≥ 0.7 mmol/L postpartum (wk 0 to 3) (McArt et al., 2013)

*** P < 0.001

To better understand the relationship between NEFA and BHB, we examined the distribution of the day of maximum NEFA and day of maximum BHB **Table 5.2**. Notably, the mean and median days of maximum BHB concentrations occurred earlier than the mean and median days of maximum NEFA concentrations. However, the large degree of variation in the days of peak BHB and NEFA concentrations prevented accurate interpretation of data distribution. Neither parity nor litter size significantly affected the mean day of peak concentrations of NEFA and BHB.

We found substantial variation when we analyzed the mean (± SD) day of maximum NEFA and BHB concentrations (BHB = -14 ± 7.2 and 9 ± 6.8 d, NEFA = -11 ± 6.6 and 14 ± 6.6 d, ante- and postpartum, respectively). Further analysis showed that for most goats, the day of maximum BHB was the same as the day of maximum NEFA: antepartum = 439 goats (47%); postpartum = 390 goats (46%). Interestingly, the day of maximum BHB occurred before the day of maximum NEFA in most of the remaining goats: antepartum = 372 goats (40%), postpartum = 373 goats (44%). Harmeyer and Schlumbohm (2006) have demonstrated that pregnant ewes have a reduced ability to use BHB. Additionally, Reynolds et al. (2003) observed that variations in the liver release of BHB could be attributed not only to changes in the liver removal of NEFA but also to diet-induced changes in circulating levels of other ketogenic precursors, such as butyrate and acetoacetate. Therefore, the relatively earlier peak of BHB could be related in part to BHB overproduction in times of impaired use, and to individual differences in hepatic energy metabolism, rather than solely due to increased removal of NEFA by the liver. Nevertheless, the reasons for the present findings remain speculative.

Table 5.2. Characteristics of the distribution of antepartum and postpartum day of maximum nonesterified fatty acids (NEFA) and day of maximum β-hydroxybutyrate (BHB) concentrations in Australian commercial dairy goats

	Antepartum[1]							Postpartum[2]						
Item	N	Mean	Med.	Min.	Max.	SD	Corr.[3]	N	Mean	Med.	Min.	Max.	SD	Corr.[3]
Day of max. BHB		-14	-15	-24	-1	7.2			9	8	0	24	6.8	
BHB[4]	936	0.4	0.3	0.1	5.6	0.37		847	0.7	0.6	0.2	6.7	0.56	
NEFA[4]	931	0.6	0.4	0.1	2.2	0.41	0.58***	841	1.0	0.9	0.1	2.8	0.55	0.58***
Glucose[4]	934	3.5	3.4	1.5	8.9	0.88	-0.22***	833	3.2	3.1	1.0	10.5	0.79	-0.27***
Day of max. NEFA		-11	-12	-24	-1	6.6			14	15	0	24	6.6	
NEFA[5]	936	0.7	0.5	0.1	2.3	0.39		847	1.3	1.2	0.1	2.8	0.59	
BHB[5]	935	0.3	0.3	0.1	4.7	0.31	0.64***	847	0.6	0.4	0.1	6.7	0.51	0.46***
Glucose[5]	931	3.3	3.2	1.1	9.8	0.93	-0.31***	841	3.0	2.9	1.3	10.5	0.70	-0.40***

[1]From d -24 to -1 relative to delivery.

[2]From d 0 to 24 relative to delivery.

[3]Spearman ρ correlation between BHB, NEFA, and glucose concentrations on the day of maximum BHB concentration, and between NEFA, BHB, and glucose concentrations on
the day of maximum NEFA concentration.

[4]BHB, NEFA, and glucose concentration (mmol/L) on the day of maximum BHB concentration.

[5]BHB, NEFA, and glucose concentration (mmol/L) on the day of maximum NEFA concentration.

***P < 0.001.

Table 5.3. Coefficients of determination (R^2)[1] and estimates (95% CI) for multiple linear regression model based on continuous β-hydroxybutyrate (BHB)[2] and glucose concentrations (mmol/L) for the prediction of plasma non-esterified fatty acids (NEFA)[2] concentration (mmol/L) in dairy goats

Period[3]	R^2	Constant	BHB	Glucose
d -24 to -18	19.1%***	0.05 (-0.03, 0.14) ns	0.32 (0.25, 0.39) ***	-0.09 (-0.11, -0.07) ***
d -17 to -11	36.4%***	0.40 (0.32, 0.47) ***	0.44 (0.38, 0.50) ***	-0.15 (-0.17, -0.13) ***
d -10 to -4	35.4%***	0.32 (0.25, 0.40) ***	0.42 (0.37, 0.48) ***	-0.14 (-0.16, -0.12) ***
d -3 to 3	40.4%***	0.03 (-0.02, 0.08) ns	0.60 (0.55, 0.66) ***	-0.01 (-0.02, 0.00) ns
d 4 to 10	46.6%***	0.50 (0.43, 0.57) ***	0.53 (0.48, 0.59) ***	-0.14 (-0.17, -0.12) ***
d 11 to 17	46.3%***	0.70 (0.62, 0.78) ***	0.50 (0.44, 0.55) ***	-0.18 (-0.21, -0.16) ***
d 18 to 24	38.5%***	0.67 (0.58, 0.75) ***	0.48 (0.41, 0.54) ***	-0.17 (-0.20, -0.14) ***

[1]Proportion of the variance in the dependent variable (i.e., NEFA) that is predictable from the independent variables in the model (i.e., BHB and glucose).
[2]Logged concentrations (mmol/L)
[3]Days relative to delivery. Number animals sampled in each period is presented in Table 5.1
*** P < 0.001; ns P > 0.05

Despite a considerable body of evidence linking elevated NEFA and BHB to negative downstream outcomes in dairy animals (Overton et al., 2017), recent studies reported either weak (r = 0.20; McCarthy et al., 2015) or absent (Pilotto et al., 2016) correlations between NEFA and BHB in transitioning dairy cows. In contrast, we observed a moderate to strong relationship between concentrations of NEFA and BHB (r = 0.66) and NEFA and glucose (r = -0.46) throughout the transition period (5,686 simultaneous sampling events from d -24 to 24), and on the NEFA and BHB days of peak concentration (**Table 5.2**). Similarly, moderate relationships between NEFA and BHB have been reported in healthy goats in the last week of pregnancy (r = 0.45) and in periparturient dairy ewes (r = 0.41; Karagiannis et al., 2014; Radin et al., 2015), suggesting a stronger association between BHB and NEFA in small ruminants than in dairy cows. Although the reason for the interspecies differences is unclear, recent studies have reported relevant distinctions in intermediary metabolism and mammary

lipogenesis between cows and goats (Bernard et al., 2017; Fougère et al., 2018), which could explain the different correlation strengths between BHB and NEFA reported in cows and goats.

Plasma NEFA concentration reflects the magnitude of fat mobilization from body reserves (LeBlanc, 2010). The moderately strong correlations observed between NEFA and BHB, and between NEFA and glucose, puts forward the idea that combined results from BHB and glucose tests could be a potential alternative to laboratory analysis of NEFA concentrations in estimating the rate of fat metabolism in dairy goats. Nonetheless, in the present study, variations in plasma BHB and glucose explained approximately 47% of the observed variations in NEFA concentrations from 4 to 17 DIM (**Table 5.3**). Taken together, results for weekly correlations (**Table 5.1**) and regression analysis (**Table 5.3**) clearly demonstrate that the strength of the association between the selected metabolites varies with time and that such associations are stronger during the 2 first weeks of lactation. These findings suggest that simultaneous BHB and glucose tests would provide a more accurate estimation of the actual nutritional status of dairy goats when measured within 4 to 17 DIM.

5.6 Conclusions

Our results showed a greater prevalence of goats above the cutoff for elevated BHB and NEFA after kidding, demonstrating that in dairy goats, as with dairy cows, intensive metabolic adaptations are more pronounced in early lactation than in late pregnancy. However, in contrast to reports in dairy cows, we observed a relatively strong relationship between NEFA and BHB concentrations throughout the transition period and on days of peak concentrations of NEFA and BHB. Further research is needed to determine critical NEFA and BHB thresholds as predictors of disease and milk production loss in transition dairy goats.

5.7 Acknowledgments

Fernanda Zamuner received a Ph.D. scholarship financed by the Australian Government Research Training Program (Melbourne, VIC, Australia). The authors gratefully acknowledge Meredith Dairy Ltd. (Meredith, VIC, Australia) personnel for their assistance throughout this study. The authors declare there was no conflict of interest during the entire process of this study.

5.8 References

Albay, M. K., M. Ç. Karakurum, Ş. Şahinduran, K. Sezer, R. Yıldız, and T. Büyükoğlu. 2014. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Vet. Med-Czech, 59 (7):336-342. https://doi.org/10.17221/7620-vetmed

Baumgard, L. H., R. J. Collier, and D. E. Bauman. 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. J. Dairy Sci., 100 (12):10353-10366. https://doi.org/10.3168/jds.2017-13242

Bernard, L., P. G. Toral, and Y. Chilliard. 2017. Comparison of mammary lipid metabolism in dairy cows and goats fed diets supplemented with starch, plant oil, or fish oil. J. Dairy Sci., 100 (11):9338-9351. https://doi.org/10.3168/jds.2017-12789

Doré, V., J. Dubuc, A. M. Bélanger, and S. Buczinski. 2015. Definition of prepartum hyperketonemia in dairy goats. J. Dairy Sci., 98 (7):4535-4543. http://dx.doi.org/10.3168/jds.2014-9172

Fougère, H., C. Delavaud, and L. Bernard. 2018. Diets supplemented with starch and corn oil, marine algae, or hydrogenated palm oil differentially modulate milk fat secretion and composition in cows and goats: A comparative study. J. Dairy Sci., 101 (9):8429-8445. https://doi.org/10.3168/jds.2018-14483

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci., 73 (9):2820-2833. https://doi.org/10.2527/1995.7392820x

Harmeyer, J., and C. Schlumbohm. 2006. Pregnancy impairs ketone body disposal in late gestating ewes: Implications for onset of pregnancy toxaemia. Res. Vet. Sci., 81 (2):254-264. http://dx.doi.org/10.1016/j.rvsc.2005.10.010

Johnson, M. M., and J. P. Peters. 1993. Technical note: an improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim Sci., 71 (3):753-756. http://dx.doi.org/10.2527/1993.713753x

Karagiannis, I., N. Panousis, E. Kiossis, I. Tsakmakidis, S. Lafi, G. Arsenos, and C. Boscos. 2014. Associations of pre-lambing body condition score and serum β-hydroxybutyric acid and non-esterified fatty acids concentrations with periparturient health of Chios dairy ewes. Small Rum. Res., 120 (1):164-173. http://dx.doi.org/10.1016/j.smallrumres.2014.05.001

LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Dev., 56 Suppl: S29-35. https://doi.org/10.1262/jrd.1056s29

Magistrelli, D., and F. Rosi. 2014. Trend analysis of plasma insulin level around parturition in relation to parity in Saanen goats. J. Anim. Sci., 92 (6):2440-2446. http://dx.doi.org/10.2527/jas2013-6993

Marutsova, V., and R. Binev. 2017. Body condition score, nonesterified fatty acids and beta-hydroxybutyrate concentrations in goats with subclinical ketosis. Agri. Sci. Tech., 9 (4):282-285. https://doi.org/10.15547/ast.2017.04.053

McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. J. Dairy Sci., 95 (9):5056-5066. http://dx.doi.org/10.3168/jds.2012-5443

McArt, J. A. A., D. V. Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Vet. J., 198 (3):560-570. http://dx.doi.org/10.1016/j.tvjl.2013.08.011

McCarthy, M. M., S. Mann, D. V. Nydam, T. R. Overton, and J. A. A. McArt. 2015. Concentrations of nonesterified fatty acids and β-hydroxybutyrate in dairy cows are not well correlated during the transition period. J. Dairy Sci., 98 (9):6284-6290. http://dx.doi.org/10.3168/jds.2015-9446

Ospina, P. A., J. A. McArt, T. R. Overton, T. Stokol, and D. V. Nydam. 2013. Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. Vet. Clin. N. Am. Food A., 29 (2):387-412. http://dx.doi.org/10.1016/j.cvfa.2013.04.003

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci., 93 (2):546-554. http://dx.doi.org/10.3168/jds.2009-2277

Overton, T. R., J. A. A. McArt, and D. V. Nydam. 2017. A 100-Year Review: Metabolic health indicators and management of dairy cattle. J. Dairy Sci., 100 (12):10398-10417. https://doi.org/10.3168/jds.2017-13054

Panousis, N., G. E. Valergakis, E. Kalaitzakis, N. Siachos, E. Kiosis, and G. Arsenos. 2018. Evaluation of a portable ketometer for on-site monitoring of blood β-hydroxybutyrate concentrations in dairy sheep and goats. Rev. Med. Vet., 169 (10-12):197-202.

Pichler, M., A. Damberger, T. Arnholdt, I. Schwendenwein, J. Gasteiner, M. Drillich, and M. Iwersen. 2014. Evaluation of 2 electronic handheld devices for diagnosis of ketonemia and glycemia in dairy goats. J. Dairy Sci., 97 (12):7538-7546. http://dx.doi.org/10.3168/jds.2014-8198

Pilotto, A., G. Savoini, A. Baldi, G. Invernizzi, C. De Vecchi, G. Theodorou, P. Koutsouli, and I. Politis. 2016. Associations between blood fatty acids, β-hydroxybutyrate, and α-tocopherol in the periparturient period in dairy cows: An observational study. J. Dairy Sci., 99 (10):8121-8126. http://dx.doi.org/10.3168/jds.2016-10938

Radin, L., M. Šimpraga, S. Vince, A. Kostelić, and S. Milinković-Tur. 2015. Metabolic and oxidative status of Saanen goats of different parity during the peripartum period. J. Dairy Res., 82 (4):426-433. https://doi.org/10.1017/S0022029915000552

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci., 86 (4):1201-1217. https://doi.org/10.3168/jds.S0022-0302(03)73704-7

Rook, J. S. 2000. Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. N. Am. Food A., 16 (2):293-317. https://doi.org/10.1016/S0749-0720(15)30107-9

Smith, M. C., and D. M. Sherman. 2009. Goat Medicine. 2 ed. Wiley-Blackwell. Iowa, USA.

Suthar, V. S., A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J. Dairy Sci., 96 (5):2925-2938. http://dx.doi.org/10.3168/jds.2012-6035

Vasava, P. R., R. G. Jani, H. V. Goswami, S. D. Rathwa, and F. B. Tandel. 2016. Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. Vet. World, 9 (8):869-874. https://doi.org/10.14202/vetworld.2016.869-874

Wankhade, P. R., A. Manimaran, A. Kumaresan, S. Jeyakumar, K. P. Ramesha, V. Sejian, D. Rajendran, and M. R. Varghese. 2017. Metabolic and immunological changes in transition dairy cows: A review. Vet. World, 10 (11):1367-1377. https://doi.org/10.14202/vetworld.2017

Yadav, S. N., D. N. Kalila, A. Phukan, G. Mahala, S. Tamuly, T. C. Dutta, A. Saleque, D. Barman, and P. Thakuria. 2015. Prevalence of Sub-Clinical Ketosis in Goats of Guwahati, Assam. J. Anim. Vet. Adv., 14 (13):382-385.

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia. J. Dairy Sci., 103 (1):954-964. https://doi.org/10.3168/jds.2019-17051

Chapter 6 - Endocrine and metabolic responses to glucose, insulin, and adrenocorticotropin (ACTH) infusions in early lactation dairy goats of high and low milk yield

F. Zamuner[*1], A.W.N. Cameron[†], E. K. Carpenter†*, B.J. Leury[*], K. DiGiacomo[*]

[*] Faculty of Veterinary and Agricultural Sciences - The University of Melbourne, Parkville, Victoria 3010, Australia.

[†] Meredith Dairy Pty Ltd, 106 Cameron Rd, Meredith, Victoria 3333, Australia.

6.1 Manuscript 4

Chapter 6 investigated underlying differences in the endocrine and metabolic regulation of energy metabolism in early lactation dairy goats of high and low milk yield. Goats were subjected to three metabolic challenges (glucose, insulin, and ACTH) performed at approximately six weeks postpartum to determine whether there are discernible differences in peripheral tissue responsiveness to the effects of catabolic and anabolic hormones, according to the level of milk production.

