UNIVERSIDADE ESTADUAL PAULISTA “JULIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

MACROMINERAL REQUIREMENTS FOR MAINTENANCE AND GROWTH OF SAANEN GOATS

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2017

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Dissertation submitted to the Universidade Estadual Paulista – Jaboticabal Campus, as part of the requirements to obtain the Doctor degree in Animal Science.

2017

C352m

Castillo Vargas, Julián Andrés
Macromineral requirements for maintenance and growth of Saanen goats / Julián A. Castillo Vargas. – – Jaboticabal, 2017
xi, 70 p. : il. ; 29 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2017
Orientadora: Izabelle Auxiliadora Molina de Almeida Teixeira
Banca examinadora: Luiz Fernando Costa e Silva, Nilva Kazue Sakomura, José Gilson Louzada Regadas Filho, Carla Joice Härter
Bibliografia

1. Abate comparativo. 2. Alometria. 3. Exigência mineral. 4. Saanen. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 636.087.2:636.3

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Diretoria Técnica de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

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Jaboticabal, 24 de abril de 2017

BIOGRAPHY

JULIÁN ANDRÉS CASTILLO VARGAS was born in June 15, 1982, in Bogotá D.C., Colombia. In 2008, he completed his bachelor’s degree in Chemistry at Universidad Nacional de Colombia, Bogotá, Colombia. He concluded his Master’s degree in Animal Science at Universidad Nacional de Colombia, in 2012. He focused on the understanding of the thermodynamics and kinetics of polyunsaturated fatty acid biohydrogenation in the rumen, under supervision of Prof. Dr. Martha Pabón and Prof. Dr. Juan Carulla. He worked as assistant professor of Biochemistry at Universidad Nacional de Colombia, in 2009. From 2011 to 2013, he worked as professor of organic chemistry, general chemistry, biochemistry, and biotechnology for the graduate and undergraduate programs of animal and agricultural sciences at Universidad Agraria de Colombia, Universidad Militar Nueva Granada, Universidad de Ciencias Aplicadas y Ambientales, and Servicio Nacional de Aprendizaje, in Bogotá, Colombia. In August 2013, he began his doctoral degree at the Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil, after receiving a scholarship awarded by Asociación Universitaria Iberoamericana de Postgrado. His doctoral dissertation focused on the study of sex effects on mineral requirements for maintenance and growth in Saanen goats using a meta-analysis, under supervision of Prof. Dr. Izabelle Auxiliadora Molina de Almeida Teixeira and co-supervision of Dr. Amélia Katiane Almeida.

“Y una vez que la tormenta termine, no recordarás cómo lo lograste, como sobreviviste. Ni siquiera estarás seguro si la tormenta ha terminado realmente. Pero una cosa si es segura. Cuando salgas de esa tormenta, no serás la misma persona que entró en ella. De eso se trata la tormenta”.

Haruki Murakami

ACKNOWLEDGMENTS

I would like to acknowledge the academic and technical support of the Universidade Estadual Paulista (Unesp), Jaboticabal Campus, because its professional and funding support for conducting this research.

To Asociación Universitaria Iberoamericana de Postgrado for the scholarship during my doctoral degree at Unesp.

To Fundação de Amparo e Apoio à Pesquisa do Estado de São Paulo (FAPESP, Grant #2014/14734-9) for the financial support.

To my advisor, Dr. Izabelle Teixeira for her knowledge, patient, and dearness during my doctoral studies. “Dr. Izabelle, you really entered a chemist in the animal science world”. God bless you.

To my co-advisor, Dr. Amelia Katiane Almeida, for her technical and academic support.

To the researchers who contributed with their time and effort in checking this material: Dr. Carla Härter, Dr. Nelson Peruzzi, Dr. Luciano Hauschild, and Dr. Marcia Helena Fernandes.

I would like to thank my friends and my family for their support and help during this period:

Aos meus colegas contemporâneos e amigos da equipe do Laboratório de Estudos em Caprinocultura: Ana Rebeca, José Maurício, Leandro, Marcelo, Marina, Nhayandra e Raiza. Seu apoio e carinho para esse “químico gringo” foram vitais para chegar até esse ponto. Ao professor Kléber, professora Izabelle, Márcia e Bruno, pelos grandes ensinamentos, paciência e carinho. Tenham certeza, que mais que contribuir para o meu crescimento profissional, me fizeram uma pessoa melhor. Deus os abençoe em abundância sempre.

A mis grandes amigos colombianos Alfredo (El abuelo) y Pablo (Maradona), por todo el cariño, aprendizaje y días de alegría.

A minha namorada Anaiane, pelo apoio, carinho, ensinamentos e paciência comigo durante todo esse tempo. “Você é esse anjo que chegou para tornar meus sonhos em realidade”. Obrigado do fundo do coração.

A mis padres, Martha y Flavio y a mi hermana Carolina, los cuales siempre estuvieron mandándome energía y esperanza desde la distancia, convirtiéndose en autores honoríficos de este trabajo. Esta victoria es para todos ustedes.

Ao pai celestial, por me dar a essência de existir.

SUMMARY

MACROMINERAL REQUIREMENTS FOR MAINTENANCE AND GROWTH OF SAANEN GOATS

ABSTRACT -The objective of this study was to evaluate the effect of sex on the net macromineral requirements for maintenance and growth of Saanen goats from 5 to 45 kg body weight (BW). For this purpose, three dataset were used: the first dataset was assembled to evaluate the effect of sex on the net requirements for maintenance of Ca (NCa_m), P (NP_m), Mg (NMg_m), and K (NK_m), estimated using the comparative slaughter technique (CST). This dataset was composed by 154 individual records (53 castrated males, 46 females, and 55 intact males) from three comparative slaughter studies. The second dataset, was constructed to evaluate the effect of sex on NCa_m, NMg_m, and NK_m, estimated using the minimum endogenous losses method (MEL). This dataset was assembled with 155 individual records (67 castrated males, 40 females, and 48 intact males) from four feeding trials. The third dataset was constructed to evaluate the effect of sex on the net requirements for growth of Ca (NCa_g), P (NP_g), Mg (NMg_g), Na (NNa_g), and K (NK_g) considering or not the degree of maturity of the goat on the estimations. This dataset comprised by 209 individual records (69 castrated males, 69 females, and 71 intact males) from six comparative slaughter studies. Mineral requirements for maintenance using CST were calculated from the intercept of the linear regression between mineral retention and the mineral intake. Using the MEL, mineral requirements for maintenance were calculated from the intercept of a linear regression between mineral excreted (urine and feces) and mineral intake. The estimation of NP_m using MEL was not possible, because of the lack of enough information on P excretion and intake from feeding trials, to fit equations for calculating its requirements. The NCa_g, NP_g, NMg_g, NNa_g, and NK_g were estimated by the first derivative of the logarithmized allometric equations. The studies were performed as meta-analyses, considering sex as fixed effect and study as random effect. Sex did not affect NCa_m, NP_m, and NK_m estimated using CST ($P > 0.10$). Estimated NCa_m, NP_m, and NK_m using CST were 21.1, 22.8, and 3.99 mg/(kg BW·d), respectively, from 5 to 45 kg BW. On the other hand, NMg_m of intact males (2.65 mg/(kg BW·d)) were greater than that estimated for castrated males and females (1.39 mg/(kg BW·d); $P < 0.10$). Similarly, sex did not affect NCa_m, NMg_m, and NK_m estimated by MEL ($P > 0.10$). The NCa_m, NMg_m, and NK_m values were 38.0, 7.45, and 25.2 mg/(kg BW·d) respectively, from 5 to 45 kg BW. With respect to mineral requirement for growth, without considering the degree of maturity, sex did not affect the NCa$_g$, NP$_g$, NNa$_g$, and NK$_g$ ($P > 0.10$). The NCa$_g$ and NP$_g$ remained constant, whereas NNa$_g$ and NK$_g$ decreased by 32 and 27%, respectively, from 5 to 45 kg BW. On the other hand, sex affected the NMg$_g$ ($P = 0.054$), where the NMg$_g$ of castrated and intact males were 8 and 18%, respectively greater than those female goats. The NMg$_g$ of castrated and intact males increased 8 and 15%, respectively, whereas NMg$_g$ of females decreased by 8% from 5 to 45 kg BW. Considering the degree of maturity, sex affected all net macromineral requirements for growth ($P < 0.10$). The NCa$_g$ and NP$_g$ of intact males were 5 and 2% respectively greater than those of castrated males and females. Besides, the NCa$_g$ and NP$_g$ remained constant from 5 to 45 kg BW across sexes. The NNa$_g$ of males were 6% greater than those females. Irrespective of sex, NNa$_g$ decreased by 32% from 5 to 45 kg BW. Regardless of sex, NK$_g$ decreased by 26% from 5 to 45 kg BW. The NMg$_g$ of castrated and intact males were 7 and 17%, respectively greater than those of female goats. The NMg$_g$ of castrated and intact males increased 8 and 16%, respectively, whereas NMg$_g$ of females decreased by 7% from 5 to 45 kg BW. Our studies indicate that sex influences mineral requirements for maintenance and growth as well as the mineral retention efficiency of Saanen goats. This information may be useful to design strategies for optimizing the mineral recommendations to goats.

Key words: allometry, comparative slaughter, mineral requirement, Saanen

EXIGÊNCIAS DE MACROMINERAIS PARA MANTENÇA E CRESCIMENTO DE CAPRINOS SAANEN

RESUMO - O objetivo deste estudo foi avaliar o efeito do sexo nas exigências liquidas de macrominerais para mantença e crescimento para caprinos Saanen de 5 a 45 kg de peso corporal (PC). Para esse fim, foram utilizados três bancos de dados: o primeiro banco de dados foi construído para avaliar o efeito do sexo sobre as exigências líquidas de Ca (NCa_m), P (NP_m), Mg (NMg_m) e K (Nk_m) para mantença estimadas pelo método do abate comparativo (CST). O banco de dados foi composto por 154 observações individuais (53 machos castrados, 46 fêmeas e 55 machos inteiros) provenientes de três estudos de abate comparativo. O segundo banco de dados, foi construído para avaliar o efeito do sexo sobre as NCa_m, NMg_m e NK_m no corpo de caprinos Saanen, estimadas pelo método das perdas endógenas mínimas (MEL). Este banco de dados foi composto por 155 observações individuais (67 machos castrados, 40 fêmeas e 48 machos inteiros) provenientes de quatro ensaios de alimentação. O terceiro banco de dados foi construído para avaliar o efeito do sexo sobre as exigências líquidas de Ca (NCa_g), P (NP_g), Mg (NMg_g), Na (NNa_g) e K (NK_g) para crescimento, considerando e não considerando o grau de maturidade do caprino, na estimativa destas. Este banco de dados foi composto por 209 observações individuais (69 machos castrados, 69 fêmeas e 71 machos inteiros) provenientes de seis estudos de abate comparativo. As exigências de minerais para mantença, obtidas pelo CST foram calculadas como o intercepto da regressão linear entre retenção mineral e ingestão mineral. Utilizando o MEL, as exigências de minerais para mantença foram calculadas como o intercepto da regressão linear entre mineral excretado (urina e fezes) e ingestão mineral. A estimativa de NP_m usando MEL não foi possível, devido à falta de informações suficientes de excreção e ingestão de P desde os ensaios de alimentanção, para ajustar ecuações com o intuito de calcular as suas exigências. Os valores de NCa_g, NP_g, NMg_g, NNa_g e NK_g foram estimados a partir da primeira derivada das equações alométricas logaritmizadas para cada mineral. Os estudos foram desenvolvidos como meta-análises, considerando-se o sexo como efeito fixo e estudo como efeito aleatório. Sexo não afetou as NCa_m, NP_m, e NK_m estimadas pelo CST (P > 0,10). Os valores de NCa_m, NP_m e NK_m estimados pelo CST foram de 21,1, 22,8 e 3,99 mg/(kg de PC·d), respectivamente, de 5 a 45 kg PC. Por outro lado, o valor de NMg_m de machos inteiros (2,65 mg/(kg PC·d)) foi maior que o estimado para machos castrados e fêmeas (1,39 mg/(kg PC·d); $P < 0,10$). Da mesma forma, o sexo não afetou as NCa_m, NMg_m e NK_m estimadas pelo MEL ($P > 0,10$). Os valores de NCa_m, NMg_m e NK_m foram 38,0, 7,45 e 25,2 mg/(kg de PC·d), respectivamente, de 5 a 45 kg PC. Com relação as exigências de macrominerais para crescimento, quando o grau de maturidade não foi considerado, o sexo não afetou as NCa_g, NP_g, NNa_g e NK_g ($P > 0.10$). Os valores de NCa_g e NP_g permaneceram constantes, enquanto que os de NNa_g e NK_g diminuíram em 32 e 27%, respectivamente, quando o PC aumentou de 5 para 45 kg. Por outro lado, o sexo afetou as NMg_g ($P = 0,054$), onde os valores de NMg_g de machos castrados e inteiros foram 8 e 18%, respectivamente, maiores do que os das fêmeas. Os valores de NMg_g de machos castrados e inteiros aumentaram em 8 e 15%, respectivamente, enquanto que os de NMg_g de fêmeas diminuíram 8%, quando PC aumentou de 5 para 45 kg. Quando o grau de maturidade foi considerado, o sexo influenciou as exigências para crescimento de todos os minerais ($P < 0,10$). Os valores de NCa_g e NP_g de machos inteiros foram 5 e 2%, respectivamente, maiores do que os de machos castrados e fêmeas. Além disso, os valores de NCa_g e NP_g permaneceram constantes à medida que o PC dos caprinos aumentou de 5 para 45 kg, em todos os sexos. Os valores de NNa_g de machos foram 6% maiores que os das fêmeas. Independentemente do sexo, os valores de NNa_g diminuíram em 32%, a medida que o PC aumentou de 5 para 45 kg. Independentemente do sexo, os valores de NK_g diminuíram 26%, à medida que o PC aumentou de 5 para 45 kg. Os valores de NMg_g de machos castrados e inteiros foram 7 e 17%, respectivamente maiores do que os das fêmeas. Os valores de NMg_g de machos castrados e inteiros aumentaram em 8 e 16%, respectivamente, enquanto que os valores de NMg_g de fêmeas diminuíram 7%, a medida que o PC aumentou de 5 para 45 kg. Nossos estudos indicam que o sexo afeta as exigências de macrominerais para mantença e crescimento, como também as eficiências de retenção de macrominerais no corpo de caprinos Saanen. Essas informações podem ser úteis para definição de estratégias visando otimizar as recomendações de minerais em rações para caprinos.

Palavras-chave: abate comparativo, alometria, exigência mineral, Saanen

LIST OF ABBREVIATIONS

ADG	Average daily gain
AIC_c	Corrected Akaike's Information Criterion
Al	Aluminium
As	Arsenic
ATP	Adenosine triphosphate
B	Boron
BGHE	High energy diet given to Boer goats
BGLE	Low energy diet given to Boer goats
BW	Body weight
Ca	Calcium
Ca_{int}	Calcium intake
Ca_{ret}	Calcium retention
Cd	Cadmium
Cl	Chlorine
Co	Cobalt
CST	Comparative slaughter technique
Cu	Copper
Cr	Chromium
DM	Dry matter
DG	Degree of maturity
EBW	Empty body weight
EWG	Empty weight gain
F	Fluorine
Fe	Iron
Hg	Mercury
I	Iodine
IGF-1	Insulin-like Growth Factor 1
K	Potassium
K_{int}	Potassium intake
K_{ret}	Potassium retention
Li	Lithium
Mature EBW	EBW at maturity
MEL	Minimum endogenous losses method
Mg	Magnesium
Mg_{int}	Magnesium intake
Mg_{ret}	Magnesium retention
MMHE	High energy diet given to Mutton Merinos
MMLE	Low energy diet given to Mutton Merinos
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NCa_g	Net Ca requirement for growth
NCa_m	Net Ca requirement for maintenance
Ni	Nickel
NK_g	Net K requirement for growth
NK_m	Net K requirement for maintenance
NMg_g	Net Mg requirement for growth

NMg_m	Net Mg requirement for maintenance
NNa_g	Net Na requirement for growth
NP_g	Net P requirement for growth
NP_m	Net P requirement for maintenance
P	Phosphorus
Pb	Lead
PC	Peso corporal
P_{int}	Phosphorus intake
P_{ret}	Phosphorus retention
PO_4^{3-}	Phosphate
Rb	Rubidium
RECa	Ca retention efficiency
REP	P retention efficiency
REMg	Mg retention efficiency
REK	K retention efficiency
S	Sulfur
SD	Standard deviation
Se	Selenium
Si	Silicon
Sn	Tin
Unesp	Universidade Estadual Paulista “Julio de Mesquita Filho”
V	Vanadium
WAD	West Africa Dwarf
Zn	Zinc
σ_e^2	Variance due to error
σ_s^2	Variance due to study

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 2

Chapter 3

DISSERTATION STRUCTURE

Chapter 1 is a literature review, about macromineral requirements for maintenance and growth in ruminants covering the main functions of macrominerals, the macromineral metabolic fates through maintenance and growth processes in ruminants, and the potential influence of specie, sex, and maturity stage on the macromineral requirement estimations. It was written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus.

Chapter 2 describes the results about the evaluation of sex effect on macromineral requirement for maintenance of Saanen goats from 5 to 45 kg BW. This chapter was written following the guidelines of the Journal of Animal Physiology and Animal Nutrition, except by the letter style, spaces between lines, and position of tables. The paper authors are J. A. C. Vargas, A. K. Almeida, A. P. Souza, M. H. M. R. Fernandes, K. T. Resende, and I. A. M. A. Teixeira.

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Chapter 4 describes the main implications of this study, written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus.

CHAPTER 1 – GENERAL CONSIDERATIONS

1. INTRODUCTION

The importance of goats as providers of meat and dairy products for human consumption has increased around the world, due to the beneficial nutritional properties of goat products (HAENLEIN, 2004; WEBB et al., 2005; PARK et al. 2007). This importance is reflected in the number of animals, meat, and milk production increase (47, 100, and 64%, respectively) during the last 20 years (FAOSTAT, 2016). In this regard, efficient management practices, proper selection for profitable breeding, adequate animal welfare practices, and accurate nutrition plans in production systems are needed to favor sustainability and improve goat production in the world (TEIXEIRA & RESENDE, 2005; WEBB et al., 2005).

From the nutritional standpoint, it is remarkable that there is a lack of information related to nutritional requirements of goats. It may be critical, considering that the low accuracy of nutritional requirements may affect the animal production and health (ARC, 1980).

Minerals constitute important nutrients in the feed, because of the roles in metabolic (e.g., as cofactors of organic nutrient catabolism), and physiological processes (e.g., bone formation and neuromuscular processes) in the body (SUTTLE, 2010). However, the recommendations of the most recent nutritional systems for small ruminants (CSIRO, 2007; INRA, 2007; NRC, 2007), regarding goat mineral requirements (i.e., maintenance, growth, lactation, gestation, and fiber production), usually adopt data from sheep and cattle to fit prediction equations for mineral requirements, which may lead to inaccurate recommendations for goats, considering the differences in mineral metabolism (WILKENS et al., 2012; HERM et al., 2015) and tissue mineral composition (VAN NIEKERK et al., 1990; HAENLEIN & ANKE, 2011) between ruminant species.

In addition, the current nutritional systems (CSIRO, 2007; INRA, 2007; NRC, 2007) do not address the potential effect of sex and degree of maturity of the goat on the mineral requirement estimation. It could introduce an additional inaccuracy on these values, considering that goats of different sexes exhibit different mature weights (ALMEIDA et al., 2016; MARCONDES et al., 2016) as well as different patterns of growth (GHAVI, 2015).

Thus, it is necessary to define mineral requirements for goats without extrapolating information from other species, as well as to assess the effect of sex on mineral requirements, considering the impact of degree of maturity of the goat on body composition. Therefore, the objective of this study was to evaluate the effect of sex on the net macromineral requirements of Saanen goats from 5 to 45 kg BW, considering or not the degree of maturity on their estimations.

2. LITERATURE REVIEW

2.1 CLASSIFICATION AND FUNCTIONS OF MINERALS IN THE ANIMAL

The nutrients of feeds can be classified into organic or inorganic (SUTTLE, 2010). Organic nutrients such as carbohydrates, lipids, and proteins are catabolized through digestive processes, absorbed in small intestine, and the final products oxidized in the cells to produce energy in the form of ATP, which is used for the maintenance and growth of the animal tissues (NELSON & COX, 2012), and for production (SUTTLE, 2010). On the other hand, inorganic nutrients (i.e., minerals) are neither catabolized in simpler chemical forms, nor oxidized to produced energy, being absorbed in the digestive tract to constitute body tissues and mediate metabolic processes in the cells (JOHNSON et al., 2012).

The minerals are divided in macrominerals and microminerals, according to the amounts required by the animal. The macrominerals include Ca, P, Mg, Na, K, Cl, and S while the microminerals include Co, Cu, Cr, I, Fe, Mn, Se, and Zn (NRC, 2007). Thirteen elements are considered essential for certain types of animals under some conditions: Al, As, B, Cr, F, Pb, Li, Mo, Ni, Rb, Si, Sn, and V (SUTTLE, 2010) and the elements: F, Mo, Pb, As, Al, Cd, and Hg are known for their toxic effects (NRC, 2007).

Macro- and micromineral functions are clustered into four classes: structural, physiologic, catalytic, and regulatory. The structural function involves the maintenance of activity of structural tissues (such as bone and muscle tissues), where the main minerals participating are: Ca, P, Mg, F, Si, and S (SUTTLE, 2010). The physiological function of minerals is associated to the regulation of osmotic pressure, acid-base balance, and membrane permeability, where Na, K, Cl, Ca and Mg play important roles. The catalytic function involves the role of minerals as cofactors in metabolic reactions (e.g., Fe, Cu, Zn, Mn, Mo, and Se) constituting the structural components of metalloenzymes. Finally, the regulatory function concerns the modulation of replication and differentiation processes in the cells, where Ca and Zn participate markedly (KINCAID et al., 2000; SUTTLE, 2010).

2.2 IMPORTANCE AND METABOLIC FATES OF MACROMINERALS IN THE MAINTENANCE AND GROWTH OF TISSUES IN RUMINANTS

Despite minerals cannot be transformed into energy or macromolecules in the body, they actively contribute to nutrient utilization in the cells, and consequently, in the maintenance and growth of animal tissues (NRC, 2016).

The macromineral functions in the maintenance and growth of tissues, depend on the macromineral distribution in the body tissues (PARK, 1990). For instance, Ca is the most abundant mineral in the body, where 99% of total Ca is in bones and 1% as bivalent ion, linked to seric proteins, organic acids, and inorganic acids (SUTTLE, 2010). It is involved in both maintenance and growth of tissues, supporting bone metabolism, muscular contraction, nervous tissue activity, cellular signaling, and enzymatic activity regulation (CARAFOLI, 1991; HURWITZ, 1996).