This work has been published in the Journal of Dairy Science;

Zamuner, F., A. W. N. Cameron, E. K. Carpenter, B. J. Leury, and K. DiGiacomo. 2020. Endocrine and metabolic responses to glucose, insulin, and adrenocorticotropin (ACTH) infusions in early-lactation dairy goats of high and low milk yield. J. Dairy Sci., 103 (12):12045–12058. https://doi.org/10.3168/jds.2020-18625

Therefore, the paper is included in this chapter as a manuscript.

6.2 Abstract

This experiment aimed to examine endocrine and metabolic responses to glucose, insulin, and adrenocorticotropin (ACTH) infusions, in early lactation dairy goats of different levels of milk production (LMP). Goats were grouped as either high (HY; 4.0 L/d, n = 13), or low milk yield (LY; 2.4 L/d, n = 13). Individual milk yield (L/d) and dry matter intake (DMI; kg/d) were measured daily. Concentration (mM) of glucose, fatty acids, and β-hydroxybutyrate (BHB), % of milk fat and protein, body weight (BW; kg), and body condition score (BCS) were assessed weekly (from 2 to 6 wks postpartum). An intravenous glucose tolerance test (IVGTT), an insulin tolerance test (ITT), and an ACTH stimulation test (AST) were carried out at 43, 44, and 45 ± 0.7 d in milk (DIM), respectively. The HY goats had greater milk yield (+67%), energy-corrected milk (ECM; +70%), dry matter intake (DMI; +28%), the ratio of ECM output to metabolic BW (+67%), and feed efficiency (FE; +25%), but lesser BCS than LY goats (2.4 vs. 2.6). The DMI (%BW) was moderately correlated with ECM (r = 0.70) and negatively correlated with BCS (r = -0.57). At the time of the IVGTT, HY goats had lesser basal insulin and glucose than LY goats. However, results from IVGTT and ITT indicate that the sensitivity of peripheral tissues to insulin was unaffected by LMP. Compared to LY, HY goats had lesser insulin secretion (-52%) and greater insulin clearance rate (+47%) after glucose infusion. The ITT and AST results show that both the growth hormone response to insulin and the cortisol response to ACTH were unaffected by LMP. Also, basal plasma concentrations of GH and cortisol were not correlated with glucose and fatty acids concentrations, or any performance parameters. Collectively, our results suggest that differences between HY and LY goats, concerning milk yield and FE, were probably more closely related to differences in insulin secretion and clearance, than to differences in peripheral tissue responsiveness to the effects of catabolic and anabolic hormones.

Key Words: insulin sensitivity, nutrient partitioning, energy metabolism, milk production

6.3 Introduction

Improved milk production efficiency is of great economic importance in the dairy industry. One of the most important characteristics determining the enhanced potential for milk production is the maternal ability to partition proportionately more of absorbed nutrients towards milk synthesis, and less into body reserves (Baumgard et al., 2017). Therefore, a deeper understanding of how different factors (e.g., environment, genotype, physiological state, nutrition) influence maternal nutrient partitioning is essential for developing optimized strategies that could enhance milk yield, or reduce production costs, without compromising herd health and welfare (Kiplagat et al., 2012; Friggens et al., 2013).

The regulation of nutrient partitioning is orchestrated by complex interactions between a plethora of hormones, among which insulin, growth hormone (GH), and glucocorticoids have a major impact on the regulation of nutrient supply in ruminants (Bell and Bauman, 1997; Baumgard et al., 2017). For instance, in dairy cows, greater milk yield is associated with reduced insulin and elevated GH concentrations (Bell and Bauman, 1997; Veerkamp et al., 2003; Lucy et al., 2009), decreased cortisol responses after ACTH administration (Beerda et al., 2004), intensified mobilization of body lipid stores (Veerkamp et al., 2003), and a greater degree of insulin resistance (De Koster and Opsomer, 2013; Cincović et al., 2018).

Moreover, similar to observations in dairy cows, a recent study in early lactation goats found significant differences in endocrine and metabolic profiles between dairy goats of high and low milk yield (Zamuner et al., 2020). We demonstrated that high-yielding goats have lesser concentrations of insulin and glucose, greater concentrations of GH, fatty acids, and BHB, and greater BW loss than their lower-yielding counterparts.

However, in contrast to the extensive information on endocrine regulation of nutrient partitioning in dairy cows (De Koster and Opsomer, 2013; Baumgard et al., 2017; Cincović et al., 2018), little is known about mechanisms controlling the nutrient supply to the mammary

gland in early lactation goats. Relatively few studies have examined changes in insulin responsiveness in dairy goats with respect to physiological state (Debras et al., 1989), genetic merit for milk production (Cronjé et al., 2000), and energy intake (Schmidely et al., 1999). However, underlying endocrine and metabolic mechanisms determining greater milk yield in goats of the same breed and plane of nutrition are yet to be elucidated.

Therefore, this experiment aimed to investigate metabolic and endocrine responses in early-lactation dairy goats. To determine whether there are discernible differences in metabolic responses according to the level of milk production (LMP), goats of high (HY) and low milk yield (LY) were subjected to 3 metabolic challenges performed at approximately 6 weeks postpartum; namely, an intravenous glucose tolerance test (IVGTT), an intravenous insulin tolerance test (ITT), and an intravenous ACTH stimulation test (AST). We hypothesized that, in comparison to LY, HY goats would exhibit (1) lesser circulating insulin concentrations following bolus glucose infusion, (2) reduced glucose and fatty acids responses to an insulin infusion, and (3) decreased cortisol response to ACTH administration.

6.4 Material & Methods

All experimental procedures were approved by the Faculty of Veterinary and Agricultural Sciences Animal Ethics and Welfare Committee of The University of Melbourne, Australia (No 1714287.1).

6.4.1 Animals, Nutrition, and Husbandry

This experiment was conducted at Meredith Dairy commercial farm (Meredith, Australia, 37°50′S; 144° 04'E). The experiment used 26 clinically healthy Saanen dairy goats, in second or third parity, that kidded in March 2018 (early autumn). Goats were selected based on milk yield from 0 to 10 DIM, and total milk yield in the previous lactation. Then, at the end of wk 1 (11 ± 4.2 d; mean ± SD), the 13 highest and 13 lowest-yielding goats were randomly allocated

to individual stalls in an elevated shed (north-south oriented with the open side facing east), where they were kept under natural lighting and ventilation conditions. The shed had 2 rows of 15 individual stalls (15 m^2) equipped with drinkers, wooden walls and slatted floor. Plastic feeders were attached to the metal-framed gates opening to the center aisle, from which goats could make audio-visual-olfactory contact. Goats were ad libitum fed a TMR once daily at around 0900h and had free access to water during the experimental period (from 2 to 6 wks postpartum). The proportions (DM basis) of the components in the diet were barley (37%), lupins (22%), ryegrass silage (28%), and commercial pellets (11%; 25% CP, 14 MJ of ME/kg, 29% NDF, 65 g of Ca/kg, 16 g of P/kg). The nutrient composition (per kg of DM) of the diet was 32% NDF, 17% CP, 13 MJ of ME, 9.4 g of Ca, and 4.3 g of P.

6.4.2 Measurements

Goats were milked twice-daily and individual milk volume recorded at 0800 and 1600 h, in a 36-sided herringbone system fitted with automatic cup removers and in-line electronic milk meters (MidiLine SG Parlour, DeLaval Inc., Colac, VIC, Australia). Feed offered and refused was weighed daily during the experimental period. Weekly adjustments were made to the amount of TMR offered to maintain a minimum 5% refusal rate, ensuring ad libitum feeding.

Individual milk samples (10 mL) were collected during morning milking at the beginning of wk 2, 3, 4, and 5. Samples were preserved with bronopol (Broad Spectrum Microtabs II, D&F Control Systems, Inc., Norwood, MA, USA) and transported on ice (4° C) to the laboratory at Herd Improvement Co-Operative (HICO, Colac, VIC, Australia) for determination of milk fat, protein, and lactose using a NexGen milk analyzer (Bentley, Chaska, MN, USA). Immediately after milking (and before feeding), blood samples were collected via jugular venipuncture using vacuum tubes (10 mL) coated with lithium-heparin (BD, Plymouth, Devon, UK), immediately placed on ice and centrifuged (1,250 *g*, 4° C) for 12 min within 1 h after collection. Isolated plasma was stored at -20 °C until analysis.

Goat BW and BCS were assessed on the same day of milk and blood testing, and at the beginning of wk 6 (always before feeding and after the morning milking). The BCS was scored by the same person, adopting a 6-point scale method (Villaquiran et al., 2004).

6.4.3 Intravenous Challenges

On the morning of d 42 (wk 6) after an overnight fast, a 14-gauge, 3.25-inch angiocath catheter (BD, Sydney, NSW, Australia) was inserted into the jugular vein approximately 8 cm and then secured to the skin using medical tape and superglue. A 22 cm plastic catheter extension with a Luer lock (Heidelberg extension tubing; B. Braun, Bethlehem, PA, USA) pre-filled with heparinized saline (50 U/L) was secured to the catheter. The catheter was flushed with 8-10 mL heparinized saline (50 U/L) and sealed with a Safesite® (B. Braun). When not frequently in use, the patency of the catheter was maintained by flushing with 8-10 mL heparinized saline (50 U/L) twice daily. During blood sampling procedures, the catheter was flushed with heparinized saline (25 U/L) immediately after every blood sample collection. Food was withheld for 12 h before each challenge. On d 43, a 50% glucose solution was administered intravenously at 0.3 g of glucose/kg of BW, and blood samples were collected at -30, -15, -1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 60, 90, 120, 150, 180, 210, 220, and 240 min relative to glucose infusion (Marett et al., 2015). On d 44, insulin (Actrapid, human insulin (92% homologous to caprine insulin), Novo Nordisk Pharmaceuticals Pty Ltd., Baulkham Hills, NSW, Australia) was administered intravenously at 0.125 IU/kg of BW, and blood samples were collected at -30, -15, -1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 90, 120, 150, 180, 210, 240, 270, and 300 mins relative to insulin infusion (Marett et al., 2017). On d 45, a synthetic analog of ACTH (Synacthen, Novartis, Sydney, NSW, Australia) was administered intravenously at 0.2 IU/kg of BW, and blood samples were collected at -30, -15, -1, 5, 10, 15, 20, 25, 30, 35, 45, 60, 75, 90, 120, 150, 180, and 210 mins relative to ACTH infusion (DiGiacomo et al., 2018). Catheters were flushed with 10 mL of 100 IU/L heparinized

saline at the end of each challenge period. All blood samples were collected into vacuum tubes (10 mL) coated with lithium-heparin (BD), immediately placed on ice, and centrifuged (1,250 × *g*, 4° C) for 12 min within 1h after collection. Isolated plasma was stored at -20° C until analysis.

6.4.4 Laboratory Analysis

The whole-blood concentration of BHB was measured using a hand-held meter (FreeStyle Optium Precision Neo, Abbott Diabetes Care Ltd., Witney, UK) prior to plasma separation. Plasma fatty acids concentrations were measured using a commercially available kit (NEFA-C ACS-ACOD Method [modified as per the methods of Johnson and Peters (1993)], Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma glucose concentrations were measured using a commercially available kit (Infinity Glucose Oxidase Liquid, Thermo-Scientific, Middletown, VA, USA). Plasma insulin concentrations were measured using RIA kit (Porcine Insulin Cat. # PI-12K, Millipore Corporation, Billerica, MA, USA), validated in goats by Maia-Nogueira (2015). Plasma GH concentrations (at -30, -15, -1, 5, 10, 15, 25, 35, 60, 90, 120 and 180 min relative to insulin infusion) and cortisol concentrations (at -30, -15, -1, 15, 30, 45, 60, 120 and 180 min relative to ACTH infusion) were measured using the methods described by Thomas et al. (1990). Assay sensitivity (limit of detection) ranged between 0.4 - 0.5 mU/L for insulin, 0.2 - 0.3 ng/mL for GH, and 0.13 - 0.29 ng/mL for cortisol. Every sample (except for BHB) was assayed in duplicate. Intra- and inter-assay coefficients of variation were < 7.0% and < 3.5% for glucose, < 6.0% and < 3.0% for fatty acids, < 10% and < 4.1% for insulin, < 10% and < 4.3% for GH, and < 10% and < 5.2% for cortisol.

6.4.5 Calculations and Statistical Analyses

At the end of each week, the total DMI (kg/goat), milk yield (L/goat), content (%) of milk fat and protein, and BW were used to calculated calculate average ECM, BW efficiency (BWE, %), DMI as a percentage of BW, and feed efficiency (FE, kg/kg). Average ECM (kg/d) was calculated using the equation ECM = milk (kg) × 0.3246 + (0.1356 × percentage of fat + 0.0704 × percentage of protein)] (Cai et al., 2018), BWE was calculated using the equation BWE = ECM / BW0.75 × 100 (Köck et al., 2018), DMI (% BW) was calculated as DMI (kg/d) / BW (kg), and FE ratio was calculated as ECM (kg/d) / DMI (kg/d). Because goats kidded over the course of 2 wks, DIM (in relation to each day of sampling) was determined for each goat by subtracting the date of kidding from the date of sampling.

Metabolic challenges. Baseline concentrations for the studied analytes were calculated as the mean concentration of the 3 blood samples taken before infusions. Plasma hormones and metabolite responses were analyzed for the area under the curve (AUC) using a linear trapezoidal summation between successive pairs of metabolite concentrations after correcting for baseline concentrations. Peak and nadir concentrations, percentage change from baseline, clearance rates (CR), time (T) to reach; half-life ($T_{1/2}$), peak (T_{peak}), and basal (T_{basal}) concentrations were calculated for each goat and mean values are reported for each specific treatment group. Values were calculated using the following formulas, as previously described by Pires et al. (2007):

CR = [(ln [ta] – ln [tb]) / (tb-ta)] × 100, where [ta] is the concentration of the metabolite at time a (ta) and [tb] is the concentration of metabolite at time b (tb).

$T_{1/2}$ glucose = [ln (2) / $CR_{2\text{-}30}$ glucose] × 100

T_{basal} glucose = [(ln [2 min] – ln [240 min]) / $CR_{2\text{-}30}$ glucose] × 100

T_{basal} insulin = [(ln [2 min] – ln [240 min]) / $CR_{20\text{-}120}$ insulin] × 100

Change = [(peak (or nadir) concentration - basal concentration) / basal concentration] × 100

MINMOD Parameters. Key indices of glucose-insulin dynamics were calculated using MINMOD Millenium software [MINMOD Inc., Pasadena, CA, USA; Boston et al. (2003)]. The parameters and indices that are output by the MINMOD software are:

G_B = basal glucose concentration pre-infusion (m*M*)

I_B = basal insulin concentration pre-infusion (mU/L)

S_G = glucose effectiveness (min^{-1}), which refers to the capacity of glucose to mediate its own uptake

S_I = insulin sensitivity ($(mU/L)^{-1}.min^{-1}$), which refers to the capacity of insulin to promote glucose uptake

AIR = acute insulin response to glucose $(mIU/L)^{-1}.min$), which addresses the adequacy of insulin secretion in response to a glucose bolus

DI = disposition index ($AIR \times S_I$), which represents the ability of the islet cells to secrete insulin

Other indices that are included in the MINMOD Millennium output are derived from the Homeostatic assessment model (HOMA):

IR = insulin resistance (m*M*/mU/L^{-2}), calculated by the equation: $(I_B \times G_B) / 22.5$

BCF = pancreatic β-cell function (mU/m*M*), calculated by the equation: $(20 \times I_B) / (G_B - 3.5)$

Statistical analyses were performed using Minitab software (version 18.1; Minitab Inc, State College, PA, USA). All outcome variables were screened for normality by calculation of kurtosis and skewness and by visual assessment of standardized residuals distribution. Data from repeated-measurements were analyzed using the ANOVA Mixed Effects Model (restricted maximum likelihood (REML) and 2-sided 95% CI), and data from non-repeated-measurements were analyzed using the ANOVA General linear model (2-sided 95% CI) of Minitab. All models included the fixed effect of LMP and the random effect of goat. Parity,

BW, BCS, DIM, and the time of the day when blood was collected, were tested in all models as covariates and retained when significant ($P < 0.05$). Models analyzing data derived from metabolic challenges used values of BW, BCS, and DIM measured at wk 6. Bonferroni method with 95% CI was used for pairwise comparisons. Statistical significance was declared at $P < 0.05$, and values of $P < 0.1$ were considered a trend toward significance. Spearman rho correlation was used to examine relationships between variables of interest. Correlations between variables measured weekly included 4 observations per goat. Correlations between values derived from metabolic challenges and performance data used values measured at wk 6.