Phosphorus (P) is the second most abundant mineral in the body. Nearly 80% of total P is stored in bones and teeth to maintain their structures (SUTTLE, 2010). The remainder 20% of P is in body fluids and soft tissues as part of phospholipids (i.e., lipids that maintain the membrane fluidity and nervous system melanization) and phosphate (PO_4^{3-}), which participates in osmotic pressure regulation, acid-basic equilibrium, and Na/K pump activity in the cells (NRC, 2007). Moreover, P is used for ATP synthesis, which is used in the maintenance and growth of tissues, participating in gluconeogenesis, *de novo* synthesis, protein synthesis, fatty acid transport, among other metabolic processes (TERNOUTH, 1990).

Magnesium (Mg) in the body is located into bones (60 - 70%), inside the cells (29%), and in extracellular fluids (1%; NRC, 2007). It participates in the regulation of both metabolic (e.g., Na/K pump activity, ATP synthesis, piruvate oxidation, PO_4^{3-} transfer reactions, and maintenance of membrane fluidity) and physiological (e.g., maintenance of rumen cellulolytic microflora, neuro-muscular electric conduction, and heart beats) functions in the animal (SHILS, 1997).

These metabolic and physiological functions are indistinctly associated to the maintenance and growth of tissues.

Potassium (K) in the body is the major cation in soft tissues (SUTTLE, 2010), in which the concentration of K is up to 30 times greater than in plasma (WARD, 1966). In this sense, K is required more in the maintenance of tissues than in the tissue growth, due to its role in acid-basic balance, Na^+/K^+ pump activity, and enzymatic activity involved in PO_4^{3}- transference, processes mainly linked to the maintenance of muscular tissues (PETERSEN, 1997).

Sodium (Na) and Cl are the main cation and anion, respectively, in extracellular fluids (SUTTLE, 2010). They operate as key components in mechanisms inherent to the body maintenance, controlling the cell osmolarity, and the Na/K pump activity along with K, resulting in the regulation of the glucose amino acids, and water transport through the cell membrane (KANEKO, 1989).

Sulfur (S) is essential for both the maintenance and growth of tissues, because their functions cover from protect tissues of oxidant and toxic molecules to regulate the synthesis of microbial protein in the rumen, which is the principal process for amino-acids supply to synthetize tissue proteins in the ruminant (SUTTLE, 2010).

Because of the association between macrominerals and metabolic processes that guide the maintenance and growth of tissues in the animal, it is essential to estimate accurately the macromineral requirements in the ruminant to preserve the animal health and production.

2.3 MACROMINERAL METABOLISM AND THEIR CONCENTRATIONS IN ANIMAL TISSUES DIFFER BETWEEN SHEEP AND GOATS: TWO REASONS TO DISCRIMINATE THEIR MACROMINERAL REQUIREMENTS

For a long time, equations to estimate the mineral requirements for the maintenance and growth of goats have been derived from adjustments mainly of sheep, given the scarcity in data from goats (AFRC, 1998; MESCHY, 2000; NRC, 2007). However, recent studies have demonstrated that nutrient partition between sheep and goats differ (WILKENS et al., 2012; SALAH et al., 2014; HÄRTER et al., 2016), which may lead to different concentrations of minerals in tissues and blood between sheep and goats. SHERIDAN et al., (2003) reported that goat carcasses of Boer goat kids had greater Ca, P, Mg, Na, and K levels than Merino Lamb carcasses, regardless of the diet offered. Similarly, SOWANDE et al. (2008) reported that Ca and K concentrations in plasma were greater in West Africa Dwarf (WAD) sheep than in WAD goats, whereas Mg and Na concentrations did not differ among these species, and P concentrations were greater in WAD goats than WAD sheep in dry season (Table 1). Other studies also reported differences between ruminant species with regard to milk mineral composition (KHAN et al., 2006; PARK et al., 2007; SLAČANAC et al., 2011). This indicate that the use of data of sheep to extrapolate minerals recommendations for goats may be inadequate.

MESCHY (2000) highlighted the need to make distinction among ruminant species regarding mineral requirement, because apparent absorption coefficient of P is greater for goats (70 - 75%) than other ruminants. It has been suggested that goats are less sensitive than sheep to high Cu (ZERVAS et al., 1989) and Mo (KESSLER, 1991) concentrations in diet. SCHRÖDER et al. (1999), WILKENS et al. (2010) and HERM et al. (2015) showed that sheep under Ca restriction, recover the Ca depletion in plasma increasing the Ca resorption from bone, which may be due to gastrointestinal Ca absorption in sheep is more insensitive than in goats under Ca restriction (WILKENS et al., 2011). In contrast to sheep, goats recover the Ca depletion in plasma causing the absorption of dietary Ca from the gastrointestinal tract and increasing its renal tubular reabsorption (SCHRÖDER et al., 1997). WILKENS et al. (2012) confirmed that goats are more efficient in compensating the Ca restriction than sheep, by increasing the Ca absorption and active transport in jejunum. KOHLER et al. (2013) showed that goats are less dependent of dietary Vitamin D from feedstuffs than sheep, whereas goats depend more of cutaneous synthesis of Vitamin D and HERM et al. (2015) suggested that differences in the expression of sodium/Ca exchanger type 1, Vitamin D receptors, and 24-hydrolase may potentially explain why goat have higher capacity to compensate for challenges of Ca homeostasis than sheep. Thus, it is remarkable the existing disparities in mineral metabolism and mineral concentration between sheep and goats, suggesting that the estimation of net or dietetic mineral requirements of goats adopting data from sheep may not be suitable.

Table 1. Mineral concentrations in carcass (mg/100g) and plasma (mmol/L) of goats and sheep.

Reference	Mineral	Tissue	Goats		Sheep	
			BGLE*	BGHE	MMLE	MMHE
SHERIDAN et al., (2003)	Ca	Carcass	$880.8^{ab} \pm 59.4$**	$946.5^{a} \pm 61.3$	$672.9^{c} \pm 65.8$	$723.9^{bc} \pm 59.4$
	P		$631.9^{a} \pm 37.7$	$653.7^{a} \pm 38.9$	$485.4^{b} \pm 41.8$	$510.4^{b} \pm 37.7$
	Mg		$32.51^{a} \pm 1.6$	$35.4^{a} \pm 1.6$	$24.3^{b} \pm 1.8$	$24.9^{b} \pm 1.6$
	Na		$56.7^{a} \pm 3.4$	$49.8^{ac} \pm 3.5$	$42.4^{bc} \pm 3.8$	$38.1^{b} \pm 3.4$
	K		$141.6^{a} \pm 6.5$	$130.9^{a} \pm 6.7$	$95.5^{b} \pm 7.2$	$86.2^{b} \pm 6.5$
SOWANDE et al., (2008)	Ca	Plasma	$5.01^{a} \pm 0.24$		$8.51^{b} \pm 0.19$	
	P		$3.41^{a} \pm 0.13$		$3.24^{b} \pm 0.01$	
	Mg		$0.70^{a} \pm 0.03$		$0.70^{a} \pm 0.02$	
	Na		$102.51^{a} \pm 1.91$		$101.17^{a} \pm 6.20$	
	K		$5.70^{a} \pm 0.09$		$6.02^{b} \pm 0.12$	

*BGLE: low energy diet given to Boer goats; BGHE: high energy diet given to Boer goats; MMLE: low energy diet given to Mutton Merinos; MMHE: high energy diet given to Mutton Merinos.

**Means in the same row with different superscripts are statistically different ($P < 0.05$). Tukey test was used in both publications.

2.4. SEX AND MATURITY STAGE EFFECTS ON MACROMINERAL REQUIREMENTS FOR RUMINANTS: A PERSPECTIVE

It has been reported that sex affects the secretion patterns of testosterone (from testicles) and estrogens (from ovaries) (ETHERTON & BAUMAN, 1998; PAULINO et al., 2009). The secretion of these steroids modulates the central effects of IGF-1, hormone that regulates the postnatal skeletal tissue growth and cell metabolism (WEBSTER, 1981; LAWRENCE et al., 2012). Therefore, considering that minerals play important roles in these processes, sex could affect mineral requirements for the animal.

Recent studies showed that not only sex but also maturity stage of the animal, must be considered to describe tissue growth, because they are linked each other (ALMEIDA et al., 2016; MARCONDES et al., 2016). It is known that animals of distinct mature weights exhibit different patterns of growth and body composition (OWENS et al., 1995; TEDESCHI et al., 2004). Hence, not only neglecting differences among sexes but also mature stage of the animal on nutritional requirements, may threaten the accuracy of recommendations.

Several studies have assessed the sex effect on mineral requirements in ruminant without considering the maturity stage of the animal in their calculations. PURCHAS (1991) showed that the total macromineral content of females was lower than males, suggesting that mineral requirements in cattle may differ between sexes. Similarly, FONTES (1995) showed that steers have smaller Ca and P requirements for maintenance than bulls, and GIONBELLI et al. (2010) reported net Na requirements for growth were different between males and females in Nellore and crossbred cattle. On the other hand, CHIZZOTTI et al. (2009) and MARCONDES et al., (2009) did not find evidence of sex effect on Ca, P, Mg, Na, and K requirements for growth of Nellore × Red Angus cattle.

In small ruminants, FERREIRA et al. (2016) showed that there was no effect of sex on Ca, P, Mg, and S requirements for growth of non-descript breed hair lambs (NDBL) from 15.5 to 29.2 kg BW. Similarly, MENDONÇA et al. (2017) showed that sex did not affect the Ca, P, Mg, Na, and K requirement for growth of Saanen goats from 5 to 15 kg BW. Conversely, SANTOS et al. (2016) found that intact male goats from 15 to 30 kg BW had greater net Na and K requirements for growth than castrated males and females, but sex did not affect the net Ca, P, and Mg requirements for growth, and the Ca, P, Mg, Na, and K requirements for maintenance. Similarly, SOARES (2013) showed that castrated male and intact male Saanen goats from 30 to 45 kg BW had higher net Na requirements for growth than females, however sex did not affect the net Ca, P, Mg, and K requirements for growth and the net Ca, P, Mg, Na, and K requirements for maintenance.

Therefore, published studies have found contradictory results regarding sex effect on macromineral requirements of ruminants. This controversy may be due to the incorrect comparison between animals of different mature sizes (WEBSTER, 1986), and because the existing databases of macromineral body composition of different sexes are small (GIONBELLI et al. 2010). Thus, sex effect on mineral requirements for ruminants could be addressed, predicting in a more realistic way the body mineral composition (i.e., considering the maturity stages of the animals) as well as gathering different studies through a meta-analysis. These two considerations may improve the robustness of the statistical analysis.

2.5. META-ANALYSIS AS A TOOL TO GET BETTER ESTIMATIONS OF MACROMINERAL REQUIREMENTS

The Agricultural Research Council (ARC, 1980) described the need to consider differences across studies when data are grouped to decrease the errors in macromineral requirement estimations. For example, for Ca fecal endogenous excretion in cattle (defined for this nutritional system as Ca requirement for maintenance), this pointed out that:

> *"Despite possible errors in interpretation arising <u>from systematic differences between experiments</u>, the effects on endogenous fecal excretion on the amounts of calcium ingested or absorbed, live weight and the age of the animal have been calculated from the pooled data"* (p. 185; ARC, 1980).

In the case of P requirements for maintenance in cattle, similar statement was set:

> *"the data summarized show considerable variation from experiment to experiment"* (p. 192; ARC, 1980).

For mineral requirements in sheep, the ARC, (1980), stated that:

> *"The range of sources was insufficient to justify the subdivision by breed type and sex, which was made for data on protein and fat concentration in sheep"*

To the date, any statistical technique to quantify the potential variation associated to these factors (i.e., differences across sources (experiments), breed, and sex), which may decrease the accuracy on mineral requirement estimations, was suggested. Indeed, the latest nutritional system for small ruminants (NRC, 2007) did not describe any statistical technique to isolate the potential variation across the studies from data were gathered, to estimate the mineral requirements in sheep, goats, and cervids.

Thus, it is worthwhile to apply statistical techniques when dataset from different experiments are pooled to get more accurate estimates of macromineral requirements. This effect that for our case will be defined as "study effect", is associated to several controllable and uncontrollable factors that differ across studies (ST-PIERRE, 2001; SAUVANT et al., 2008).

To overcome the barrier of differences between studies during analysis, the meta-analysis constitutes a robust statistical tool, that enable the analysis of pooled data gathered from different experiments isolating the study effect. In this regard, the meta-analysis allows the evaluation of specific factors across the studies to propose suitable generalizations to a broader range of scenarios.

While performing a meta-analysis, one should consider the mixed model structure to analyze the data. A mixed model has both fixed and random effects. Fixed effects are defined by the objectives of the study, and does not have probabilistic nature (e.g., sex, type of feed, level of nutrient inclusion, nutritional management factors, etc.). On the other hand, a random effect is defined as a random sample of possible factors (with probabilistic nature), when the best example is the study effect, because to conduct the meta-analysis is necessary to take a probabilistic sample of studies from a large population of studies related to the research objective (ST-PIERRE, 2001). Thus, a mixed model is mathematically defined as follows:

$$Y = X\beta + Zu + e$$

where:

Y is the vector of observations, X is the incidence matrix of known fixed effects, β is the vector of unknown fixed effects, Z is the incidence matrix of known random effects, u is the vector of unknown random effects, and e is the vector of random errors.

According to the model, the study effect (Zu) can be isolated and quantified to avoid its potential effect on the response variable, while evaluating the real effects of the objective factors (fixed effects) of the research. Therefore, the isolation of the study effect increases the accuracy on parameter estimation of mathematical models.

In this sense, meta-analysis permits to isolate the variation due to differences across studies. For additional details related to how to conduct a meta-analysis, ST-PIERRE (2007) and SAUVANT et al. (2008) describe several considerations to take into account before, during, and after its execution in animal science studies.

There are several examples of meta-analyses conducted to estimate nutrient requirements in ruminants. CHIZZOTTI et al. (2008) determined the net energy and protein requirements of growing bulls, steers, and heifers of Nellore purebred and Nellore × Bos Taurus cross breed using comparative slaughter studies, and SALAH et al. (2014) evaluated the potential differences between ruminant species, and estimated the net protein and energy requirements of sheep, goats, and cattle in warm climate changes. More recently, HÄRTER et al. (2016) conducted a meta-analysis to estimate the net energy and protein requirements of pregnant goats and sheep, indicating that protein and energy requirements for pregnancy differ between these species.

Regarding to macrominerals, meta-analysis evaluating potential animal factors (e.g., sex, breed, specie, etc) on their requirements in cattle and small ruminants have not been conducted until now. The use of meta-analysis to derive prediction equations of macromineral requirements for ruminants could be advantageous considering the lesser dataset availability of macromineral body composition data, where meta-analysis may permit to pool data from several studies, and consequently increase the accuracy on parameter estimations of prediction equations (ST-PIERRE, 2001).

For goats, there is an additional interest in using the meta-analysis as summarization technique to macromineral requirement estimation, because of the absence of substantial scientific reports on the mineral requirements in comparison to sheep and cattle. Thus, meta-analysis could lead to produce more accurate prediction equations for macromineral requirements for goats avoiding the use of data from cattle and sheep, as was in the past (ARC, 1980; AFRC, 1998; INRA, 2007; CSIRO, 2007; NRC, 2007).

In this sense, we evaluate the effect of sex on the net macromineral requirements of Saanen goats from 5 to 45 kg BW, considering or not the degree of maturity on the estimations, using the meta-analysis as a tool. Moreover, the effect of sex on macromineral retention efficiency was evaluated.

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CHAPTER 2 - SEX EFFECTS ON MACROMINERAL REQUIREMENTS FOR MAINTENANCE IN SAANEN GOATS: A META-ANALYSIS

ABSTRACT: The objective of this study was to evaluate the effect of sex on net Ca (NCa_m), P (NP_m), Mg (NMg_m), and K (NK_m) requirements for maintenance in Saanen goats from 5 to 45 kg BW. For this purpose, two datasets were used: the first dataset was composed by 154 individual records (53 castrated males, 46 females, and 55 intact males) from three comparative slaughter studies, being used to evaluate the effect of sex on NCa_m, NP_m, NMg_m, NK_m using the comparative slaughter method (CST). The second dataset was assembled with 155 individual records (67 castrated males, 40 females, and 48 intact males) from four feeding trials, being used to evaluate the effect of sex on NCa_m, NMg_m, and NK_m estimated using the minimum endogenous losses method (MEL). Both meta-analyses were performed using a mixed model, in which sex was considered as fixed effect, while study as random effect. Sex did not affect NCa_m, NP_m, and NK_m of Saanen goats from 5 to 45 kg BW, when estimated using CST ($P > 0.10$). The daily NCa_m, NP_m, and NK_m were 21.1, 22.8, and 3.99 mg/kg BW, respectively. On the other hand, sex affected the NMg_m of Saanen goats ($P = 0.077$). The daily NMg_m for castrated males and female Saanen goats were 1.39 mg/kg BW, while for intact males were 2.65 mg/kg BW. Sex did not affect NCa_m, NMg_m, and NK_m for Saanen goats from 5 to 45 kg BW when estimated using MEL ($P > 0.10$). The daily NCa_m, NMg_m, and NK_m values were 38.0, 7.45, and 25.2 mg/kg BW respectively. Thus, our results support that, when formulating diets for growing Saanen goat, Mg requirements for maintenance need to be adjusted based on sex, but not Ca, P, and K requirements for maintenance.

Key words: comparative slaughter, mineral requirement, minimum endogenous losses, Saanen, sex.

1. INTRODUCTION

Saanen goats breed is the most used in the world for goat milk production, with 900,301 animals around the world (FAOStat, 2017). Despite its worldwide importance, information on macromineral requirements for maintenance as well as the factors that may affect them is still scarce. Nowadays, the recommendations of the National Research Council (NRC, 2007) and Agricultural and Food Research Council (AFRC,

1998) are predominantly used to formulate macrominerals in the diet of Saanen goats. However, the equations proposed by these feeding systems for estimating the macromineral requirements for maintenance were fitted, mostly using data from sheep and cattle, which may not be suitable, because mineral body composition, absorption, and metabolism differ among ruminant species (Meschy, 2000; Sheridan et al., 2003; Wilkens et al., 2014).

In addition, due to the lack of studies on goat mineral requirements for maintenance, NRC and AFRC also did not consider on the estimations, the effect of breed and sex, which may affect the mineral requirements for maintenance as well. The breed or genotype of the goat affects the energy or protein requirements (Sahlu et al., 2004; NRC; 2007). In consequence, it is also expected a breed effect on mineral requirements. With respect to sex, it is expected that it affects mineral requirements as well, because males have greater testosterone levels than castrated males and females, and testosterone may modulate the mineral dynamics in the body (Lawrence et al., 2012). Thus, there is a need to accurately assess the mineral requirements for maintenance of Saanen goats, and to evaluate if sex may affect them.

Independent studies of macromineral requirements for maintenance with Saanen goats of different sexes and BW ranges were conducted at out institution. Gathering these studies and analyzing them through meta-analysis, may allow us to develop prediction equations with a broader BW range of application than individual studies (St-Pierre, 2001) and to accurately analyze the potential effect of sex on the macromineral requirements for maintenance of Saanen goats.

The objective of this study was to evaluate, through two meta-analyses, the effect of sex on the net Ca (NCa_m), P (NP_m), Mg (NMg_m), and K (NK_m) requirements for maintenance of Saanen goats from 5 to 45 kg BW. We hypothesize that macromineral requirements for maintenance of Saanen goats from 5 to 45 kg BW differ from those recommended by the conventional feeding system for goats (AFRC, 1998; NRC, 2007) as well as that sex affects the macromineral requirements for maintenance for Saanen goats.

2. MATERIALS AND METHODS

To conduct this study, the approval of the ethics committee on animal use was not necessary, because the data were collected from previously published sources

Development of the datasets

Two datasets were developed: the first dataset was used to evaluate the effect of sex on NCa_m, NP_m, NMg_m, and NK_m of Saanen goats, as well as on the retention efficiency of Ca (RECa), P (REP), Mg (REMg), and K (REK) (Costa e Silva et al., 2016), using the comparative slaughter method (CST) (Lofgreen and Garret, 1968). This dataset was assembled collecting data from three comparative slaughter studies (Soares, 2013; Mendonça, 2013; Santos, 2016), resulting in a dataset with 154 individual animal records (53 castrated males, 46 females, and 55 intact males; Table 1). The variables included were: sex (i.e., castrated male, female, and intact male), animal code, level of feed restriction (i.e., ad libitum, and 25 and 50% of feed restriction), block (i.e., each block in each study was formed by 3 goats submitted to 0 (i.e., ad libitum), 25 and 50% of feed restriction), days on feed, initial and final body weight (iBW and BW, respectively), empty BW (EBW), average daily gain (ADG), average BW ((iBW + BW)/2), Ca, P, Mg, and K retained in the body (Ca_{ret}, P_{ret}, Mg_{ret}, and K_{ret}, respectively), and the Ca, P, Mg, and K intakes (Ca_{int}, P_{int}, Mg_{int}, and K_{int}, respectively; Table 2). The Ca, P, Mg, and K composition of the diets fed to the animals ranged from 1.01 to 1.80 g Ca/kg DM, from 0.45 to 1.00 g P/kg DM, from 0.18 to 0.35 g Mg/kg DM, and from 1.37 to 2.39 g K/kg DM. The ratio of roughage to concentrate used was 50:50.

The second dataset was used to evaluate the effect of sex on the NCa_m, NMg_m, and NK_m in Saanen goats estimated using the minimum endogenous losses method (MEL) (Fernandes et al., 2012). This dataset was developed collecting data from four feeding trials (Ferreira, 2003; Mendonça, 2013; Soares, 2013; Santos, 2016), resulting in a dataset with 155 individual records (67 castrated males, 40 females, and 48 intact males; Table 3). The variables included were: study identification, author name, sex (i.e., castrated male, female, and intact male), animal code, restriction level (i.e., ad libitum, and 25, 30, 50, and 60% of feed restriction), and block (i.e., as defined previously for the first dataset), days in trial, BW, EBW, amounts of Ca, Mg, and K in feces and urine, as well as Ca_{int}, Mg_{int}, and K_{int} (Table 4).

Table 1. Summary of the dataset used in the meta-analysis developed, for estimating mineral requirements for maintenance and mineral retention efficiency in Saanen goats by the comparative slaughter method.

Reference	n[1]	Sex	Phase[2]	BW[3] (kg)	EBW (kg)	Age (days)
Mendonça (2013)	14	Castrated male	Suckling	6.18-16.7	4.11-13.7	102-114
	13	Female	Suckling	8.42-16.4	6.58-13.4	86-114
	19	Intact Male	Suckling	7.99-16.6	5.09-12.8	73-159
Soares (2013)	21	Castrated male	Weaned	28.0-47.4	23.0-39.7	297-431
	16	Female	Weaned	29.0-44.9	23.9-38.1	348-426
	18	Intact Male	Weaned	29.9-46.6	24.0-39.7	228-422
Santos (2016)	18	Castrated male	Weaned	19.1-32.6	14.9-26.4	149-204
	17	Female	Weaned	17.6-31.8	14.7-26.4	160-213
	18	Intact Male	Weaned	17.3-34.0	14.3-28.4	167-242

[1] Total number of animal records included in each study.