Data Presentation. Results for log-transformed variables were reported after back-transformation. Data are presented as means ± SEM unless declared otherwise.

6.5 Results

The HY goats had substantially greater milk yield than LY goats (4.0 vs. 2.4 L/d; P < 0.001). However, for all variables, no interaction effect of LMP × week was detected. Thus, the main effect of LMP on average performance and metabolite concentrations from wk 2 to 5 are depicted in **Table 6.1.**

The HY and LY goats were homogeneous for parity (2.5 vs. 2.5; P = 0.855) and age (34.7 vs. 34.2 months; P = 0.869). However, average DIM (at wk 2) was 4 d greater in HY than LY goats (15 vs. 11 DIM; P = 0.008). The HY goats had greater ECM (+70%; P < 0.001), greater DMI both on a kg (+28%; P < 0.001) and % BW basis (+30%; P < 0.001), greater BWE (+67%; P < 0.001), and greater FE (+25%; P = 0.001) than LY goats. Mean BCS was greater in LY than HY goats (2.6 vs. 2.4; P = 0.016), and although the ADG from wk 2 to 6 tended to be lesser in HY than in LY goats (ADG; -96 vs. -12 g/d; P = 0.086), we did not observe significant changes in BW for both HY and LY goats during the same period (HY = 67.2 vs. 66.8 kg; P > 0.1, and LY = 66.7 vs. 67.2 kg; P > 0.1, for wk 2 vs. wk 6, respectively). The HY goats had

greater milk protein than LY goats (3.3 vs. 3.1 %; P = 0.020), but average milk fat (4.6 ± 0.19%) and lactose (5.2 ± 0.06%) were similar between groups. Plasma fatty acids concentration was greater (+34%; P < 0.001) in HY than LY goats, but glucose and BHB were not affected by LMP.

Correlations between performance variables are listed in **Table 6.2.** Despite ECM being correlated with DMI (r = 0.70), and DMI being correlated with BWE (r = 0.74), BW was not correlated with ECM. We also observed a negative relationship between BCS and DMI, ECM, FE, and BWE. Concentrations of BHB and fatty acids were positively correlated (P < 0.01) to ECM (r = 0.33 and 0.28), FE (r = 0.38 and 0.25), and BWE (r = 0.33 and 0.27), and were negatively correlated (P < 0.01) to BCS (r = -0.32 and -0.22). Fatty acids concentration was correlated with glucose (r = -0.26; P < 0.01) and BHB (r = 0.32; P < 0.01).

Table 6.1. Effects of level of milk production on performance traits and metabolite concentrations in high-yielding (HY; n = 13), and low-yielding (LY; n = 13) commercial dairy goats from 14 to 42 DIM[1]

				P-value		
Item	HY	LY	SED	Level (L)	Week (W)	L×W
Milk						
Yield (L/d)	4.0	2.4	0.18	< 0.001	0.073	0.193
Fat (%)	4.6	4.6	0.19	0.986	0.776	0.839
Protein (%)	3.3	3.1	0.06	0.012	< 0.001	0.056
Lactose (%)	5.2	5.2	0.06	0.473	0.728	0.123
ECM (kg/d)	4.6	2.7	0.20	< 0.001	0.073	0.193
DMI (kg/d)	2.3	1.8	0.14	< 0.001	1.016	0.984
DMI (% BW)	3.5	2.7	0.20	< 0.001	0.012	0.970
FE (kg/kg)	2.0	1.6	0.12	0.001	< 0.001	0.685
BW (kg)	66	67	2.9	0.688	0.057	0.343
BCS	2.4	2.6	0.08	0.016	0.075	0.974
BWE (%)	20	12	0.9	< 0.001	0.017	0.079
ADG (g/d)	-96	-12	46.5	0.086	< 0.001	0.194
Fatty acids (mM)	0.5	0.4	0.05	0.021	< 0.001	0.199
Glucose (mM)	3.4	3.5	0.14	0.532	0.295	0.486
BHB (mM)	0.4	0.4	0.04	0.168	0.076	0.096

[1]Values are restricted maximum likelihood (REML) means

ECM = energy-corrected milk [milk (kg) × (0.3246 + (0.1356 × fat (%) + 0.0704 × protein (%)) (Cai et al., 2018)], DMI = dry matter intake, FE = feed efficiency rate [ECM (kg/d) / DMI (kg/d)], BW = body weight, BCS = body condition score, BWE = bodyweight efficiency [ECM (kg/d) / BW0.75 (kg) × 100 (Köck et al., 2018)], ADG = average daily gain, BHB = β-hydroxybutyrate, SED = standard error of differences

Table 6.2. Spearman rho correlations between performance traits in commercial dairy goats from 14 to 42 DIM

	ECM	DMI	FE	BWE	BW
DMI (%BW)	0.70***				
FE (kg/kg)	0.61***	ns			
BWE (%)	0.95***	0.74***	0.65***		
BW (kg)	ns	-0.17†	-0.19†	-0.18†	
BCS	-0.62***	-0.57***	-0.33**	-0.66***	ns

ECM = energy-corrected milk [milk (kg) × (0.3246 + (0.1356 × fat (%) + 0.0704 × protein (%)) (Cai et al., 2018)], DMI = dry matter intake, FE = feed efficiency rate [ECM (kg/d) / DMI (kg/d)], BW = body weight, BCS = body condition score, BWE = bodyweight efficiency [ECM (kg/d) / $BW^{0.75}$ (kg) × 100 (Köck et al., 2018)]
*** P < 0.001; ** P < 0.01; * P < 0.05; † P < 0.1; ns P > 0.1Intravenous Glucose

6.5.1 Intravenous Glucose Tolerance Test (IVGTT)

Response curves for insulin, glucose, and fatty acids concentrations after a glucose infusion in HY and LY goats are presented in **Figure 6.1**. Results of the comparisons of analyzed IVGTT variables between HY and LY goats are given in **Table 6.3**. Before the IVGTT, LY had greater basal glucose (+13%; $P = 0.046$) and insulin (+130%; $P = 0.008$) than HY goats, but there was no difference in basal fatty acids. We observed an LMP × time interaction ($P < 0.001$) for both glucose and insulin concentration. Glucose concentration was greater ($P < 0.05$) in LY than in HY goats from 2 to 40 min post-infusion. Insulin was greater ($P < 0.05$) in LY than in HY goats from 2 to 10 and from 50 to 240 min post-infusion.

Table 6.3. Plasma glucose, insulin, and fatty acids responses to a glucose infusion (0.3 g/kg of BW) performed in high (HY; 4.0 L/d, n = 13) and low yielding (LY; 2.4 L/d, n = 13) dairy goats at ~ 43 DIM[1]

Item	HY	LY	SED	P-value
Glucose				
Basal (mM)	3.0	3.4	0.18	0.046
Peak (mM)	15.6	17.2	0.66	0.009
Nadir (mM)	2.1	2.3	0.19	0.250
Change[2] (%)	418	426	27.0	0.775
Recovery[3] (mM)	2.9	3.3	0.19	0.013
$T_{1/2}$[4] (min)	30	32	1.7	0.385
T_{basal}[5] (min)	80	84	4.2	0.567
CR $_{2-30}$ (%/min)	2.4	2.3	0.16	0.598
CR $_{30-60}$ (%/min)	2.1	2.0	0.24	0.859
AUC_{0-60} (mM.min)	316	361	20.4	0.012
AUC_{0-120} (mM.min)	324	394	45.5	0.068
Insulin				
Basal (mU/L)	5.6	12.9	2.51	0.008
Peak (mU/L)	78.1	115.2	24.7	0.097
Nadir (mU/L)	3.3	7.9	1.87	0.016
Change[2] (%)	4695	1354	2387	0.174
Recovery[3] (mU/L)	4.3	10.3	1.85	0.004
T_{peak}[6] (min)	19	20	1.4	0.782
T_{basal}[5] (min)	117	119	6.5	0.842
CR $_{30-60}$ (%/min)	2.8	1.9	0.43	0.042
AUC_{0-60} (mU/L.min)	3453	4974	667.0	0.120
AUC_{0-120} (mU/L.min)	3919	6203	819.9	0.061
Fatty acids				
Basal (mM)	0.6	0.5	0.15	0.376
Peak (mM)	1.2	0.9	0.20	0.188
Nadir (mM)	0.3	0.2	0.04	0.088
Change[2] (%)	127	102	25.2	0.283
Recovery[3] (mM)	1.0	0.7	0.19	0.137
AUC_{0-60} (mM.min)	-6.3	-3.9	2.9	0.568
AUC_{0-120} (mM.min)	-11.7	-8.2	7.0	0.728

[1]Values are restricted maximum likelihood (REML) means

[2]Maximum increase relative to baseline concentration

[3]Concentration at time 240 min relative to glucose infusion

[4]Time to reach half-life concentration after the glucose infusion

[5]Time to reach basal concentration after the glucose infusion
[6]Time to reach peak concentration after the glucose infusion
[7]Time to reach nadir concentration after the glucose infusion
CR = clearance rate, AUC = area under the response curve, SED = standard error of differences

Plasma glucose concentration peaked immediately following infusion (2 min) in both groups. The HY goats had reduced peak glucose (-9%; $P = 0.009$), lesser glucose concentration at 240 min (recovery) (-14%; $P = 0.013$), lesser glucose AUC_{0-60} (-12%; $P = 0.012$), lesser nadir insulin (-58%; $P = 0.016$), greater insulin CR from 30-60 min (+47%; $P = 0.005$), lesser insulin concentration at 240 min (-140%; $P = 0.004$), and tended to have lesser peak insulin (-32%; $P = 0.097$), lesser glucose AUC_{0-120} (-18%; $P = 0.068$) and lesser insulin AUC_{0-120} (-37%; $P = 0.061$) than LY goats. The glucose $T_{1/2}$, was similar ($P > 0.1$) between groups (31 ± 1.2 min), as were glucose T_{basal} (82 ± 3.7 min), insulin T_{peak} (20 ± 1.1 min) and insulin T_{basal} (118 ± 4.4 min). There were no significant differences between HY and LY goats for fatty acids data derived from IVGTT.

The indices derived from the MINMOD software are presented in **Table 6.4**. Basal plasma insulin concentrations were 2.4-fold greater in LY ($P = 0.008$) than in HY goats, but there was no difference for basal glucose. Compared to HY goats, LY goats had 1.5-fold greater AIR ($P = 0.047$), 3.3-fold greater IR ($P = 0.01$), and 7.5-fold greater BCF ($P = 0.006$), but there were no differences in S_G, S_I, and DI between groups.

Table 6.4. Glucose and insulin MINMOD[1] parameters derived from intravenous glucose tolerance tests performed in high (HY; 4.0 L/d, n = 13) and low yielding (LY; 2.4 L/d, n = 13) dairy goats at ~ 43 DIM

Item	HY	LY	SED	P-value
G_B[2] (mM)	2.8 (2.4, 3.4)	3.0 (2.8, 3.3)	-	0.443
I_B (mU/L)	5.3	12.5	2.45	0.008
S_G[2] (mM/min)	0.02 (0.01, 0.02)	0.01 (0.01, 0.02)	-	0.229
S_I (mU/L. min^{-1})	3.4	4.4	1.51	0.505
AIR (μU/L.min^{-1})	352	536	87.5	0.047
DI	2903	1578	1501.2	0.409
IR[2] (mM.mU/L^{-2})	0.4 (0.2, 0.9)	1.3 (0.8, 2.2)	-	0.010
BCF[2] (mU/mM)	83 (33, 213)	619 (208, 1847)	-	0.006

[1]MINMOD Millennium, a windows-based software developed to mathematically describe the dynamic relationship between insulin and glucose during an intravenous glucose tolerance test (Boston et al., 2003). Values are restricted maximum likelihood (REML) means.

[2]Back-transformed values with 95% CI presented in parentheses (lower limit, upper limit)

GB = basal glucose, IB = basal insulin, SG = glucose effectiveness, SI = insulin sensitivity, AIR = acute insulin response to glucose, DI = disposition index, IR = insulin resistance, BCF = pancreatic β-cell function, SED = standard error of differences.

Correlations between IVGTT and MINMOD parameters and goat performance data are reported in **Table 6.5**. There were no significant associations between values of glucose $T_{1/2}$, glucose $CR_{2\text{-}20}$, or any IVGTT derived value for fatty acids and performance parameters. Basal insulin was negatively correlated with DMI, ECM, and BWE. Although basal glucose was not correlated with any performance parameter, glucose $AUC_{0\text{-}120}$ was negatively associated with ECM, FE, and BWE. Greater insulin CR was associated with greater ECM, FE, and BWE. Greater S_I, DI, and BCF were associated with greater ECM, while greater AIR and IR were associated with reduced ECM, DMI, and BWE.

Table 6.5. Spearman rho correlations between performance traits and glucose and insulin parameters derived from an intravenous glucose tolerance test performed in commercial dairy goats at ~ 43 DIM

Item	ECM (kg/d)	DMI (%BW)	FE (kg/kg)	BWE (%)	BW (kg)	BCS
Basal						
Glucose (mM)	ns	ns	ns	ns	ns	0.34†
Insulin (mM)	-0.54**	-0.55**	ns	-0.54**	ns	0.59**
Change[1]						
Glucose (%)	ns	ns	ns	ns	ns	ns
Insulin (%)	ns	ns	ns	ns	ns	ns
AUC_{0-120}						
Glucose (mM.min)	-0.44*	ns	-0.52**	-0.53**	0.40*	ns
Insulin (mM.min)	-0.54**	-0.62**	ns	-0.56**	ns	0.66***
CR_{30-60}						
Glucose (%/min)	ns	ns	0.35†	ns	-0.36†	ns
Insulin (%/min)	ns	0.36†	0.37†	0.48*	-0.65**	-0.41*
Glucose/Insulin ratio	0.51*	0.56**	ns	0.52**	ns	-0.61**
Fatty acids/Insulin ratio	0.35†	0.35†	ns	0.36†	ns	-0.51**
MINMOD						
S_G (mM/min)	0.49*	ns	0.33†	0.54**	ns	-0.42*
S_I (mU/L.min^{-1})	ns	0.33†	ns	ns	ns	ns
AIR (mU/L.min^{-1})	-0.41*	-0.51**	ns	-0.37†	ns	0.54**
DI	0.45*	ns	0.59**	0.52**	ns	ns
IR (mM.mU/L^{-2})	-0.44*	-0.48*	ns	-0.45*	ns	0.55**
BCF (mU/mM)	0.41*	0.42*	ns	0.44*	ns	-0.42*

[1]Maximum increase relative to the basal concentration

MINMOD = a windows-based software developed to mathematically describe the dynamic relationship between insulin and glucose during an intravenous glucose tolerance test (Boston et al., 2003). ECM = energy-corrected milk, DMI = dry matter intake, FE = feed efficiency rate (ECM (kg/d) / DMI (kg/d)), BWE = bodyweight efficiency (ECM (kg/d) / $BW^{0.75}$ (kg) × 100), BW = body weight, BCS = body condition score, AUC = area under the curve, CR = clearance rate, G_B = basal glucose, I_B = basal insulin, S_G = glucose effectiveness, S_I = insulin sensitivity, AIR = acute insulin response to glucose, DI = disposition index, IR = insulin resistance, BCF = pancreatic β-cell function.