[2] Suckling refers to goats that were fed both milk and solid diet and weaned refers to goats that were fed just solid diet.

[3] BW = body weight; EBW = Empty BW.

Table 2. Descriptive statistics of dataset used, for estimating mineral requirements for maintenance and mineral retention efficiency in Saanen goats, using the comparative slaughter method.

	Mean	Min.	Max.	SD
Castrated males (n = 53)				
BW (kg)	26.3	6.18	47.4	11.6
EBW (kg)	21.8	4.11	39.7	10.0
ADG (g/d)	80.9	-16.8	216.8	56.6
Retained macrominerals (g/d)				
Ca	0.85	-0.27	3.32	0.86
P	0.42	-2.13	2.21	0.80
Mg	0.011	-0.038	0.070	0.024
K	0.16	-0.10	0.60	0.15
Macromineral intake (g/d)				
Ca	8.24	1.42	20.2	5.36
P	3.78	1.69	7.13	1.55
Mg	1.62	0.40	4.07	1.04
K	9.17	1.94	18.0	4.45
Females (n = 46)				
BW (kg)	25.8	8.42	44.9	10.5
EBW (kg)	21.4	6.58	38.1	9.4
ADG (g/d)	83.9	-9.34	183.7	48.1
Retained macrominerals (g/d)				
Ca	0.69	-0.76	3.31	0.75
P	0.31	-1.76	1.60	0.64
Mg	0.012	-0.022	0.068	0.019
K	0.15	-0.050	0.37	0.093
Macromineral intake (g/d)				
Ca	7.59	1.69	20.2	5.00
P	3.71	1.63	7.14	1.46
Mg	1.50	0.43	4.28	0.98
K	8.82	2.87	17.3	3.71
Intact males (n = 55)				
BW (kg)	25.6	7.99	46.6	11.6
EBW (kg)	21.1	5.09	39.7	10.2
ADG (g/d)	90.8	-13.6	198.8	52.1
Retained macrominerals (g/d)				
Ca	0.87	-0.17	4.50	0.76
P	0.54	-1.20	2.22	0.53
Mg	0.012	-0.087	0.061	0.029
K	0.19	-0.042	0.71	0.13
Macromineral intake (g/d)				
Ca	7.41	1.34	19.79	5.49
P	3.67	1.62	7.17	1.44
Mg	1.47	0.34	3.93	1.03
K	8.54	2.40	16.9	3.73

BW = body weight; EBW = empty BW; ADG = average daily gain; Min = minimum; Max = maximum; SD = standard deviation.

Table 3. Summary of the dataset used in the meta-analysis developed, for estimating mineral requirements for maintenance in Saanen goats by the minimum endogenous losses method.

Reference	n[1]	Sex	Phase[2]	BW[3] (kg)	EBW (kg)
Ferreira (2003)	18	Castrated male	Weaned	20.5-33.9	16.4-28.1
Mendonça (2013)	12	Castrated male	Suckling	7.75-12.9	6.55-10.4
	7	Female	Suckling	8.00-12.4	6.74-10.0
	12	Intact Male	Suckling	7.40-12.4	6.29-10.1
Soares (2013)	19	Castrated male	Weaned	27.3-40.3	22.3-33.6
	16	Female	Weaned	28.0-39.7	22.9-33.1
	18	Intact Male	Weaned	26.3-42.1	21.4-35.2
Santos (2016)	18	Castrated male	Weaned	16.2-25.5	12.7-20.8
	17	Female	Weaned	15.2-24.6	11.8-20.0
	18	Intact Male	Weaned	15.5-27.5	12.0-22.5

[1] Total number of animal records included in each study.

[2] Suckling refers to goats that were fed both milk and solid diet and weaned refers to goats that were fed just solid diet.

[3] BW = body weight; EBW = Empty BW.

Table 4. Descriptive statistics of dataset used for estimating mineral requirements for maintenance in Saanen goats using the minimum endogenous losses estimated by feeding trials.

	Mean	Min.	Max.	SD
Castrated males (n = 67)				
BW (kg)	25.2	7.75	40.3	9.94
EBW (kg)	19.5	6.55	33.6	7.46
Macromineral excretion (g/d)				
Ca	5.42	0.47	15.9	4.23
Mg	1.00	0.11	2.46	0.62
K	3.20	0.12	14.4	3.81
Macromineral intake (g/d)				
Ca	6.58	0.72	37.4	6.04
Mg	1.56	0.13	9.23	0.76
K	4.85	0.24	17.3	4.46
Females (n = 40)				
BW (kg)	26.2	8.00	39.7	11.3
EBW (kg)	19.3	6.74	33.1	7.74
Macromineral excretion (g/d)				
Ca	7.79	0.24	17.3	4.40
Mg	0.94	0.11	3.30	0.62
K	3.59	0.10	13.3	3.31
Macromineral intake (g/d)				
Ca	7.32	0.44	14.0	3.91
Mg	1.44	0.13	3.16	0.76
K	4.76	0.22	15.2	4.71
Intact males (n = 48)				
BW (kg)	24.6	7.40	42.1	12.8
EBW (kg)	18.8	6.29	35.2	8.77
Macromineral excretion (g/d)				
Ca	7.09	0.23	16.5	4.79
Mg	1.00	0.13	2.36	0.58
K	3.62	0.28	14.5	3.31
Macromineral intake (g/d)				
Ca	6.97	0.68	16.6	4.82
Mg	1.43	0.21	4.09	0.90
K	4.66	0.22	15.0	4.71

BW = body weight; Min = minimum; Max = maximum; SD = standard deviation.

The Ca, Mg, and K composition of the diets fed to the animals ranged from 0.53 to 1.80 g Ca/kg DM, from 0.15 to 0.35 g Mg/kg DM, and from 0.51 to 2.39 g K/kg DM. The ratio of roughage to concentrate used was 50:50. Because of the small number of data of P excretion and intake, as well as its high variation, it was not possible to fit reliable equations for describing P excretions on the intake. Therefore, the effect of sex on NP_m using the MEL was not analyzed.

Data Analysis and Calculations

Net Macromineral Requirements for Maintenance using the CST

The initial EBW from initial BW were estimated using the equations proposed by Souza et al., (2017), while the initial body Ca, P, Mg, and K amounts were estimated using the following equations, as described by Vargas et al., (2017):

$$Ca = 10^{0.987(\pm 0.0386)} \times EBW^{0.997(\pm 0.0291)} \text{ (All sexes)} \quad [1]$$

$$P = 10^{0.892(\pm 0.0383)} \times EBW^{0.998(\pm 0.0273)} \text{ (All sexes)} \quad [2]$$

$$Mg = 10^{-0.381(\pm 0.0917)} \times EBW^{1.035(\pm 0.0478)} \text{ (Castrated males)} \quad [3]$$

$$Mg = 10^{-0.376(\pm 0.0885)} \times EBW^{1.066(\pm 0.0445)} \text{ (Intact males)} \quad [4]$$

$$Mg = 10^{-0.289(\pm 0.0924)} \times EBW^{0.961(\pm 0.0490)} \text{ (Females)} \quad [5]$$

$$Na = 10^{0.330(\pm 0.0698)} \times EBW^{0.815(\pm 0.0401)} \text{ (All sexes)} \quad [6]$$

$$K = 10^{0.386(\pm 0.0564)} \times EBW^{0.851(\pm 0.0394)} \text{ (All sexes)} \quad [7]$$

The final macromineral body composition of each animal in all experiments was calculated from the chemical composition of the empty body. For this purpose, body samples were digested using a nitric-perchloric acid digestion (AOAC, 1990; method number 935.13); after, the amounts of Ca, Mg, and K were evaluated using atomic absorption spectroscopy (AOAC, 1990; method number 935.13), and the P content was quantified using colorimetry (AOAC, 1990; method number 965.17).

Macromineral body retention was calculated as the difference between the final macromineral body composition and the initial macromineral body composition. Net macromineral requirements for maintenance were assumed to be the intercept of the linear regression between the daily Ca_{ret}, P_{ret}, Mg_{ret}, and K_{ret} (g/kg EBW) and daily Ca_{int}, P_{int}, Mg_{int}, and K_{int} (g/kg EBW), respectively.

Net Macromineral Requirement for Maintenance using the MEL

A linear regression between the daily Ca, Mg, and K excreted in urine and feces (g/kg EBW) and daily Ca_{int}, Mg_{int}, and K_{int} (g/kg EBW) was used to calculate the net macromineral requirements for maintenance. The intercept of the regression equation was considered as the endogenous and metabolic losses of macromineral, which is assumed to represent the net macromineral requirements for maintenance.

Macromineral Retention Efficiencies

The RECa, REP, REMg, and REK were calculated by the ratios: Ca_{ret}/Ca_{int}, P_{ret}/P_{int}, Mg_{ret}/Mg_{int}, and K_{ret}/K_{int}, respectively. The daily Ca_{ret}, P_{ret}, Mg_{ret}, and K_{ret} (g/kg EBW) as well as the daily Ca_{int}, P_{int}, Mg_{int}, and K_{int} (g/kg EBW) were calculated as described in the comparative slaughter method section. In addition, the RECa, REP, REMg, and REK were regressed against average BW as well as were regressed against ADG, and sex effect on the parameter estimations for each model was evaluated, as described in the statistical analysis section.

Statistical Analysis

A mixed model was used assuming sex as fixed effect, while block nested, in study and sex crossed (block (study × sex)) was used as random effect (Littell et al., 2006).

The general statistical model was:

$$Y_{ijk} = a_0 + a_1X_{ijk} + a_2S_{k(ij)} + a_3S_{k(ij)}X_{ijk} + e_{ijk}, \qquad [8]$$

where Y_{ijk} = the dependent variable Y at sex j and block k in the study i; a_0 = the overall intercept; a_1 = the overall slope that result from regressing Y and X across all studies; X_{ijk} = the observed value X at sex j and block k in the study i; a_2 = the effect of block k nested in, study i and sex j crossed ($S_{k(ij)}$) on the intercept; a_3 = the effect of block k nested in, study i and sex j crossed ($S_{k(ij)}$) on the slope of the regression of Y on X; e_{ijk} = the random error.

An initial linear regression analysis was conducted using the MIXED procedure of SAS (SAS Inst. Inc., Carry, NC). From the Studentized residual plot of macromineral retentions, we observed that the assumption of homoscedasticity variance was not suited for representing the variability observed in the daily Ca_{ret}, P_{ret}, and Mg_{ret} (g/kg

EBW) (i.e., used in macromineral requirement estimation using CST), and for representing the variability observed in the daily Ca excretion (g/kg EBW) (i.e., used in macromineral requirement estimation using MEL). To solve this, we challenged the conventional assumption of homoscedasticity using the NLMIXED procedure of SAS (SAS Inst. Inc., Carry, NC; Appendix 1), testing the mathematical functions proposed by Araujo et al. (2015), to accommodate the heteroscedasticity of daily Ca_{ret}, P_{ret}, Mg_{ret}, and daily Ca excretion:

$$\sigma_e^2 = \sigma^2, \quad [9]$$

$$\sigma_e^2 = (\sigma_0^2) \times \exp(c \times \text{mineral intake}), \quad [10]$$

$$\sigma_e^2 = (\sigma^2) \times \mu^{2\varphi}, \quad [11]$$

where σ^2 is the homogeneous residual variance (Eq. [9]). The exponential variance (Eq. [10]) contains the starting residual variance (σ_0^2) which increases exponentially at a rate c proportional to mineral intake. Equation [11] represents the residual variance ($\sigma_e^2 = \sigma^2$), scaled by a power (φ) function of the expected mean, μ.

After analysis, the error variances (σ_e^2) of daily Ca_{ret}, P_{ret}, and Mg_{ret} were fitted to Eq. [10] whereas the σ_e^2 of daily Ca excretion was fitted to Eq. [11], selecting the equation with the lowest AIC_c in each case, according to recommendations provided by Araujo et al., (2015). On the other hand, the σ_e^2 of daily K_{ret} (i.e., used in macromineral requirement estimated by CST), daily Mg and K excretions (i.e., used in macromineral requirement estimated by MEL), as well as RECa, REP, REMg, and REK, were homogeneous, and therefore, the analysis were conducted using the MIXED procedure, as conventionally stated for linear mixed models.

3. RESULTS

Sex did not affect the intercept and the slope of the equations used to estimate NCa_m, NP_m, and NK_m by the CST (*P* > 0.10; Table 5). Thus, irrespective of sex, daily NCa_m, NP_m, and NK_m were 21.1, 22.8, and 3.99 mg/kg BW, respectively. On the other hand, sex affected the intercept (*P* = 0.045) and slope (*P* = 0.077) of the equations used to estimate the NMg_m (Table 5). From the pairwise comparisons, the slopes (*P* = 0.077) but not the intercept (*P* = 0.102) were different between intact males and females, the slope (*P* = 0.028) and intercept (*P* = 0.077) were different between intact males and castrated males, and the slope (*P* = 0.557) and intercept (*P* = 0.636) were not different between castrated males and females. Hence, we proposed one equation to estimate the NMg_m for castrated males and females, and other equation to estimate the NMg_m for intact males. The daily NMg_m for castrated males and females were 1.39 mg/kg BW, while for intact males were 2.65 mg/kg BW.

Sex did not affect the intercept and slope of the equations used to estimate NCa_m, NMg_m, and NK_m by the MEL ($P > 0.10$). Irrespective of sex, the daily NCa_m, NMg_m, and NK_m were 38.0, 7.45, and 25.2 mg/kg BW, respectively (Table 5).

It was found that RECa, REP (Figure 1) as well as REMg and REK (Figure 2) were linearly proportional to average BW ($P < 0.01$), and sex did not affect these relationships ($P > 0.10$). Similarly, RECa and REP (Figure 3), as well as REMg and REK (Figure 4) were linearly proportional to ADG ($P < 0.01$), and sex did not affect these relationships as well ($P > 0.10$). The resultant regression equations of RECa, REP, REMg, and REK against ADG, allowed to predict the macromineral retention efficiencies as a function of ADG, irrespective of sex (Figures 3 and 4). Additionally, it was found that RECa, REP, REMg, and REK at maintenance level in Saanen goats were 9.91 (±2.14), 15.0 (±3.77), 0.35 (±0.16), and 0.93 (±0.21) % (i.e., intercept of equations of mineral retention efficiency prediction from ADG; Figures 3 and 4). The RECa, REP, REMg, and REK at maintenance level were calculated, assuming that an animal is in a maintenance level when ADG = 0 (Luo et al., 2004).

4. DISCUSSION

The current feeding systems for goats (AFRC, 1998; NRC, 2007) provides mineral requirement for maintenance recommendations, adopting data from sheep and cattle, which may compromise the accuracy of the value reported for goats. Also, they do not discriminate between stage of development, sex, and breed. Therefore, the major contributions of this study are the estimation of mineral requirements for maintenance of Saanen goats using animals from early to mature stage of development (i.e., from 5 to 45 kg BW) and two methods of estimation (i.e, CST and MEL), as well as the evaluation of sex effects on the macromineral requirements for maintenance.

Table 5. Daily net macromineral requirements for the maintenance of Saanen goats of different sexes using the comparative slaughter (CST) and minimum endogenous losses (MEL) methods.

Item[1]	b_0	b_1	Daily net requirement (mg/kg EBW)	Daily net requirement[3], (mg/kg BW)	σ_e^2[4]	*P*-value (Sex effect)	
						a	b
	CST[1]						
Ca	-0.0253 (±0.0134)	0.202 (±0.039)	25.3	21.1	Eq. [10]	0.779	0.929
P	-0.0274 (±0.0113)	0.418 (±0.085)	27.4	22.8	Eq. [10]	0.871	0.937
Mg					Eq. [10]	0.045	0.077
Castrated males and Females	-0.00167 (±0.0005)	0.0299 (±0.0060)	1.67	1.39			
Intact males	-0.00318 (±0.0006)	0.0490 (±0.0074)	3.18	2.65			
K	-0.00479 (±0.0016)	0.0278 (±0.0023)	4.79	3.99	3.00×10^{-5}	0.292	0.200
	MEL[2]						
Ca	0.0456 (±0.0205)	0.592 (±0.080)	45.6	38.0	Eq. [11]	0.571	0.861
Mg	0.00894 (±0.0050)	0.639 (±0.067)	8.94	7.45	1.38×10^{-4}	0.138	0.111
K	0.0303 (±0.0171)	0.566 (±0.039)	30.3	25.2	6.55×10^{-3}	0.176	0.268

[1] Daily Mineral retention (g mineral/kg EBW) = $b_0 + b_1 \times$ daily mineral intake (g mineral/kg EBW), where EBW = empty BW. Intercepts and slopes were significant at $P < 0.01$.

[2] Feces + urine mineral excretion (g mineral/kg EBW) = $b_0 + b_1 \times$ mineral intake (g mineral/kg EBW). Intercepts and slopes were significant at $P < 0.01$.

[3] Conversion factor from EBW to BW units = Net requirement in EBW units × 83.3% (Souza et al., 2017).

[4] Mathematical functions used for modeling the error variance (σ_e^2) structure: Eq. [10] $\sigma_e^2 = (\sigma_0^2) \times \exp(\mathrm{c} \times \text{mineral intake})$, and Eq. [11] $\sigma_e^2 = (\sigma_0^2) \times \mu^{2\varphi}$, where σ_0^2, c, and φ are parameters.

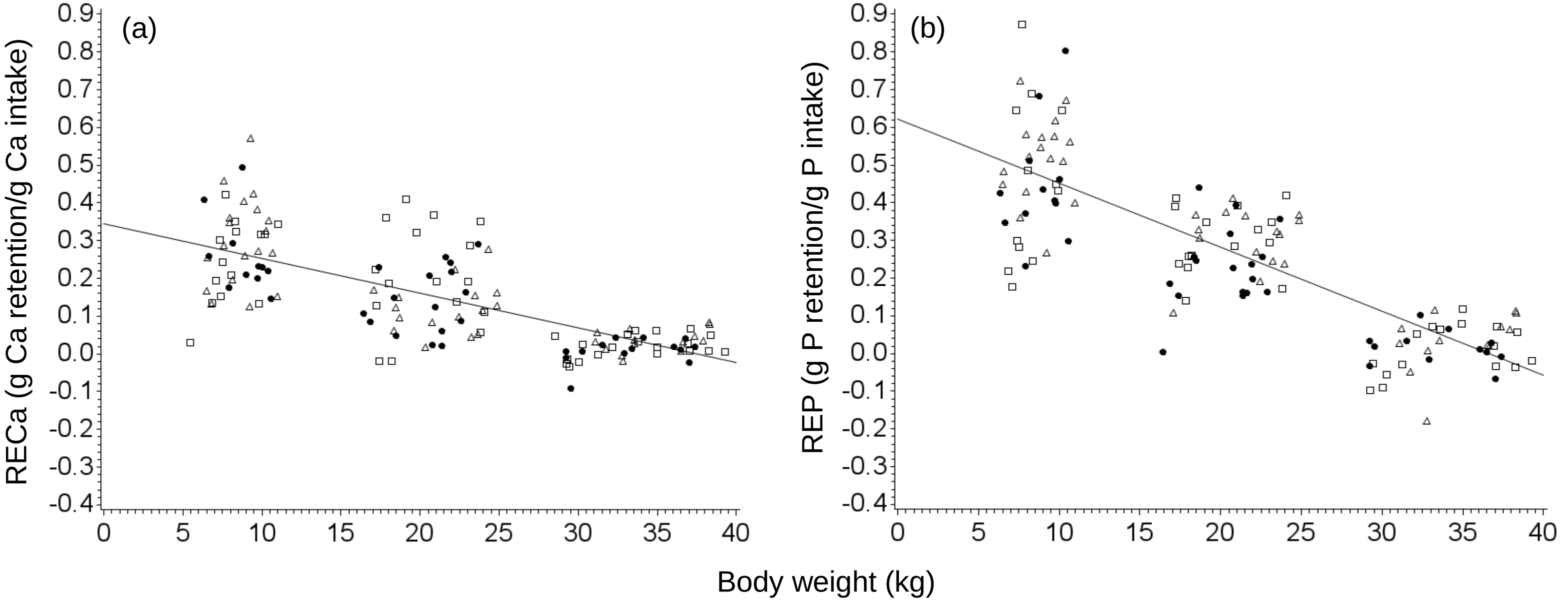

Figure 1. Relationship between retention efficiencies of Ca (RECa) and P (REP) (g mineral retention/g mineral intake) and average body weight (kg) in growing Saanen goats of different sexes (□ castrated males, ∆ intact males, and ● females). Intercepts and slopes were significantly different from zero (*P* < 0.01). For both macrominerals, intercepts and slopes were not different between sexes (*P* > 0.10): a) for Ca, RECa = 0.323 (±0.0201) - 0.00872 (±0.000665) × average body weight (RMSE = 0.169), and b) for P, REP = 0.609 (±0.0300) - 0.0167 (±0.00112) × average body weight (RMSE = 0.242). The observations were adjusted by the study effect.

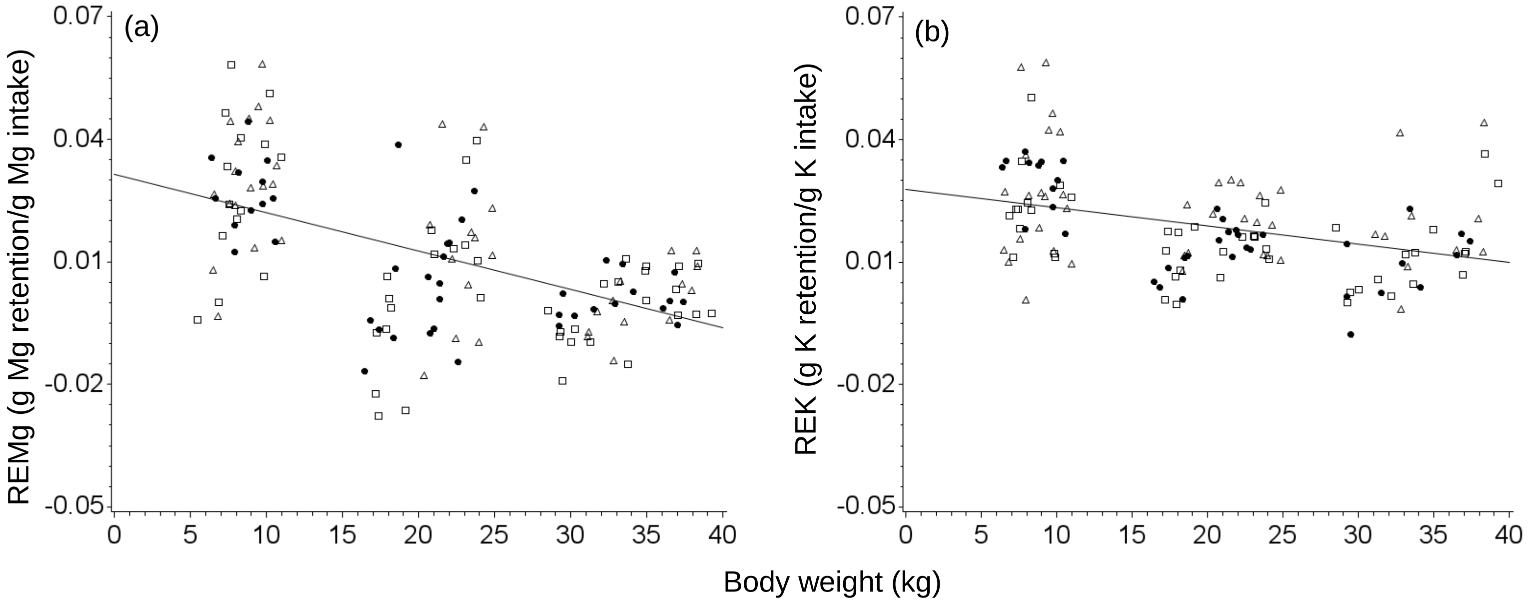

Figure 2. Relationship between retention efficiencies of Mg (REMg) and K (REK) (g mineral retention/g mineral intake) and average body weight (kg) in growing Saanen goats of different sexes (□ castrated males, Δ intact males, and ● females). Intercepts and slopes were significantly different from zero (*P* < 0.01). For both macrominerals, intercepts and slopes were not different between sexes (*P* > 0.10): a) for Mg, REMg = 0.0296 (±0.00329) - 0.00085 (±0.000112) × average body weight (RMSE = 0.0279), and b) for K, REK = 0.0240 (±0.00260) - 0.00034 (±0.000115) × average body weight (RMSE = 0.0199). The observations were adjusted by the study effect.