*** P < 0.001; ** P < 0.01; * P < 0.05; † P < 0.1; ns P > 0.1

6.5.2 Insulin tolerance test (ITT)

Response curves for glucose, GH, and fatty acids concentrations after an insulin infusion in HY and LY goats are presented in **Figure 6.2**. Results of the comparisons of analyzed ITT variables between HY and LY goats are given in **Table 6.6**. There were no differences in basal glucose, GH, or fatty acids concentrations between groups. There were no differences between HY and LY goats for glucose, GH, and fatty acids concentrations throughout the ITT or for ITT derived data. Glucose concentration began to decrease immediately after the administration of insulin, reaching 50% of basal concentration ($T_{1/2}$) in 26 ± 2.0 min post-infusion. Glucose concentration started to increase after 30 min, returning to near baseline values (~ 3.5 m*M)* within 120 min post-infusion. Fatty acids increased sharply from 30 to 60 min, reaching a peak of 0.6 ± 0.05 m*M* before decreasing to a plateau of ~ 0.4 m*M* at around 90 min post-infusion. Plasma GH concentrations fluctuated over time, and there was a large degree of variation in the GH $AUC_{0\text{-}120}$ in both groups. However, the relative increase in GH concentration was almost 4-fold greater (P = 0.021) in HY compared to LY goats. Basal GH was positively correlated with basal glucose (r = 0.42; P = 0.033), and GH_{peak} was positively correlated with DMI (r = 0.47; P = 0.016), but basal GH was not correlated with fatty acids or any other performance parameters.

Table 6.6. Plasma glucose, growth hormone and fatty acids responses to insulin infusion (0.125 IU/kg of BW) performed in high (HY; 4.0 L/d, n = 13) and low yielding (LY; 2.4 L/d, n = 13) dairy goats at ~ 44 DIM[1]

Item	HY	LY	SED	P-value
Growth Hormone				
Basal (ng/mL)	1.4	1.3	0.29	0.707
Peak (ng/mL)	3.6	3.5	0.65	0.900
Nadir (ng/mL)	0.5	0.4	0.20	0.717
Change[2] (%)	400	112	116.7	0.021
T_{peak}[3] (min)	69	98	18.5	0.123
AUC_{0-120} (ng/mL.min)	33.2	55.7	39.12	0.571
Glucose				
Basal (mM)	4.1	3.8	0.26	0.515
Peak (mM)	4.5	4.3	0.39	0.729
Nadir (mM)	1.6	1.4	0.19	0.580
Change[2] (%)	-62	-63	3.4	0.818
$T_{1/2}$[4] (min)	27	26	3.9	0.866
CR_{5-30} (%/min)	3.0	2.9	0.27	0.785
AUC_{0-120} (mM.min)	-146.4	-121.0	18.71	0.232
Fatty acids				
Basal (mM)	0.3	0.3	0.05	0.723
Peak (mM)	0.7	0.6	0.07	0.647
Nadir (mM)	0.2	0.2	0.03	0.712
Change[2] (%)	147	140	41.1	0.882
T_{peak}[3] (min)	56	67	12.4	0.398
AUC_{0-120} (mM.min)	7.0	7.3	4.12	0.947

[1]Values are restricted maximum likelihood (REML) means

[2]Maximum increase or decrease relative to baseline concentration

[3]Time to reach peak concentration after the insulin infusion

[4]Time to reach half-life concentration after the insulin infusion

CR = clearance rate, AUC = area under the response curve, SED = standard error of differences

6.5.3 Adrenocorticotropin (ACTH) Stimulation Test (AST)

The cortisol response curve following the ACTH infusion is presented in **Figure 6.3 a**, and the AUCs for glucose, fatty acids, and cortisol responses at 60, 120, and 180 min after ACTH infusion are presented in **Figure 6.3 b**. Results of the comparisons of analyzed AST parameters between HY and LY goats are given in **Table 6.7.** Basal cortisol was highly variable within LMP (range; 3.0 - 17.7 and 1.8 - 20.4 ng/mL, for HY and LY goats, respectively). There were no differences in basal cortisol, glucose, or fatty acids concentrations between groups (**Table 6.7**). Also, there was no effect of LMP or LMP × time interaction on cortisol response (**Figure 6.3 a**) or on cortisol-stimulated responses on glucose and fatty acids concentrations after ACTH infusion (**Figure 6.3 b**). In both groups, ACTH infusion provoked a 10-fold increase in cortisol concentration within 15 min. Cortisol concentration remained elevated until 60 min, when it started to decrease gradually, returning to near baseline values 180 min post-infusion. The ACTH infusion also caused an increase in glucose and fatty acids concentrations to a peak of 4.4 ± 0.14 and 0.6 ± 0.06 mM, respectively. Both glucose and fatty acid concentrations fluctuated over time, reaching a maximum increase of 13% and 100% for glucose and fatty acid, respectively, but returned to values similar to basal concentrations at the end of the AST (210 min). Basal cortisol was not correlated with basal glucose, fatty acids, or any performance parameters. Cortisol Tpeak was positively correlated with FE ($r = 0.42$; $P = 0.034$).

6.6 Discussion

The central aim of our experiment was to determine whether differential productivity in dairy goats is related to differences in some aspects of the regulation of energy metabolism. Our experiment employed 3 metabolic challenges (glucose, insulin, and ACTH) to examine interrelationships between hormones and metabolite concentration and production performance in early lactation dairy goats.

Table 6.7. Plasma cortisol, glucose, and fatty acids responses to an adrenocorticotropic hormone (ACTH) infusion (0.2 IU/kg of BW) performed in high (HY; 4.0 L/d, n = 13) and low yielding (LY; 2.4 L/d, n = 13) dairy goats at ~ 45 DIM[1]

Item	HY	LY	SED	P-value
Cortisol				
Basal (ng/mL)	6.8	8.0	2.1	0.557
Peak (ng/mL)	103.4	102.5	10.1	0.933
Nadir (ng/mL)	12.2	14.2	2.3	0.464
Change[2] (%)	2048	1871	602.0	0.755
T_{peak}[3] (min)	48	42	5.9	0.237
Glucose				
Basal (mM)	3.8	4.0	0.30	0.357
Peak (mM)	4.3	4.5	0.28	0.618
Nadir (mM)	3.2	3.4	0.19	0.231
Change[2] (%)	16	12	3.3	0.250
Fatty acids				
Basal (mM)	0.3	0.3	0.08	0.723
Peak (mM)	0.7	0.6	0.11	0.662
Nadir (mM)	0.2	0.2	0.05	0.808
Change[2] (%)	136	128	42.9	0.848

[1]Values are restricted maximum likelihood (REML) means
[2]Maximum increase relative to baseline concentration
[3]Time to reach peak concentration after the ACTH infusion
SED = standard error of differences

Our results demonstrate that along with the increased milk yield, HY goats also had significantly greater DMI and, most importantly, improved FE. However, because FE is described as kg of DMI divided by kg of milk produced per day, FE can be overestimated when body reserves are being mobilized to sustain increased milk production (Bach et al., 2019). According to Dunshea et al. (1990), fatty acids concentration provides a better estimate of body-fat loss in early lactation than BW variations alone because, as lactation advances, goats increase DMI, thereby increasing gut fill such that total BW might not change. Although we did not observe significant differences in ADG or BW between groups, the inferior BCS and

greater fatty acids concentration in HY goats suggests greater lipid mobilization in HY than LY goats, which might have contributed (to some extent) to the improved FE and BWE observed in HY goats.

The ability to direct proportionally more of absorbed nutrients towards milk synthesis, and less to body reserves, is one of the most critical mechanisms determining improved milk yield (Baumgard et al., 2017; Bach et al., 2019). Insulin action and glucose transporters (GLUT) play a pivotal role in the regulation of nutrient partitioning during lactation (Bell and Bauman, 1997). For instance, the expression GLUT-1 (non-insulin-dependent), which is the predominate glucose carrier in the mammary gland of lactating cows and rodents (Zhao and Keating, 2007), is substantially increased (4-fold) in mammary epithelial cells of early compared to late lactation goats (Na et al., 2009). Whereas, the expression of GLUT-4 (insulin-dependent), which virtually disappears in mammary epithelial cells of lactating cows (Zhao and Keating, 2007), is also reportedly downregulated in skeletal muscle of lactating goats (Balage et al., 1997). Therefore, a state of decreased pancreatic insulin secretion or reduced sensitivity of peripheral tissues to the metabolic actions of insulin would result in reduced glucose utilization by extra-mammary tissues, thereby favoring substrate availability for milk synthesis (Jaakson et al., 2013; Cincović et al., 2018). Based on these principles, we hypothesized that HY goats would exhibit a greater degree of insulin resistance than LY goats.

According to De Koster and Opsomer (2013), based on data from an IVGTT, insulin resistance can be identified when glucose CR is low, glucose AUC is high, and the glucose $T_{½}$ and T_{base} are high. Interestingly, even though HY goats had significantly lesser basal glucose and insulin concentration than LY goats, values of glucose for CR, $T_{½}$, T_{base}, and maximum change (%) from basal concentrations were comparable between groups. These results were further supported by results from MINMOD showing that, despite the observed positive association between ECM and S_G ($r = 0.49$) and between ECM and DI ($r = 0.45$), there were

no measurable differences in S_I, S_G, and DI between groups, which is inconsistent with our first hypothesis that HY goats would have a lesser glucose-induced insulin secretion during the IVGTT. Moreover, the lesser calculated IR (-69%; $P = 0.010$), and lesser glucose AUC_{0-60} (-12%; $P = 0.012$) in HY goats are indicative of a less insulin-resistant state in this group, which contrasts with what has generally been accepted in high-yielding dairy cows (De Koster and Opsomer, 2013; Baumgard et al., 2017; Cincović et al., 2018).

However, it should be emphasized that glucose disappearance during the IVGTT is the result of both glucose uptake by the insulin-sensitive tissues (e.g., muscle and adipose tissue), and by insulin-insensitive tissues (e.g., mammary gland, brain, kidneys, immune cells) (De Koster and Opsomer, 2013; Kvidera et al., 2017). Thus, these apparently conflicting results may be attributable to the greater demand for glucose placed by the mammary gland of HY compared to LY goats, which would consequently influence the glucose dynamics during the IVGTT. Moreover, a proper interpretation of an IVGTT presupposes similar insulin secretion between animals (De Koster and Opsomer, 2013), which does not seem to be the case in our study given the observed differences in basal insulin, AIR, and BCF. Hence, based on the IVGTT results only, no conclusions could be drawn on whether HY and LY goats exhibit a different degree of insulin resistance.

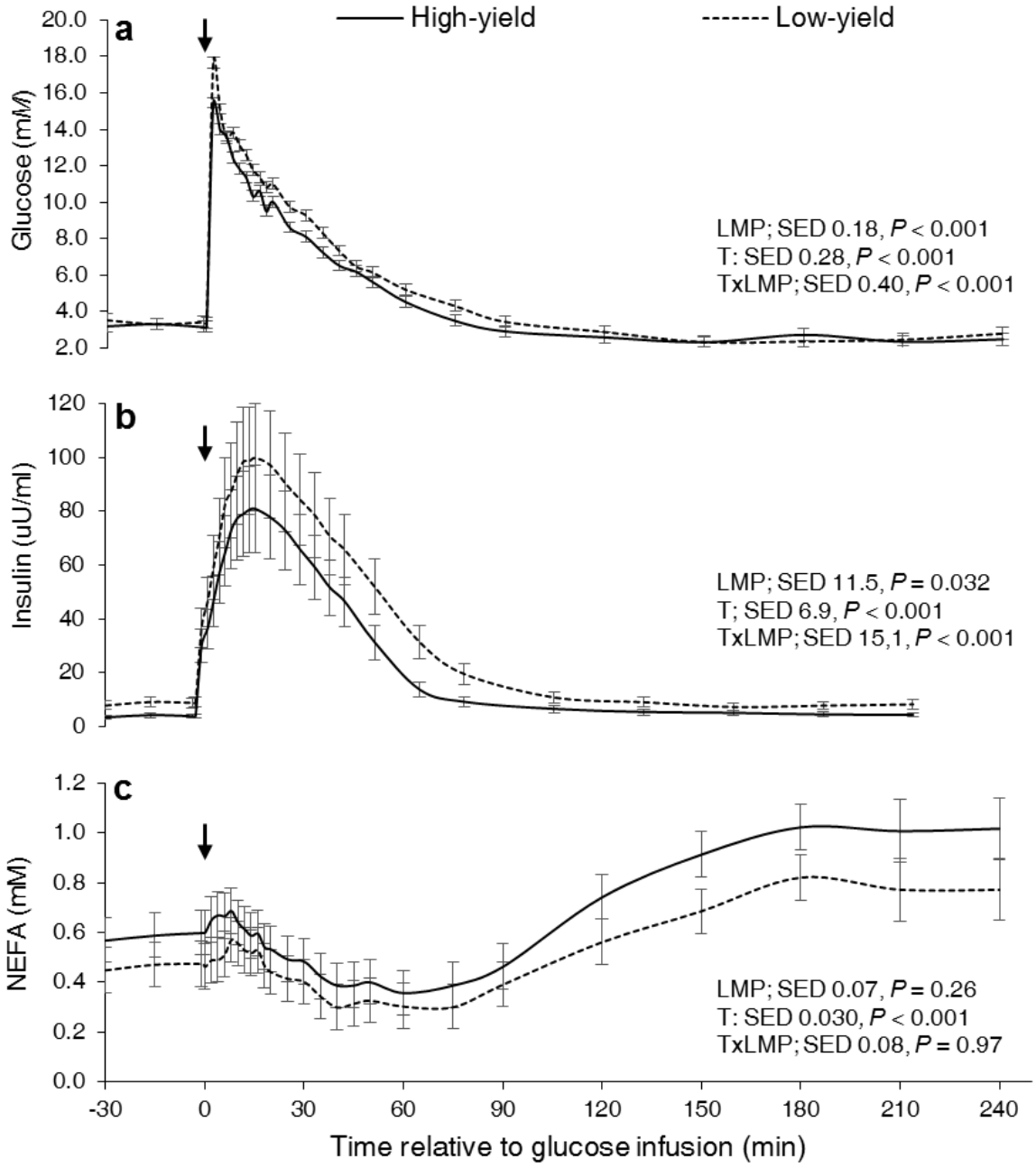

Figure 6.1. Responses in (a) plasma glucose, (b) insulin, and (c) fatty acids concentrations to a glucose infusion (0.3 g 50% glucose/kg of BW) at time zero (🡻) in high-yielding (4.0 L/d, n = 13) and low-yielding (2.4 L/d, n = 13) dairy goats at 43 ± 0.7 DIM. Values are restricted maximum likelihood (REML) means, and error bars represent standard errors of means. LMP = level of milk production, T = time of sampling, LMP × T = joint effect of LMP and T, SED = pooled standard errors of differences.

The ITT is commonly used to evaluate insulin sensitivity of insulin-responsive tissues (Wilcox, 2005; Davis et al., 2017; Marett et al., 2017), but is also useful for assessing GH responses to insulin-induced hypoglycemia (Marett et al., 2014). In this study, results from the ITT indicate similar responses to insulin in both groups, which is inconsistent with our second hypothesis that HY goats would exhibit lesser glucose and fatty acids responses during the ITT. These results suggest that the lesser milk production in LY goats may be more closely related to greater insulin secretion than to differences in whole-body sensitivity to insulin between LY and HY goats. This is supported by the negative association between EMC, and basal insulin, insulin $AUC_{0\text{-}120}$, and AIR (r = -0.41 to -0.54), and is accordance with findings of Hammon et al. (2007), in which milk yield from crossbred dairy heifers (at 30 DIM) was negatively correlated with both basal insulin (r = -0.30) and insulin AUC (r = -0.35). Similarly, Shingu et al. (2002) demonstrated that dairy cows have markedly lesser glucose-induced insulin secretion than beef cows, regardless of the stage of lactation. Present and previous results endorse an inverse relationship between milk yield and insulin secretion, regardless of whether the increased milk yield is due to greater genetic merit between or within breeds.

Previous work in humans (Wilcox, 2005; Keane and Newsholme, 2014), and in dairy cows (De Koster and Opsomer, 2013; Cincović et al., 2018) have reported a negative association between fatty acids concentration and pancreatic insulin secretion. In the present study, differences in insulin secretion could not be explained by differences in fatty acids concentration because basal fatty acids concentration was comparable between HY and LY goats on the day of IVGTT, ITT, and AST. However, Keane and Newsholme (2014) stated that prolonged exposure to elevated concentrations of fatty acids might chronically decrease glucose-stimulated insulin secretion in rats and human pancreatic islets. Considering that HY goats had greater plasma fatty acids concentration during the weeks preceding the metabolic challenges, the lesser BCF and AIR in HY goats might reflect long-term effects of increased

fatty acids concentration on pancreatic insulin secretory capacity. Nevertheless, the reasons for the present findings remain speculative.

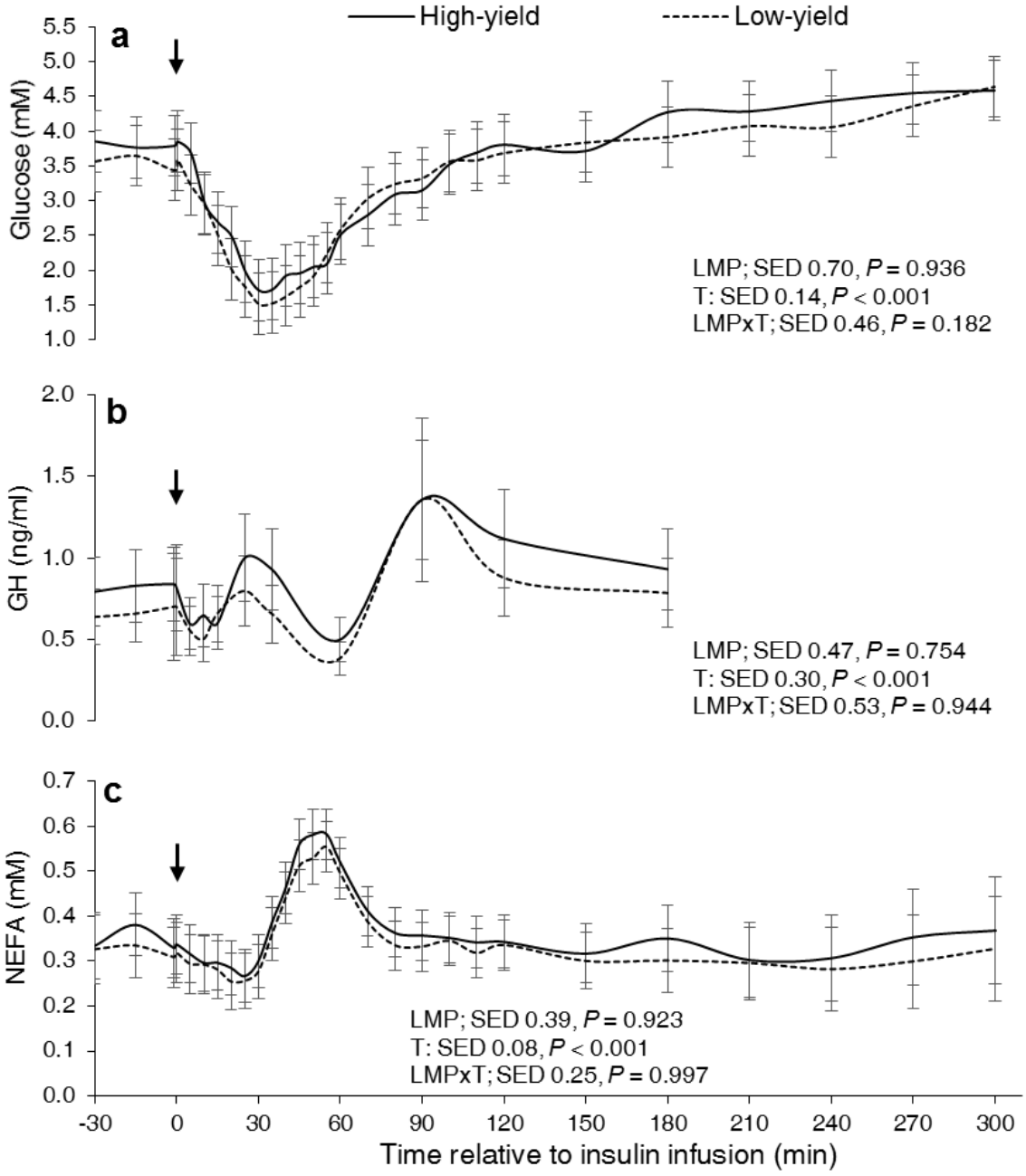

Figure 6.2. Responses in (a) plasma glucose, (b) growth hormone (GH), and (c) fatty acids concentrations to an insulin infusion (0.125 IU/kg of BW)) at time zero (🡻), in high-yielding (4.0 L/d, n = 13) and low-yielding (2.4 L/d, n = 13) dairy goats at 45 ± 0.7 DIM. Values are restricted maximum likelihood (REML) means, and error bars represent standard errors of means. LMP = level of milk production, T = time of sampling, LMP × T = joint effect of LMP and T, SED = pooled standard errors of differences.

Data from the IVGTT show that in addition to the lesser AIR, insulin CR in HY was 1.5-fold greater than in LY goats, which indicates that the lesser insulin concentration in HY goats could also be a consequence of the increased insulin degradation in this group. According to Lacasse and Prosser (2003), variations in milk yield are usually accompanied by a similar variation in mammary and hepatic blood flow in goats. Therefore, it is plausible that increased milk yield might have contributed to greater insulin CR in HY goats by accelerating insulin clearance by the liver (Reynolds et al., 2003).

Further, counter-regulatory responses to insulin-induced hypoglycemia (e.g., glucagon, cortisol, or GH secretion) may affect metabolic responses to an ITT (Malaisse, 2015; Davis et al., 2017; Marett et al., 2017). For instance, GH has insulin-antagonistic effects both in hepatic and peripheral tissues, stimulating lipolysis and gluconeogenesis, and inhibiting lipogenesis and glucose uptake, which results in increased nutrient supply to the mammary gland (Bell and Bauman, 1997). Not surprisingly, in dairy cows, increased milk production is associated with elevated basal GH, greater insulin resistance, (Bell and Bauman, 1997; Lucy et al., 2009), and treatment with exogenous GH has been shown to increase milk yield in dairy cows and goats (Chadio et al., 2000; Baldi et al., 2002; Baumgard et al., 2017). Moreover, Marett et al. (2019) demonstrated that GH responses to insulin-induced hypoglycemia tend to be greater in high- than low-yielding cows, and a recent study in male goats has shown that insulin infusion increased plasma GH concentration up to 3-fold, shortly after infusion (Nishihara et al., 2017). Given the lesser insulin concentration and the greater milk yield in HY goats, we expected that GH responses to an ITT would be greater in this group. This hypothesis was supported by the greater increase in GH concentration after the insulin infusion in HY compared to LY goats (5.0 vs. 2.1-fold; $P = 0.021$, respectively). However, there was no other evident effect of LMP on GH response to the ITT.

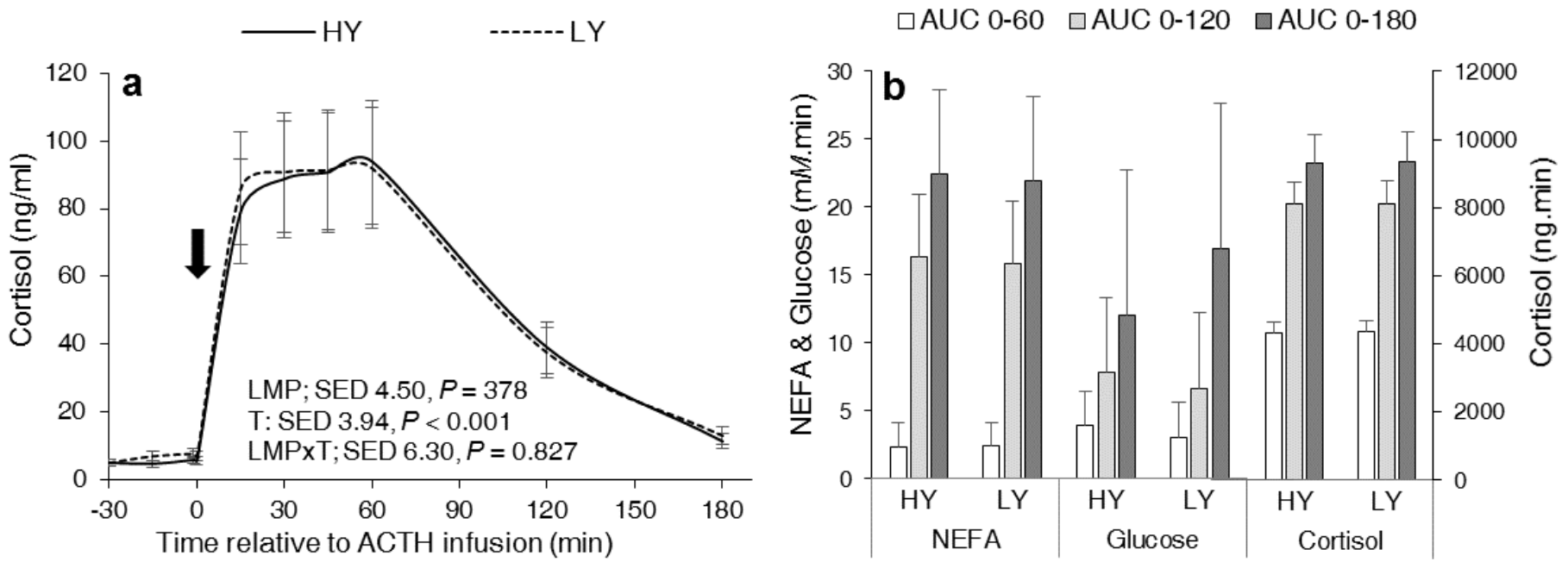

Figure 6.3. Responses in (a) plasma cortisol to an adrenocorticotropic hormone (ACTH) infusion (0.2 IU/kg of BW) at time zero (⬇), and (b) area under the curve (AUC) for fatty acids, glucose and cortisol at 60, 120 and 180 min post-infusion in high-yielding (HY; 4.0 L/d, n = 13) and low-yielding (LY; 2.4 L/d, n = 13) dairy goats at 46 ± 0.7 DIM. Values are restricted maximum likelihood (REML) means, and error bars represent standard errors of means. The P-values for AUC 0-60min, 0-120 min and 0-180min were: fatty acids; 0.98, 0.93, and 0.95, glucose; 0.82, 0.88, and 0.75, and cortisol; 0.93, 0.99, and 0.96. LMP = level of milk production, T = time of sampling, LMP × T = joint effect of LMP and T, SED = pooled standard errors of differences

In a recent study (Zamuner et al., 2020), we observed that greater milk yield (2-fold) in early lactation (wk 0 to 3) was associated with greater GH/insulin ratio (3-fold) and greater plasma levels of fatty acids and BHB (2-fold) during the same period, and to a greater BW loss from wk 0 to 6 (7.0 vs. 1.0 % of BW loss; $P < 0.01$, for HY vs. LY, data not shown), which suggests greater catabolic state and degree of negative energy balance in HY compared to LY goats. Comparatively, the present data show that HY had greater milk yield (1.7-fold) and slightly greater plasma fatty acids concentration (1.3-fold) during the weeks preceding the challenges. However, at the time of the ITT (wk 6), basal plasma fatty acids were comparable in HY and LY, suggesting similar energy balance across groups. The literature shows that, in lactating dairy cows, both basal GH and GH secretory responses increase with increasing merit for milk yield (Shingu et al., 2002; Lucy et al., 2009) and increasing negative energy balance (Bradford and Allen, 2008), but decrease with increasing DIM (Shingu et al., 2002; Marett et al., 2014). Therefore, the fact that in the present study goats were tested later in lactation [wk 6 compared to wk 3 of Zamuner et al. (2020)] may have hindered our ability to detect differences in GH responses between HY and LY goats.

In addition, recent research in dairy cows suggests that decreased ACTH-induced cortisol responses are associated with greater FE (DiGiacomo et al., 2018), and with greater milk yield (Beerda et al., 2004; Gross et al., 2018). Thus, we hypothesized that, compared to LY goats, HY goats would have a lesser cortisol response to AST. However, in the present experiment, cortisol responses to ACTH administration (e.g., AUC, peak concentration, the time of peak, decline pattern) was not related to LMP. These results substantiate previous findings in dairy goats, in which ACTH administration (0.6 - 2.5 IU/kg BW) were not related to changes in milk yield (Shamay et al., 2000; Bomfim et al., 2018). Innate differences in metabolic responsiveness to the catabolic signals of cortisol may affect nutrient partitioning, thereby influencing FE (DiGiacomo et al., 2018). Nevertheless, despite the positive relationship

between Tpeak cortisol and FE (r = 0.42), we found no measurable difference in fatty acids and glucose responses to AST according to LMP, indicating that milk yield was not associated with differential effects of cortisol on carbohydrate and lipid metabolism in HY and LY goats.

6.7 Conclusions

The current data show that LY goats exhibit significantly greater pancreatic insulin secretion than HY goats. On the other hand, there was no evident difference between groups in peripheral tissue responses to glucose, insulin, or ACTH infusion. Hence, the presented results suggest that production efficiency, in early lactation dairy goats, is more closely related to differences in the regulation of insulin release and degradation than to differences in peripheral tissue responsiveness to the effects of catabolic and anabolic hormones.

The present findings provide insights into the mechanisms that contribute to improved milk yield in dairy goats. However, similar investigations should be carried out at varying stages of lactation in order to confirm mechanisms of nutrient partitioning responsible for increased milk yield throughout lactation.

6.8 Acknowledgments

Fernanda Zamuner received a Ph.D. scholarship financed by the Australian Government Research Training Program (Melbourne, VIC, Australia). The authors gratefully acknowledge Meredith Dairy Ltd. (Meredith, VIC, Australia) personnel for their assistance throughout this study. The authors declare there was no conflict of interest during the entire process of this study.

6.9 References

Bach, A., M. Terré, and M. Vidal. 2019. Symposium review: Decomposing efficiency of milk production and maximizing profit. J. Dairy Sci., 103 (6):5709–5725. https://doi.org/10.3168/jds.2019-17304

Balage, M., J. F. Hocquette, B. Graulet, P. Ferré, and J. Grizard. 1997. Skeletal muscle glucose transporter (GLUT-4) protein is decreased in lactating goats. Anim. Sci. J., 65 (2):257-265. https://doi.org/10.1042/bj3050465

Baldi, A., S. Modina, F. Cheli, F. Gandolfi, L. Pinotti, L. B. Scesi, F. Fantuz, and V. Dell'Orto. 2002. Bovine somatotropin administration to dairy goats in late lactation: effects on mammary gland function, composition and morphology. J. Dairy Sci., 85 (5):1093-1102. http://dx.doi.org/10.3168/jds.S0022-0302(02)74170-2

Baumgard, L. H., R. J. Collier, and D. E. Bauman. 2017. A 100-Year Review: Regulation of nutrient partitioning to support lactation. J. Dairy Sci., 100 (12):10353-10366. https://doi.org/10.3168/jds.2017-13242

Beerda, B., J. E. Kornalijnslijper, J. T. N. Van der Werf, E. N. Noordhuizen-Stassen, and H. Hopster. 2004. Effects of milk production capacity and metabolic status on HPA function in early postpartum dairy cows. J. Dairy Sci., 87 (7):2094-2102. https://doi.org/10.3168/ jds.S0022-0302(04)70027-2

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia, 2 (3):265-278. https://doi.org/10.1023/ A:1026336505343

Bomfim, G. F., G. K. F. Merighe, S. A. Oliveira, and J. A. Negrao. 2018. Effect of acute stressors, adrenocorticotropic hormone administration, and cortisol release on milk yield, the expression of key genes, proliferation, and apoptosis in goat mammary epithelial cells. J. Dairy Sci., 101 (7):6486-6496. https://doi.org/10.3168/jds.2017-14123

Boston, R. C., D. Stefanovski, P. J. Moate, A. E. Sumner, R. M. Watanabe, and R. N. Bergman. 2003. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol. Ther., 5 (6):1003-1015. https://doi.org/10.1089/152091503322641060

Bradford, B. J., and M. S. Allen. 2008. Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. Domest. Anim. Endocrinol., 34 (2):196-203. https://doi.org/10.1016/j.domaniend.2007.02.002

Cai, J., F. Q. Zhao, J. Liu, and D. Wang. 2018. Local mammary glucose supply regulates availability and intracellular metabolic pathways of glucose in the mammary gland of lactating dairy goats under malnutrition of energy. Front. Physiol., 9 (1467):1-13. https://doi.org/10.3389/fphys.2018.01467

Chadio, S. E., G. Zervas, K. Kiriakou, C. Goulas, and J. Menegatos. 2000. Effects of recombinant bovine somatotropin administration to lactating goats. Small Rum. Res., 35 (3):263-269. https://doi.org/10.1016/S0921-4488(99)00104-2