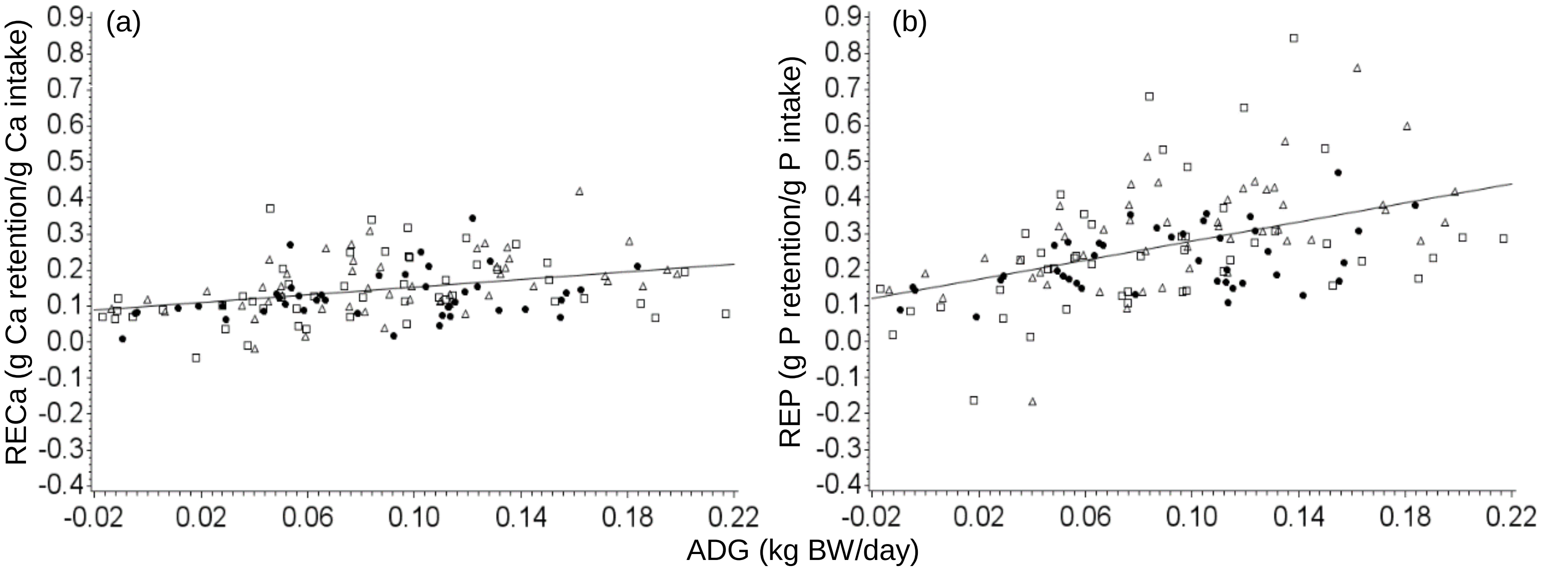

Figure 3. Relationship between retention efficiencies of Ca (RECa) and P (REP) (g mineral retention/g mineral intake) and average daily gain (ADG; kg BW/day) in growing Saanen goats of different sexes (□ castrated males, ∆ intact males, and ● females). Intercepts and slopes were significantly different from zero (*P* < 0.01). For both macrominerals, intercepts and slopes were not different between sexes (*P* > 0.10): a) for Ca, RECa = 0.0991 (±0.0214) + 0.511 (±0.167) × ADG (RMSE = 0.0888), and b) for P, REP = 0.150 (±0.0377) + 1.245 (±0.299) × ADG (RMSE = 0.155). The observations were adjusted by the study effect.

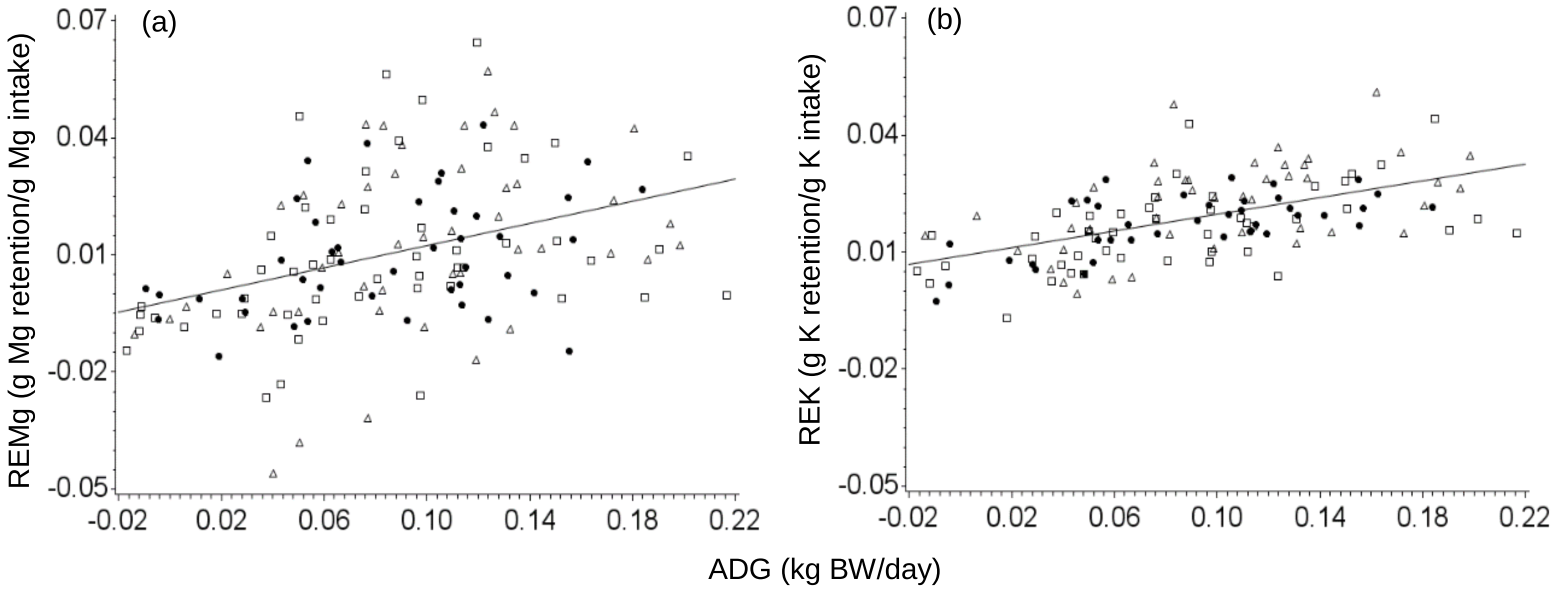

Figure 4. Relationship between retention efficiencies of Mg (REMg) and K (REK) (g mineral retention/g mineral intake) and average daily gain (ADG; kg BW/day) in growing Saanen goats of different sexes (□ castrated males, Δ intact males, and ● females). Intercepts and slopes were significantly different from zero (*P* < 0.01). For both macrominerals, intercepts and slopes were not different between sexes (*P* > 0.10): a) for Mg, REMg = -0.00347 (±0.00161) + 0.0833 (±0.0159) × ADG (RMSE = 0.0354), and b) for K, REK = 0.00928 (±0.00209) + 0.103 (±0.0183) × ADG (RMSE = 0.00964). The observations were adjusted by the study effect.

Our study showed that sex did not influence the NCa_m, NP_m, and NK_m regardless of estimation method used (i.e., CST or MEL) as well as the NP_m, estimated using CST, for Saanen goats from 5 to 45 kg BW. This absence of sex effect, may be due to the specific location of Ca, P, and K in the body and their function in the maintenance of tissues. The Ca and P are mainly located into bones (99 and 80%, respectively), interacting in a dynamic state to maintain biomembrane structures, and to regulate enzymatic activity into the cell (Lehninger et al., 2005; Wilkens et al., 2012). On the other hand, K is mainly located into intracellular fluids in the cells, regulating the osmolarity and acid-basic balance regulation, as well as in the activity of PO_4^{3-} transference enzymes (Petersen, 1997). Nowadays, it has not been suggested in the literature that sex influence the mentioned functions during growing phase, which agrees with our results of the lack of sex effect on NCa_m, NP_m, and NK_m.

On the other hand, a potential link between the role of Mg in energetic metabolism and differences between sexes in energy requirements for maintenance, may explain sex effect on NMg_m in Saanen goats when estimated by CST. The Mg is mainly involved into the activity of around 300 enzymes used for ATP-dependent membrane transport and production, supporting the maintenance of muscular tissues (Shils, 1997). According to NRC (2007), intact males have a 15% greater energy requirement for maintenance than females, being associated it to body protein of intact males that is greater than of females (Webster, 1981; Geay, 1984). Considering the role of Mg in energy metabolism into the cell, we may suggest that NMg_m of intact males was greater than those of castrated males and females due to the differences in body protein contents between sexes.

We may expect that if sex affects NMg_m estimated using CST then it should also affect NMg_m estimated using MEL, but contrary to our expectations, sex did not influence it. We suggest that this difference between estimation methods, may be due to the uncertainty of Mg excretion (i.e., variable used in NMg_m estimated using MEL) is greater than of Mg retention in the body (i.e., variable used in NMg_m estimated using CST). This assumption is in accordance with ARC, (1980), which reported that determination of Mg excretion may be subjected to high variation, due to Mg low amounts excreted in ruminants. Thus, the use of CST for evaluating the effect of sex on NMg_m in ruminants could be better than the use of MEL, and could be better to test the sex effects on other mineral requirements for maintenance as well. However, additional studies are necessary to confirm these assumptions.

Differences between mineral requirement estimation methods have not been explored in goats from early to mature life, despite the need to define mineral requirements accurately for this specie. We found that NCa_m, NMg_m, and NK_m estimated using MEL were greater than those estimated using CST. Like our results, Fernandes et al. (2012) found for intact male ¾ Boer × ¼ Saanen goat from 20 to 35 kg BW, that NCa_m, NMg_m, and NK_m estimated using MEL were greater than those estimated using CST. These differences in mineral requirement estimates between methods, may be due to endogenous losses are constituted by the sum of fecal and urinary fractions. In consequence, it is necessary to take in mind that the mineral endogenous losses may represent not only the net requirements for maintenance, but may include an excretory component represented by mineral excess absorbed by the animal (Meschy, 2000). Therefore, requirements obtained by MEL method need to be carefully interpreted.