Cincović, M. R., R. Đoković, B. Belić, I. Lakić, N. Stojanac, O. Stevančević, and N. Staničkov. 2018. Insulin resistance in cows during the periparturient period. Acta agric. Serb., 23 (46):233-245. https://doi.org/10.5937/AASer1846233C

Cronjé, P. B., M. De Jager, and E. Vlok. 2000. Nutrient partitioning and response to insulin challenge at different planes of nutrition during lactation in goats of high vs. low milk production potential. S. Afr. J. Anim. Sci., 30 (3-4):178-185. https://doi.org/10.4314/sajas.v30i3.3850

Davis, A. N., J. L. Clegg, C. A. Perry, and J. W. McFadden. 2017. Nutrient restriction increases circulating and hepatic ceramide in dairy cows displaying impaired insulin tolerance. Lipids, 52 (9):771-780. https://doi.org/10.1007/s11745-017-4287-5

De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. Food Anim., 29 (2):299-322. http://dx.doi.org/10.1016/j.cvfa.2013.04.002

Debras, E., J. Grizard, E. Aina, S. Tesseraud, C. Champredon, and M. Arnal. 1989. Insulin sensitivity and responsiveness during lactation and dry period in goats. Am. J. Physiol. Endocrinol. Metab., 256 (2):E295-E302. https://doi.org/10.1152/ajpendo.1989.256.2.E295

DiGiacomo, K., E. Norris, F. R. Dunshea, B. J. Hayes, L. C. Marett, W. J. Wales, and B. J. Leury. 2018. Responses of dairy cows with divergent residual feed intake as calves to metabolic challenges during midlactation and the nonlactating period. J. Dairy Sci., 101 (7):6474-6485. https://doi.org/10.3168/jds.2017-12569

Dunshea, F., A. Bell, and T. Trigg. 1990. Body composition changes in goats during early lactation estimated using a two-pool model of tritiated water kinetics. Br. J. Nutr., 64 (1):121-131. https://doi.org/10.1079/BJN19900015

Friggens, N. C., L. Brun-Lafleur, P. Faverdin, D. Sauvant, and O. Martin. 2013. Advances in predicting nutrient partitioning in the dairy cow: recognizing the central role of genotype and its expression through time. Animal, 7 (s1):89-101. https://doi.org/10.1017/S1751731111001820

Gross, J. J., R. S. Zbinden, M. F. Dohme, and R. M. Bruckmaier. 2018. Adrenal cortex reactivity in dairy cows differs between lactational stages and between different feeding levels. J. Anim. Physiol. Anim. Nutr., 102 (1):e309-e315. https://doi.org/10.1111/jpn.12746

Hammon, H. M., O. Bellmann, J. Voigt, F. Schneider, and C. Kühn. 2007. Glucose-dependent insulin response and milk production in heifers within a segregating resource family population. J. Dairy Sci., 90 (7):3247-3254. https://doi.org/10.3168/jds.2006-748

Jaakson, H., K. Ling, J. Samarütel, A. Ilves, T. Kaart, O. Kärt, and M. Ots. 2013. Blood glucose and insulin responses during the glucose tolerance test in relation to dairy cow body condition and milk yield. Vet. Zootech. Lith., 62 (84):28-35.

Johnson, M. M., and J. P. Peters. 1993. An improved method to quantify nonesterified fatty acids in bovine plasma. J. Anim. Sci., 71 (3):753-756. https://doi.org/10.2527/1993.713753x

Keane, K., and P. Newsholme. 2014. Metabolic regulation of insulin secretion. Vitam. Horm., 95:1-33. https://doi.org/10.1016/B978-0-12-800174-5.00001-6

Kiplagat, S. K., M. K. Limo, and I. S. Kosgey. 2012. Genetic improvement of livestock for milk production. Pages 77-96 in Milk production - advanced genetic traits, cellular mechanism, animal management and health. N. Chaiyabutr. Intech Publishers. http://dx.doi.org/10.5772/50761

Köck, A., M. Ledinek, L. Gruber, F. Steininger, B. Fuerst-Waltl, and C. Egger-Danner. 2018. Genetic analysis of efficiency traits in Austrian dairy cattle and their relationships with body condition score and lameness. J. Dairy Sci., 101 (1):445-455. https://doi.org/10.3168/jds.2017-13281

Kvidera, S., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. S. Fernandez, and L. H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci., 100 (3):2360-2374. https://doi.org/10.3168/jds.2016-12001

Lacasse, P., and C. G. Prosser. 2003. Mammary blood flow does not limit milk yield in lactating goats. J. Dairy Sci., 86 (6):2094-2097. https://doi.org/10.3168/jds.S0022-0302(03)73798-9

Lucy, M. C., G. A. Verkerk, B. E. Whyte, K. A. Macdonald, L. Burton, R. T. Cursons, J. R. Roche, and C. W. Holmes. 2009. Somatotropic axis components and nutrient partitioning in genetically diverse dairy cows managed under different feed allowances in a pasture system. J. Dairy Sci., 92 (2):526-539. https://doi.org/10.3168/jds.2008-1421

Maia-Nogueira, D. 2015. The meat goat industry in Australia: geographical, seasonal and nutritional influences on reproduction in female goats. (PhD thesis), James Cook University, Australia. http://researchonline.jcu.edu.au/41271/

Malaisse, W. J. 2015. Insulin secretion: functional biochemical aspects. Pages 95-99 in Encyclopedia of endocrine diseases, 2nd Ed. I. Huhtaniemi and L. Martini. Academic Press. Oxford, UK. https://doi.org/10.1016/B978-0-12-801238-3.98737-3

Marett, L. C., M. J. Auldist, P. J. Moate, W. J. Wales, K. L. Macmillan, F. R. Dunshea, and B. J. Leury. 2015. Response of plasma glucose, insulin, and nonesterified fatty acids to intravenous glucose tolerance tests in dairy cows during a 670-day lactation. J. Dairy Sci., 98 (1):179-189. http://dx.doi.org/10.3168/jds.2014-8205

Marett, L. C., M. J. Auldist, W. J. Wales, K. L. Macmillan, K. DiGiacomo, and B. J. Leury. 2014. Evaluation of growth hormone response to insulin-induced hypoglycaemia in dairy cattle during a 670-day lactation. Anim. Prod. Sci., 54 (9):1323-1327. http://dx.doi.org /10.1071/AN14224

Marett, L. C., M. J. Auldist, W. J. Wales, K. L. Macmillan, F. R. Dunshea, and B. J. Leury. 2017. Responses of plasma glucose and nonesterified fatty acids to intravenous insulin tolerance tests in dairy cows during a 670-day lactation. J. Dairy Sci., 100 (4):3272-3281. https://doi.org/10.3168/jds.2016-11985

Marett, L. C., M. J. Auldist, W. J. Wales, K. L. Macmillan, F. R. Dunshea, and B. J. Leury. 2019. Responses to metabolic challenges in dairy cows with high or low milk yield during an extended lactation. J. Dairy Sci., 102 (5):4590-4605. https://doi.org/10.3168/jds.2018-15513

Na, Z., L. Qingzhang, G. Xuejun, N. Xuemei, Y. Hongbo, and L. Chun. 2009. Expression and localisation of glucose transporter 1 (GLUT1) in dairy goat mammary gland at different physiological stages. Can. J. Anim. Sci., 89 (4):475-480. https://doi.org/10.4141/CJAS09038

Nishihara, K., R. Kobayashi, Y. Suzuki, K. Sato, K. Katoh, and S. Roh. 2017. Post-prandial decrease in plasma growth hormone levels is not related to the increase in plasma insulin levels in goats. Asian-Australas. J. Anim. Sci., 30 (12):1696. https://doi.org/10.5713/ajas.16.0965

Pires, J., A. Souza, and R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci., 90 (6):2735-2744. https://doi.org/0.3168/jds.2006-759

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci., 86 (4):1201-1217. https://doi.org/10.3168/jds.S0022-0302(03)73704-7

Schmidely, P., M. Lloret-Pujol, P. Bas, A. Rouzeau, and D. Sauvant. 1999. Influence of feed intake and source of dietary carbohydrate on the metabolic response to propionate and glucose challenges in lactating goats. J. Dairy Sci., 82 (4):738-746. https://doi.org/10.3168/jds.S0022-0302(99)75291-4

Shamay, A., S. J. Mabjeesh, F. Shapiro, and N. Silanikove. 2000. Adrenocorticotrophic hormone and dexamethasone failed to affect milk yield in dairy goats: comparative aspects. Small Rum. Res., 38 (3):255-259. https://doi.org/10.1016/s0921-4488(00)00152-8

Shingu, H., K. Hodate, S. Kushibiki, Y. Ueda, A. Watanabe, M. Shinoda, and M. Matsumoto. 2002. Breed differences in growth hormone and insulin secretion between lactating Japanese Black cows (beef type) and Holstein cows (dairy type). Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 132 (4):493-504. https://doi.org/10.1016/s1532-0456(02)00115-1

Veerkamp, R. F., B. Beerda, and T. Aan der Lende. 2003. Effects of genetic selection for milk yield on energy balance, levels of hormones, and metabolites in lactating cattle, and possible links to reduced fertility. Livest. Prod. Sci., 83 (2-3):257–275. https://doi.org/10.1016/S0301-6226(03)00108-8

Villaquiran, M., T. A. Gipson, R. C. Merkel, A. L. Goetsch, and T. Sahlu. 2004. Body condition scores in goats. American Institute for Goat Research. Langston University, OK, USA. Accessed on Accessed Mar. 18, 2016. http://www.luresext.edu/sites/default/ files/BCS_factsheet.pdf

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. Rev., 26 (2):19-39. https://www.ncbi.nlm.nih.gov/pubmed/16278749

Zamuner, F., K. DiGiacomo, A. W. N. Cameron, and B. J. Leury. 2020. Endocrine and metabolic status of commercial dairy goats during the transition period. J. Dairy Sci., 103 (6):5616-5628. https://doi.org/10.3168/jds.2019-18040

Zhao, F.-Q., and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. J. Dairy Sci., 90:E76-E86. https://doi.org/10.3168/jds.2006-470

Chapter 7 - General Discussion

7.1 Introduction

This suite of experiments aimed to fill gaps in knowledge regarding the regulation of energy metabolism during the transition period and to add to the information available regarding the effects of nongenetic factors on the productive performance of commercial dairy goats in Australia. Due to the absence of reliable data on Australian dairy goats, a large-scale study was undertaken in the first part of this research to collect lactation performance and metabolic profile information from 940 commercial dairy goats. Such information was then explored in great detail in Chapters 3, 4, and 5. The first experimental chapter (Chapter 3) investigated the combined effects of some of the main nongenetic factors influencing goat dairy production systems and estimated their effect on milk yield, milk quality, lactation length, and lactation curve. The two subsequent chapters (Chapter 4 and 5) provided an overview of temporal variations in concentrations of selected hormones and metabolites involved in the regulation of energy metabolism and examined how the level of milk yield, parity number, and litter size affect the metabolic profile of commercial dairy goats during the transition period. Furthermore, a fourth experimental chapter (Chapter 6) was conducted to test the hypothesis that differences in the regulation of energy metabolism could be responsible for the distinct milk yield variation between goats of high and low milk yield.

7.2 Influence of nongenetic factors on goat milk yield and composition

Chapter 3 describes the effects of month of kidding, parity number, and litter size on milk yield of Saanen dairy goats managed under an intensive system and fed a TMR year-round. The information collected in this study was predominantly in agreement with previous studies that analyzed the lactation curves of Spanish (León et al., 2012) and French dairy herds (Arnal

et al., 2018). These authors reported increasing milk yield with increasing parity, increasing litter size, and for goats kidding during warmer months (Spring-Summer). Interestingly, authors also demonstrated that the month of kidding could have a significant impact on the shape and scale of the lactation curve of dairy herds regardless of whether goats are managed in extensive, semi-intensive (León et al., 2012), or intensive systems (Arnal et al., 2018).

Our results are not only consistent with previous findings from European studies, but most importantly, the large number of goats enrolled in the present study has enabled the identification of significant interactions between the month of kidding and parity number. Data presented in Chapter 3 shows that the effects of the month of kidding on milk yield increase with increasing parity and are accentuated during mid-lactation. These results indicate the potential benefits of including relationships between month of kidding and parity number into mathematical models to predict lactation performance. Such novel information would assist farmers with estimating the total lactation yield, forecasting individual and herd production at a given time, and making strategic decisions such as feeding, joining, and culling (Solis-Ramirez, 2014).

Also, several authors have demonstrated that light manipulation is a cost-effective method to significantly increase milk production in goats (Garcia-Hernandez et al., 2007; Flores et al., 2011; Russo et al., 2013). Results presented in **Table 3.4** and **Figure 8.1** demonstrate that the lactation curves of primiparous goats are the least affected by the month of kidding (and environmental changes along the lactation period), indicating that if resources are limited, multiparous should have priority over primiparous goats in protocols of light manipulation for increased milk production.

Additionally, knowledge of the shape and scale of the lactation curve and factors affecting the curve (e.g., parity, season, nutritional management) also help with the optimization of genetic selection schemes targeting improved production efficiency. That is because

nongenetic factors may interfere with the animal's true genetic potential and create a bias in the selection process (Djemali and Berger, 1992; Solis-Ramirez, 2014). The presented results (Chapter 3) indicate the need for adjustments for parity number and month of kidding when estimating the genetic factor in milk yield.

This is the first study describing the lactation performance of dairy goats from a commercial herd in Australia. The large number of goats enrolled in this study allowed for a thorough analysis of the influence of various factors on goats' performance traits and it contributed to the accuracy and robustness of the findings. Overall, this study highlights the substantial influence of the month of kidding on variations in milk yield and quality. This information is highly relevant to the local industry because, in Australia, approximately 85% of dairy goat farms are located below latitude 30° south (Zalcman and Cowled, 2018), thereby facing similar environmental conditions than those of the present study. Thus, the novel information presented here have potential applications in the optimization of farming practices and to augment the accuracy of selection in breeding/genetic schemes for the improvement of Australian dairy goats.

Nevertheless, changes in environmental conditions other than photoperiod (e.g., temperature, solar radiation, humidity, wind speed) can have a significant impact on goat lactation performance and milk physicochemical properties (Salama et al., 2014). For instance, several studies in dairy goats have shown that elevated temperature-humidity index is positively correlated with respiratory and heart rates, and rectal temperature (Todaro et al., 2015), and negatively correlated with DMI, milk production, and contents of fat and protein (Hamzaoui et al., 2013; Salama et al., 2014; Zhu et al., 2020). Moreover, other studies indicate that goats' response to heat stress might vary according to breed (Salama et al., 2014), farm management practices (Di Grigoli et al., 2009), and genetic merit for milk (Menéndez-Buxadera et al., 2012). Given the Australian climate and landscape diversity, further

investigation into the lactational performance of dairy goats in different regions and dairy farming systems would be a valuable contribution to the Australian dairy goat industry.

7.3 Endocrine and metabolic regulation of energy metabolism during the transition period and early lactation

Data presented in Chapters 4 and 5 are complementary to Chapter 3 by providing a clear demonstration of how parity, litter size, and milk production can affect the metabolic profile of periparturient goats. One of the most relevant results from this study was that increased milk yield had the most significant influence on the magnitude of body tissue mobilization, as evidenced by the increasing NEFA, BHB, and PUN concentrations with increasing milk yield. Our results support the notion that genetic selection for increased milk yield is likely to increase the incidence of peripartum disorders as a consequence of intensified metabolic burden in high-yielding goats (Cannas and Pulina, 2008; Celi et al., 2008; Matthews, 2016).

Another insight to emerge from this research (Chapter 4) is that pregnancy and lactation are less able to elicit lipid mobilization in primiparous compared with multiparous goats (**Table 4.5** and **Figure 8.2**). The substantially lower NEFA, BHB, and PUN concentration in primiparous goats might be explained by their inferior milk production, thereby lesser nutritional requirements compared to multiparous goats. However, the same rationale does not explain the effects of litter size on lipid mobilization. Our results show that not only the metabolic profile of primiparous goats was unaffected by litter size, but also that multiparous goats had greater NEFA and BHB concentrations than did primiparous goats of equal litter size. Together, these results suggest that primiparous goats were partitioning nutrients towards their growth, most likely in detriment of fetal development. However, to my knowledge, this is the first study describing how litter size affects the energy metabolism of multiparous and primiparous goats over the transition period.

It is important to note that the study described in Chapter 4 was carried out under commercial conditions and involved a large number of goats. Thus, it is necessary to have some of the advantages and limitations of large-scale studies in mind when analyzing present results. For instance, the fact that all goats were exposed to identical husbandry conditions, regardless of parity, litter size, milk production, BW, BCS, etc., has enabled a more realistic estimation of the metabolic burden experienced by periparturient dairy goats in farm conditions. On the other hand, such a setup might have favored some goats while preventing others from expressing their true genetic potential for milk production. That is because the group-feeding approach is likely to result in under or overfeeding in some cohorts of the herd (Cabrera and Kalantari, 2016; Kalantari et al., 2016) while managing large and heterogeneous groups is likely to increase feed competition and aggressive behavior (Goetsch et al., 2010).

Furthermore, the increasing NEFA and BHB with increasing milk yield, and the greater ratios of glucose, NEFA, and BHB to insulin observed in high-yielding goats indicate a homeorhetic shift in metabolism toward catabolic pathways, suggesting that high-yielding goats have a greater propensity to divert nutrients towards milk production and away from body reserves than their lower-yielding counterparts. It is also worth noting that, in addition to the expressive differences in NEFA and BHB concentrations between goats of high and low milk yield, we also found a considerable variation among high-yielding goats (CV (range) for weekly measurements was 42 - 77% for BHB, and 46 - 62 % for NEFA). In other words, despite the increasing NEFA and BHB concentrations with increasing milk yield, there were a substantial number of high-yielding goats that did not show elevated NEFA and BHB concentrations during the transition period despite their high level of milk production. Such results indicate an opportunity for using a metabolic profile to generate EBVs for metabolic resilience.

However, because feed intake was not measured in this study (Chapter 4), it was not possible to estimate the relative contribution of DMI and milk yield to postpartum levels of NEFA and BHB in high and low-yielding goats. Observations from the first experiment were further investigated in an additional experiment (Chapter 6), which aimed to determine whether there are discernible differences in the regulation of energy metabolism of high and low-yielding goats. Three different metabolic challenges were employed to compare the metabolic response to glucose, insulin, and ACTH infusions. It was found that, despite the substantially greater DMI, high-yielding goats exhibited lower basal insulin, greater NEFA, and lower BCS than low-yielding goats. This was consistent with results obtained in Chapter 4 and provided further support for an increased overall catabolic activity favoring substrate availability for milk synthesis in high-yielding goats in comparison to their lower-yielding counterparts. Further, the responses of the goats to glucose infusion revealed a reduced glucose-induced insulin response and a faster insulin clearance in high-yielding goats. However, there were no measurable differences between high and low-yielding goats for glucose and NEFA responses to either insulin or ACTH infusions.

Overall, the results from Chapter 6 did not support our hypothesis that goats would behave similarly to observations made in dairy cows (Bell, 1995; De Koster and Opsomer, 2013; Cincović et al., 2018), whereby increased milk yield would be associated with increased insulin resistance. Instead, data collected in this experiment indicated that differential milk production is primarily linked to differences in insulin secretion and degradation, rather than to differences in peripheral tissue responsiveness to the effects of catabolic and anabolic hormones (namely insulin and ACTH). Nevertheless, although results within this thesis provide some valuable insight into the regulation of energy metabolism in peripartum goats, the underlying mechanisms driving nutrients towards milk synthesis remain to be clarified.

7.4 Association between NEFA and BHB concentrations, milk yield, and culling rates

Nutritional and metabolic diseases are the dominant pathology of intensive dairy goat farming (Bousquet, 2005). However, only a few studies have explored the link between elevated blood levels of NEFA and BHB and NEB-related diseases in goats. Although the study described in Chapter 4 was not intended nor designed to be an epidemiologic study, it resulted in a large dataset consisting of a total of 940 goats and more than 5,800 blood samples. Thus, it was pertinent to use this data to calculate the prevalence of goats with elevated NEFA and BHB during the transition period, and to estimate associations between elevated NEFA and BHB concentrations and performance traits such as milk yield, and culling rates.

According to several authors, ketosis is one of the most common diseases affecting goat herds worldwide (Rook, 2000; Bousquet, 2005; Matthews, 2016). Despite its potential negative economic impact, there are only a few studies describing the prevalence of ketosis in dairy goats, which can be as high as 33% in some herds (Doré et al., 2015; Ismail et al., 2015; Yadav et al., 2015). In contrast to the higher prevalence of ketosis ante- than postpartum reported in Beetal and Baladi goats (Ismail et al., 2015; Yadav et al., 2015), the present investigation revealed that the average weekly prevalence of goats with BHB $\geq$ 0.8 mmol/L was approximately 6 times greater post- than antepartum. The discrepancy between studies is probably related to energy demands for lactation. Baladi and Beetal goats are dual-purpose breeds with average milk production considerably inferior to Saanen goats, thus comparatively less prone to intensive NEB in early lactation.

Moreover, supplementary analysis (**Figure 8.3**) has shown an increased prevalence of goats with BHB $\geq$ 0.8 mmol/L in multiparous goats (4-fold greater than primiparous), and in goats carrying triplets (10-fold greater than singles). These results highlight the importance of performing the calculation separately for each group (multiparous and primiparous); otherwise, the prevalence of goats with BHB $\geq$ 0.8 mmol/L could be underestimated among

multiparous, and also that it might be worthwhile separating the herd according to litter size, which can help to reduce feed competition and to make it easier to identify sick animals within the herd.

Despite the considerable number of goats with elevated NEFA and BHB (**Table 5.1**, **Figure 8.3**, and **Figure 8.4**), only 2% of the goats were removed from the milking herd between -21 to 21 DIM. Also, contrary to studies in dairy cows, we observed a positive relationship between plasma NEFA and milk yield. Although no direct evidence has been demonstrated yet, present and previous studies (Bousquet, 2005; Doré et al., 2015) suggest that dairy goats might be less sensitive to the elevated concentrations of NEFA and BHB than dairy cows.

7.5 Recommendations for Further Research

While this thesis provides extensive and robust information on both the productive performance and the metabolic status of Australian dairy goats, it has also uncovered many areas that would benefit from further research, including:

- The effects of prepartum photoperiod on mammary development and subsequent milk production in dairy goats.
- Further investigation into the lactational performance of dairy goats and dairy farming systems (e.g., extensive, semi-extensive feeding systems) in different regions of Australia.
- The effects of litter size on the metabolic profiles of primiparous goats and their kid's performance in early life.
- In-depth exploration (preferably including data from several herds) of relationships between plasma NEFA and BHB and performance traits, as well as estimation of

optimal NEFA and BHB thresholds for predicting productive performance in dairy goats.

- Underlying mechanisms driving differences in nutrient partitioning in dairy goats of high and low milk yield (e.g., abundancy and binding affinity of hormone receptors in multiple target tissues, glucose transporters, enzymatic factors involved in lipogenesis and lipolysis).

7.6 Conclusions

This thesis provides the first comprehensive overview of the metabolic status of Australian dairy goats during the transition period. Overall, the research within this thesis has not only broadened our knowledge of physiological mechanisms underlying the regulation of energy metabolism of transition goats but also provided broad insight into the productive performance of commercial dairy goats raised in intensive husbandry systems and managed in multiple kidding seasons per year.

Collectively, the novel findings presented here contribute invaluable information for the development of effective management and breeding plans, which, in turn, can accelerate increments in the national herd productivity. Looking ahead, our results could also guide future research that aims to improve the use of endocrine and metabolic profiles as diagnostic tools in nutritional evaluations and in predicting potential risk for peripartum diseases in dairy goats.

7.7 References

Arnal, M., C. Robert-Granié, and H. Larroque. 2018. Diversity of dairy goat lactation curves in France. J. Dairy Sci., 101 (12):11040-11051. https://doi.org/10.3168/jds.2018-14980

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci., 73 (9):2804-2819. http://dx.doi.org/10.2527/1995.7392804x

Bousquet, C. 2005. Goat pathology in the Deux Sèvres region: prevalence and impact on culling and mortality levels. (PhD Thesis), Université Paul-Sabatier de Toulouse, Toulouse, France. https://oatao.univ-toulouse.fr/1159/1/debouch_1159.pdf

Cabrera, V. E., and A. S. Kalantari. 2016. Economics of production efficiency: Nutritional grouping of the lactating cow. J. Dairy Sci., 99 (1):825-841. http://dx.doi.org/10.3168/jds.2015-9846

Cannas, A., and G. Pulina. 2008. Dairy goats feeding and nutrition. CABI International. Bologna, Italy. https://doi.org/10.1079/9781845933487.0000

Celi, P., A. Di Trana, and A. Quaranta. 2008. Metabolic profile and oxidative status in goats during the peripartum period. Aust. J. Exp. Agr., 48 (7):1004-1008. https://doi.org/10.1071/ea07410

Cincović, M. R., R. Đoković, B. Belić, I. Lakić, N. Stojanac, O. Stevančević, and N. Staničkov. 2018. Insulin resistance in cows during the periparturient period. Acta agric. Serb., 23 (46):233-245. https://doi.org/10.5937/AASer1846233C

De Koster, J. D., and G. Opsomer. 2013. Insulin resistance in dairy cows. Vet. Clin. Food Anim., 29 (2):299-322. http://dx.doi.org/10.1016/j.cvfa.2013.04.002

Di Grigoli, A., M. Todaro, G. Di Miceli, L. M. Alicata, G. Cascone, and A. Bonanno. 2009. Milk production and physiological traits of ewes and goats housed indoor or grazing at

different daily timing in summer. Ital. J. Anim. Sci., 8 (sup2):616-618. https://doi.org/10.4081/ijas.2009.s2.616

Djemali, M., and P. J. Berger. 1992. Yield and reproduction characteristics of Friesian cattle under North African conditions. J. Dairy Sci., 75 (12):3568-3575. http://dx.doi.org/10.3168/jds.S0022-0302(92)78133-8

Doré, V., J. Dubuc, A. M. Bélanger, and S. Buczinski. 2015. Definition of prepartum hyperketonemia in dairy goats. J. Dairy Sci., 98 (7):4535-4543. http://dx.doi.org/10.3168/jds.2014-9172

Flores, M. J., J. A. Flores, J. M. Elizundia, A. Mejía, J. A. Delgadillo, and H. Hernández. 2011. Artificial long-day photoperiod in the subtropics increases milk production in goats giving birth in late autumn. J. Anim. Sci., 89 (3):856-862. https://doi.org/10.2527/jas.2010-3232

Garcia-Hernandez, R., G. Newton, S. Horner, and L. C. Nuti. 2007. Effect of photoperiod on milk yield and quality, and reproduction in dairy goats. Livest. Sci., 110 (3):214-220. https://doi.org/10.1016/j.livsci.2006.11.005

Goetsch, A. L., T. A. Gipson, A. R. Askar, and R. Puchala. 2010. Invited review: Feeding behavior of goats. J. Anim. Sci., 88 (1):361-373. https://doi.org/10.2527/jas.2009-2332

Hamzaoui, S., A. A. K. Salama, E. Albanell, X. Such, and G. Caja. 2013. Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. J. Dairy Sci., 96 (10):6355-6365. http://dx.doi.org/10.3168/jds.2013-6665

Ismail, Z. B., O. Al-Rawashdeh, A. M. Al-Majali, and F. Amireh. 2015. Prevalence and risk factors for pregnancy toxemia of goats in Jordan. Anim. Biol. Anim. Husb. (ABAH), 7 (1):53-59. http://www.abah.bioflux.com.ro/docs/2015.53-59.pdf

Kalantari, A. S., L. E. Armentano, R. D. Shaver, and V. E. Cabrera. 2016. Economic impact of nutritional grouping in dairy herds. J. Dairy Sci., 99 (2):1672-1692. https://doi.org/10.3168/jds.2015-9810

León, J. M., N. P. P. Macciotta, L. T. Gama, C. Barba, and J. V. Delgado. 2012. Characterization of the lactation curve in Murciano-Granadina dairy goats. Small Rum. Res., 107 (2-3):76-84. http://dx.doi.org/10.1016/j.smallrumres.2012.05.012

Matthews, J. G. 2016. Diseases of the goat. 4th Ed. John Wiley & Sons Ltd. Chelmsford, Essex, UK. https://doi.org/10.1002/9781119073543.ch4

Menéndez-Buxadera, A., A. Molina, F. Arrebola, I. Clemente, and J. M. Serradilla. 2012. Genetic variation of adaptation to heat stress in two Spanish dairy goat breeds. J. Anim. Breed. Genet., 129 (4):306-315. https://doi.org/10.1111/j.1439-0388.2011.00984.x

Rook, J. S. 2000. Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. Food Anim., 16 (2):293-317. https://doi.org/10.1016/S0749-0720(15)30107-9

Russo, V. M., A. W. N. Cameron, F. R. Dunshea, A. J. Tilbrook, and B. J. Leury. 2013. Artificially extending photoperiod improves milk yield in dairy goats and is most effective in late lactation. Small Rum. Res., 113 (1):179-186. https://doi.org/10.1016/j.smallrumres.2013.01.002

Salama, A. A. K., G. Caja, S. Hamzaoui, B. Badaoui, A. Castro-costa, M. M. Guilhermino, and R. Bozzi. 2014. Different levels of response to heat stress in dairy goats. Small Rum. Res., 121:73-79. https://doi.org/10.1016/j.smallrumres.2013.11.021

Solis-Ramirez, J. 2014. A genetic improvement programme for New Zealand dairy goats, PhD Thesis. Massey University. Palmerston North, New Zealand. Accessed on 05/10/2018. https://mro.massey.ac.nz/handle/10179/5623

Todaro, M., M. Dattena, A. Acciaioli, A. Bonanno, G. Bruni, M. Caroprese, M. Mele, A. Sevi, and M. T. Marinucci. 2015. Aseasonal sheep and goat milk production in the Mediterranean area: Physiological and technical insights. Small Rum. Res., 126:59-66. http://dx.doi.org/10.1016/j.smallrumres.2015.01.022

Yadav, S. N., D. N. Kalila, A. Phukan, G. Mahala, S. Tamuly, T. C. Dutta, A. Saleque, D. Barman, and P. Thakuria. 2015. Prevalence of sub-clinical ketosis in goats of Guwahati, Assam. J. Anim. Vet. Adv., 14 (13):382-385. http://dx.doi.org/10.36478/javaa.2015.382.385

Zalcman, E., and B. Cowled. 2018. Farmer survey to assess the size of the Australian dairy goat industry. Aust. Vet. J., 96 (9):341-345. https://doi.org/10.1111/avj.12734

Zhu, X., J. Wen, and J. Wang. 2020. Effect of Environmental Temperature and Humidity on Milk Production and Milk Composition of Guanzhong Dairy Goats. Vet. Anim. Sci.:100121. https://doi.org/10.1016/j.vas.2020.100121

Appendices

8.1 Appendices to Chapter 3

8.1.1 Drying-off Protocol

Meredith Dairy uses a non-antibiotic approach for drying-off pregnant goats. As per usual farm procedure, the drying-off process starts 9-10 weeks before the expected kidding date by adjusting the ration and reducing the milking frequency. On day 1 the TMR fed to pregnant milking goats is replaced by cereal straw (without the addition of any concentrated feed). From day 2 onwards the milking frequency is reduced from twice a day to once a day, and as soon as there is a significant drop in milk production (which usually takes 2-3days) milk frequency is reduced from once a day to every second day. Goats are dried-off when average production falls below ~ 0.3 L/d, which usually happens within one week after the drying-off protocol was initiated.

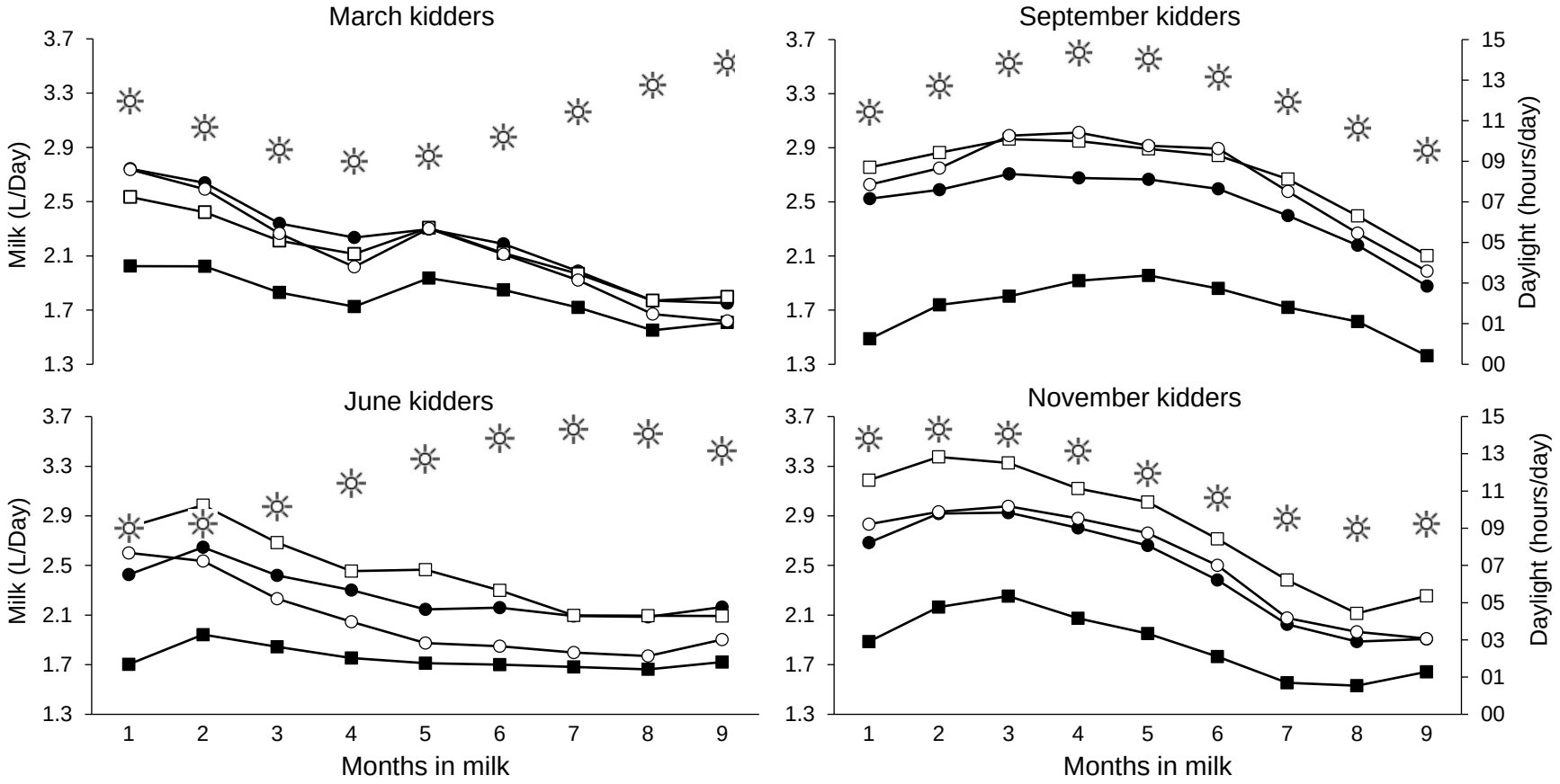

Figure 8.1. Monthly variations in daylight length (hours of light/d; sun symbol), and lactation curves of milk yield (L/d) of goats in parity 1 (■; n = 319), parity 2 (●; n = 244), parity 3 (□; n = 237), and parity 4+ (○; n = 140) at Meredith Dairy (Meredith, VIC, Australia) from June 2016 to December 2017

8.2 Appendices to Chapter 4

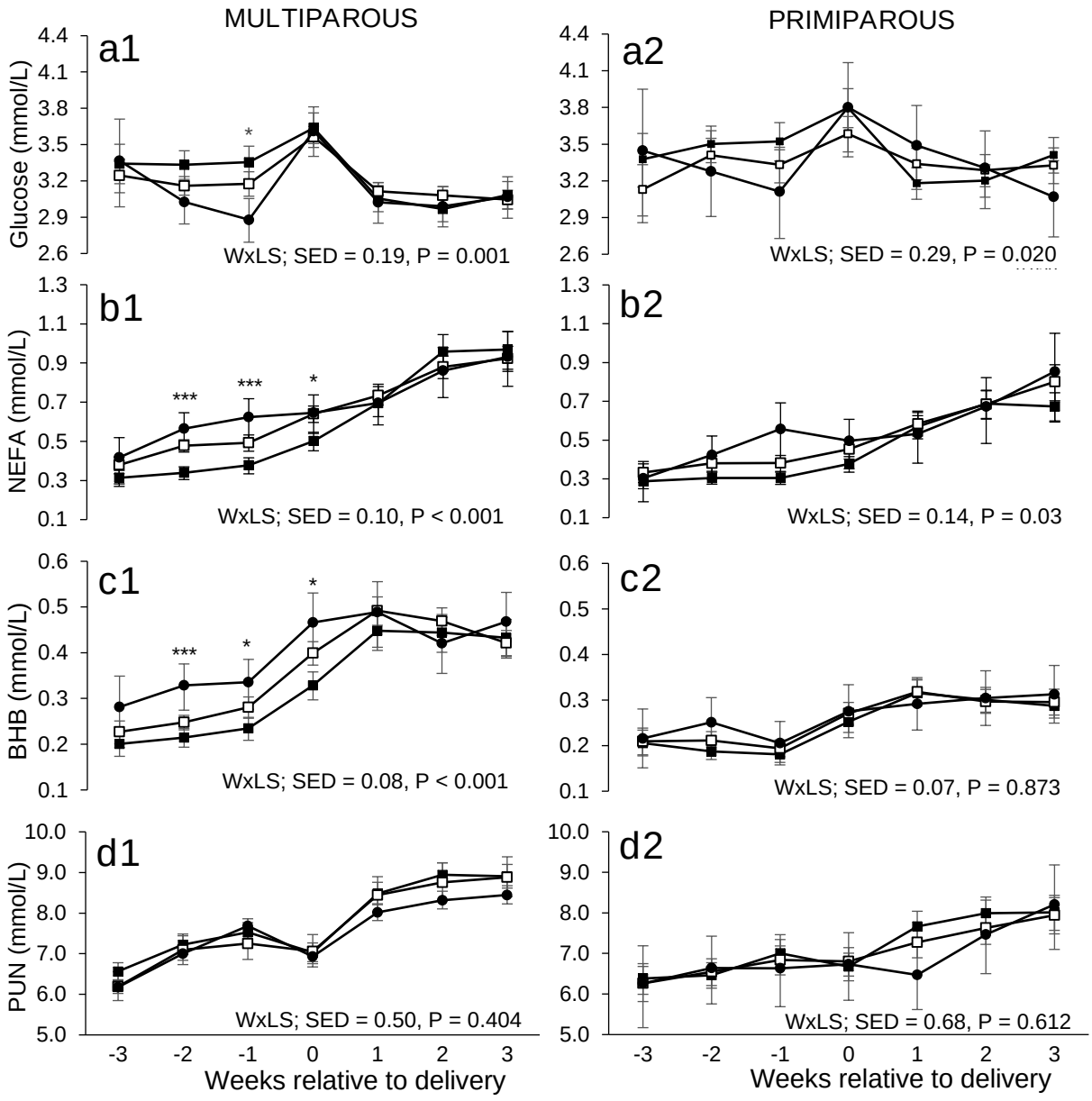

Figure 8.2. Effects of litter size (LS; 1 (■), 2(□), and 3 (●)) on weekly (W) variations in concentrations of (a1-2) glucose, (b1-2) non-esterified fatty acids (NEFA), (c1-2) β-hydroxybutyrate (BHB), and (d1-2) plasma urea nitrogen (PUN) in multiparous and primiparous periparturient dairy goats. Values are back-transformed means; error bars represent 95% CI. SED = standard error of differences. Weekly comparisons between groups; *** P < 0.001; ** P < 0.01; * P < 0.05. The number of goats tested each week is presented in Table 4.1

8.3 Appendices to Chapter 5

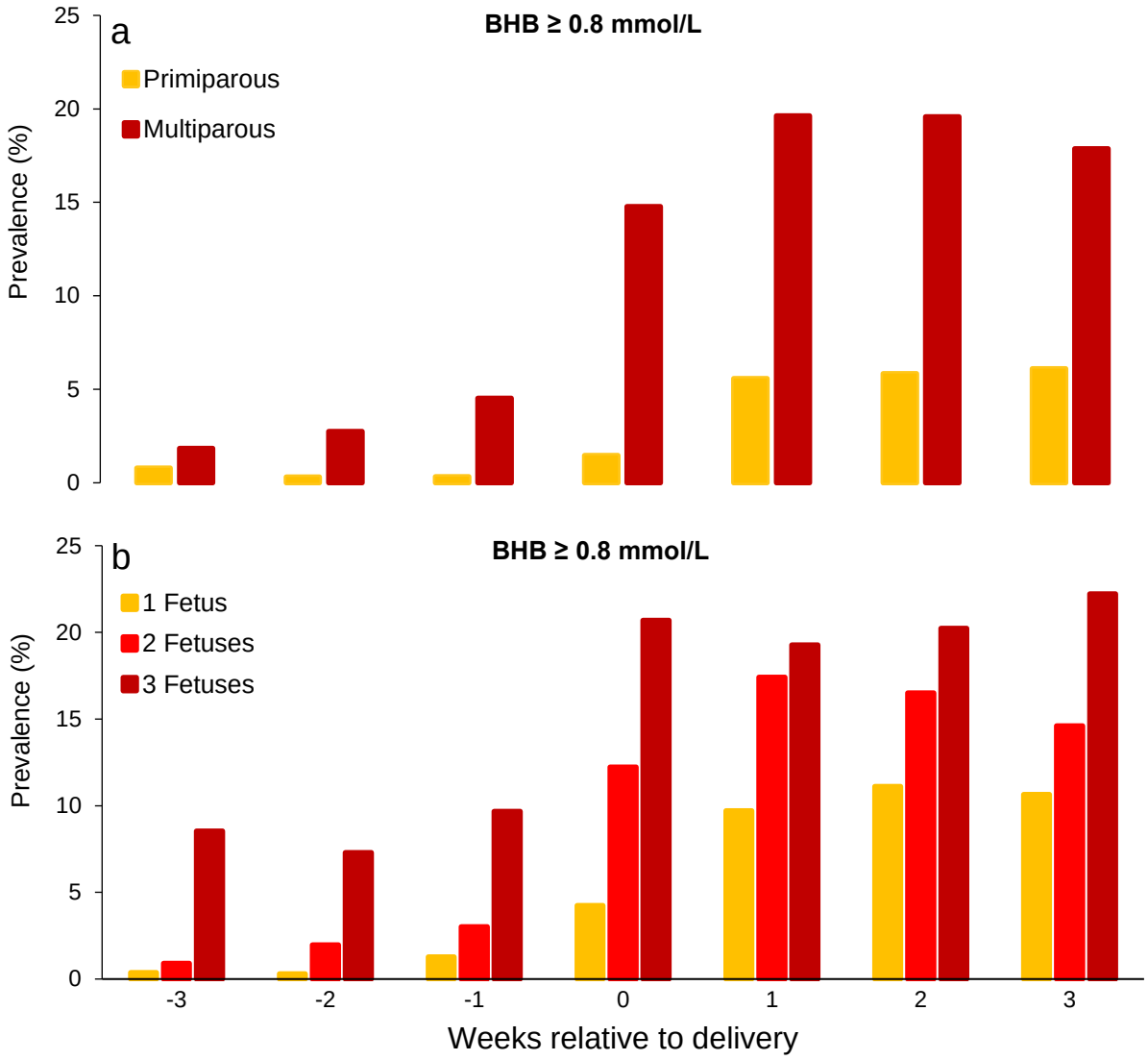

Figure 8.3. Weekly prevalence of goats above critical levels for BHB according to (a) parity, and (b) litter size, in commercial dairy goats in Australia (n = 940). Goats were sampled weekly (always before feeding and after the morning milking) and grouped by 'weeks from delivery' based on their kidding dates. The number of goats tested in each week (by factor combination) is presented in Table 4.1.

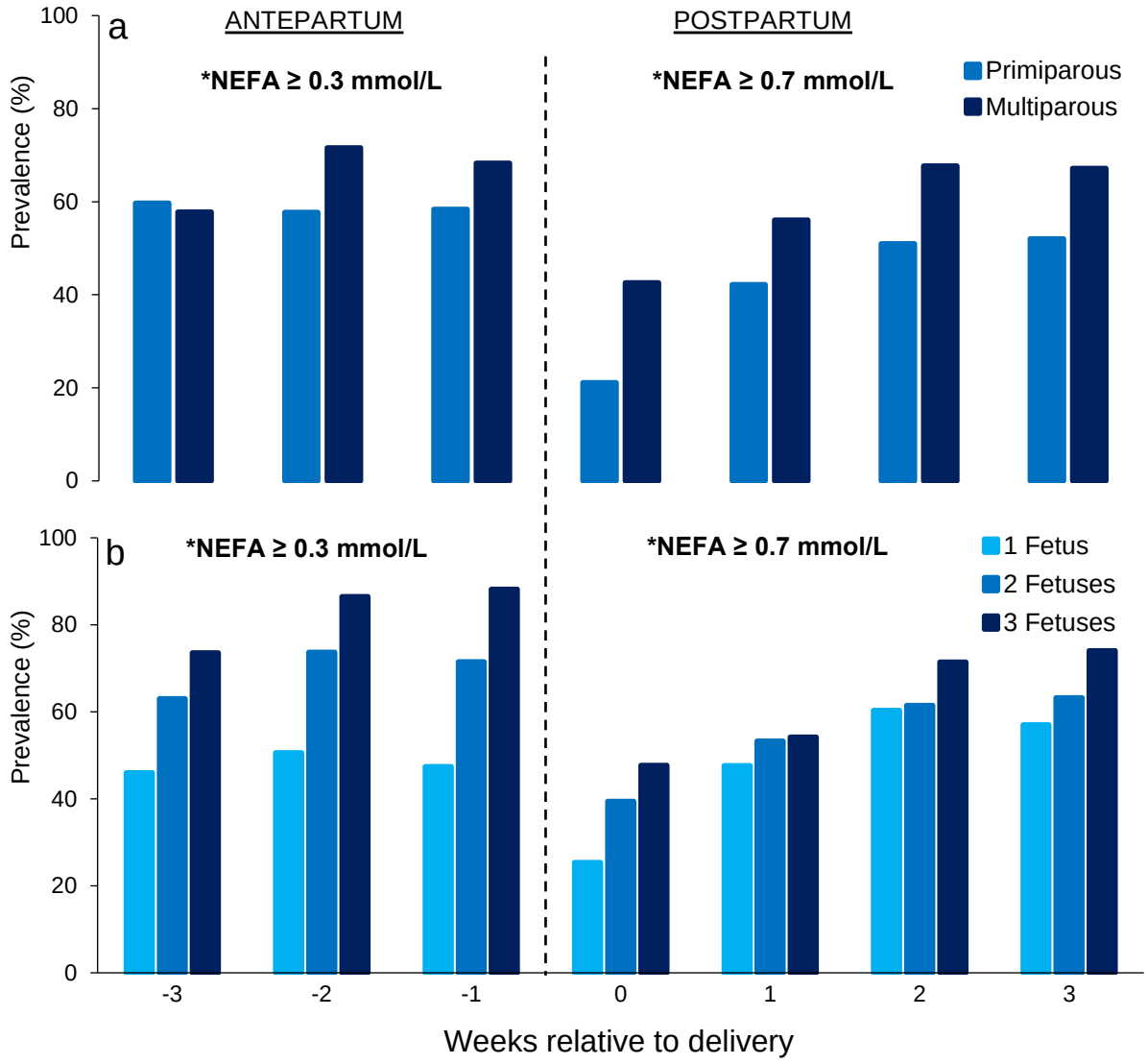

Figure 8.4. Ante- and postpartum prevalence of goats with elevated NEFA concentration, according to (a) parity, and (b) litter size, in Australian dairy goats (n = 940). Goats were sampled weekly (always before feeding and after the morning milking) and grouped by 'weeks from delivery' based on their kidding dates. The number of goats tested in each week (by factor combination) is presented in Table 4.1. *Cutpoints for elevated NEFA concentration in dairy cows; NEFA ≥ 0.3 mmol/L antepartum (wk -3 to -1) and ≥ 0.7 mmol/L postpartum (wk 0 to 3) (McArt et al., 2013