Regarding feeding systems, the AFRC (1998) and NRC (2007) assume that NCa_m and NP_m of sheep may be extrapolated to goats. However, Kohler et al. (2013), Wilkens et al. (2014), and Herm et al. (2015) demonstrated that Ca and P metabolism differs between sheep and goats, which explain why NCa_m regardless of estimation method used as well as the NP_m estimated using CST were greater and lower, respectively, than AFRC (1998) and NRC (2007) recommendations (20 mg Ca/kg BW; 30 mg P/kg BW). Similarly, NMg_m and NK_m regardless of estimation method used, were different from the general values suggested by AFRC (1998) and NRC (2007) of 3.5 mg Mg/kg BW and 38 mg K/kg BW which were estimated using studies with cattle, sheep, and goats. These differences may originate from the fact that Mg and K concentrations in plasma and carcass differ between cattle, sheep, and goats (Sheridan et al., 2003; Sowande et al., 2008), suggesting potential differences in Mg and K metabolism between ruminant species as well. This further demonstrates the need of reports of macromineral requirements for maintenance using exclusively data from goats.

The most recent nutritional systems for goats (AFRC, 1998; NRC, 2007) describe the mineral utilization (i.e., absorption coefficients) for this specie based on values of mineral excretion and intake from feeding trials. However, feeding trials present important limitations, including possible mineral intake overestimation and output underestimation, as well as the influence of environmental conditions (Kopple, 1987). On the other hand, mineral body composition is less affected by mechanical or environmental factors such as diet and production system (Williams et al., 1983). In consequence, mineral retention efficiency (i.e., mineral retention/mineral intake) in the body may help to describe more accurately the mineral utilization in goats.

We found that all mineral retention efficiencies, decreased linearly with the increase of average BW, regardless of sex. It is known that there is a directly proportional relation between the age and BW. Therefore, our results suggest that young animals have a greater mineral retention efficiency than old animals. In consequence, dietetic macromineral requirements should be adjusted considering the animal age. Similarly, we found that all mineral retention efficiencies increased linearly with the increase of ADG, regardless of sex. Hence, animals with greater ADG presented greater mineral retention efficiency. It makes biological sense, considering that mineral demands in the body increase as an animal growth (Suttle, 2010). Thus, our prediction equations of mineral retention efficiency may be used for evaluating the mineral utilization at different average BW (i.e., proportional to age) and ADG as well as in formulation programs to convert net mineral requirements for maintenance into dietetic mineral requirements for maintenance, reducing not only production cost but also the excretion of inorganic elements to the environment, without detrimental effects on animal production (Chizzotti et al., 2009; Suttle, 2010).

The AFRC (1998) and NRC (2007) do not discriminate mineral utilization between maintenance, gain, or lactation, reporting the absorption coefficient as a unique value. However, we found from the relation between the RECa, REP, REMg, and REK and ADG, that a retention efficiency for each macromineral at maintenance stage can be calculated, which were lower than those predicted when ADG > 0. It suggests that contrary to AFRC (1998) and NRC (2007) assumptions, mineral utilization in the body may differ between the productive stages in the animal (e.g., maintenance, growth, lactation, gestation or wool production), which would be necessary to consider for producing better mineral requirement recommendations. Thus, further studies should be conducted to better estimate the mineral utilization of goats at different productive stages.

To our knowledge, our study performed the first two meta-analyses to estimate macromineral requirements for maintenance of Saanen goats from 5 to 45 kg BW by the comparative slaughter and minimum endogenous losses methods. Therefore, net requirements for maintenance found in the present study may be relevant to optimize mineral nutrition management in early and growing phase of Saanen goats.

Our results support the hypothesis that macromineral requirements for maintenance of Saanen goats are different from those recommended by AFRC (1998) and NRC (2007). Also, that the Ca, P, and K requirements for maintenance must not be adjusted based on sex. Conversely, Mg requirements differ between sexes. In addition, Ca, P, Mg, and K retention efficiency can be predicted using average BW and ADG as a regressors, regardless of sex in growing Saanen goats.

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CHAPTER 3 - SEX EFFECTS ON MACROMINERAL REQUIREMENTS FOR GROWTH IN SAANEN GOATS: A META-ANALYSIS

ABSTRACT: The aim of this study was to investigate the effects of sex on the net requirements of growth for Ca (NCa_g), P (NP_g), Na (NNa_g), K (NK_g), and Mg (NMg_g) in Saanen goats from 5 to 45 kg body weight (BW), with or without consideration of the degree of maturity. A database containing 209 individual records for Saanen goats (69 castrated males, 71 intact males, and 69 females) was generated from six comparative slaughter studies. Total amounts of Ca, P, Na, K, and Mg in the body were fitted to logarithmized allometric equations using empty BW (EBW) or degree of maturity (EBW/mature EBW) as regressors. The equations were fitted using a mixed model, where sex was considered a fixed effect and study was considered a random effect. Net requirements were estimated by the first derivative of the logarithmized allometric equations. Then, a Monte Carlo simulation was used to assess the 95% confidence limits of macromineral requirements. Without considering the degree of maturity, sex did not affect the NCa_g, NP_g, NNa_g, and NK_g ($P > 0.10$). The NCa_g and NP_g remained constant, whereas NNa_g and NK_g decreased by 32 and 27%, respectively, from 5 to 45 kg BW. Conversely, sex affected the NMg_g ($P = 0.054$), where that of castrated and intact males was 8 and 18%, respectively, greater than that of female goats. The NMg_g of castrated and intact males increased by 8 and 15%, respectively, whereas that of females decreased by 8% from 5 to 45 kg BW. Considering the degree of maturity, sex affected all net macromineral requirements for growth ($P < 0.10$). The NCa_g and NP_g of intact males were 5 and 2%, respectively, greater than those of castrated males and females. Additionally, the NCa_g and NP_g remained constant from 5 to 45 kg BW across sexes. The NNa_g of males was 6% greater than that of females. Irrespective of sex, NNa_g decreased by 32% from 5 to 45 kg BW. Regardless of sex, NK_g decreased by 26% from 5 to 45 kg BW. The NMg_g of castrated and intact males was 7 and 17%, respectively, greater than that of female goats. The NMg_g of castrated and intact males increased 8 and 16%, respectively, whereas that of females decreased by 7% from 5 to 45 kg BW. The results of this study indicate that consideration of maturity highlights differences across sexes in the net macromineral requirements for growth in goats. Elucidation of sex effects on macromineral requirements for growth may be useful for improving the accuracy of recommendations for mineral requirements for dairy goats.

Key words: allometry, comparative slaughter, mature weight, mineral requirement, uncertainty.

1. INTRODUCTION

Animal production, reproduction, and immunity are negatively affected when the mineral status in the body falls outside the adequate range (NRC, 2007); therefore, a proper assessment of the mineral requirements of goats is crucial. However, information regarding the mineral requirements for growth in goats is scarce, leading to the use of recommendations based on cattle and sheep (ARC, 1980; AFRC, 1998; NRC, 2007). This may not be suitable, because mineral body composition, absorption, and metabolism differ among ruminant species (Meschy, 2000; Sheridan et al., 2003; Wilkens et al., 2014).

Besides species, sex may influence the mineral requirements because of its influence on body composition (NRC, 2007). Regarding the effect of sex on the mineral requirements of goats, contradictory results have recently been reported. Mendonça et al. (2017) reported the absence of sex effects on Ca, P, Na, K, and Mg requirements for growth in young Saanen goat kids. Conversely, Santos et al. (2016) reported that sex affected the Na and K requirements for growth, but not the Ca, P, and Mg requirements in Saanen goats at 15 to 30 kg BW. These contradictory results may be due to comparisons between sexes being performed using animals at different stages of maturity.

Different sexes of Saanen goats present different mature weights (Almeida et al., 2016). Mature weight may be used to scale animal growth, as patterns of growth and body composition differ between animals at distinct stages of maturity (Tedeschi et al., 2004). Hence, consideration of maturity stage on mineral requirements may yield more suitable comparisons between sexes, which may be critical for clarifying sex effects on the mineral requirements of goats. Therefore, the aim of this study was to investigate the effects of sex on the net requirements for growth of Ca (**NCa_g**), P (**NP_g**), Na (**NNa_g**), K (**NK_g**), and Mg (**NMg_g**) for Saanen goats from 5 to 45 kg BW, with or without consideration of the degree of maturity.

2. MATERIALS AND METHODS

Data Collection

A database containing 209 individual records from six comparative slaughter studies (Ferreira, 2003; Figueiredo, 2011; Gomes et al., 2011; Soares, 2013; Santos et al., 2016; Mendonça et al., 2017; Table 1) was constructed. The studies were conducted at the goat facilities of the Universidade Estadual Paulista (Unesp, Jaboticabal Campus, SP, Brazil). Data on the mineral body composition of castrated male (n = 69), intact male (n = 71), and female (n = 69) Saanen goats fed ad libitum were used to estimate net mineral requirements (Table 2). In all studies, the empty BW (**EBW**) for each animal was calculated by subtracting the weight of the gastrointestinal, bladder, and gallbladder contents from the BW. Body samples were digested by nitric-perchloric acid wet digestion (AOAC, 1990; method number 935.13), the contents of Ca, Na, K, and Mg were determined by atomic absorption (AOAC, 1990; method number 935.13), and the contents of P were determined by colorimetry (AOAC, 1990; method number 965.17).

The energy, protein, Ca, P, Na, K, and Mg composition of the diets fed to the animals ranged from 2.4 to 2.7 Mcal/kg ME, from 147 to 204 g CP/kg DM, from 0.77 to 1.90 g Ca/kg DM, from 0.41 to 1.00 g P/kg DM, from 0.30 to 0.62 g Na/kg DM, from 0.66 to 1.53 g K/kg DM, and from 0.18 to 0.35 g Mg/kg DM. The ratio of roughage-to-concentrate ranged between 25:75 and 50:50. All procedures used across studies were reviewed by the University's Animal Care Committee (Comissão de Ética e Bem-Estar Animal – CEBEA).

Statistical Analyses and Parameter Estimation

Net Macromineral Requirements for Growth (Classical Approach). Total mineral contents (Ca, P, Na, K, and Mg) in the EBW were fitted to the allometric equation (Eq. [1]; Huxley, 1932), and logarithmized allometric equation (Eq. [2]; Zar, 1968), with the %NLINMIX macro and MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.4), respectively.

$$Y_{ijk} = B_{0_i} \times EBW_{ijk}^{B_{1_i}} , \qquad [1]$$

$$Log_{10}Y_{ijk} = B^{*}_{0_i} + B_{1_i} \times Log_{10}EBW_{ijk} , \qquad [2]$$

where, Y_{ijk} is the total body content of Ca, P, Na, K, or Mg expressed in grams for the k^{th} animal of the i^{th} sex in the j^{th} study, B_{0i}, B^{*}_{0i}, and B_{1i} are the parameters to be estimated for each of the i = 1, 2, 3 sexes. The ‘asterisk’ symbol was used in Eq. [2] to clarify that, although the B_{0i} and B^{*}_{0i} are mathematically equivalent in both equations, their estimates are not statistically equal.

We selected Eq. [2] to fit the Ca, P, Na, K, and Mg mineral contents in the body, because Eq. [2] presented a better normal distribution of the errors and a more homogeneous pattern of standardized conditional residuals than Eq. [1]. In addition, we improved the homoscedasticity of standardized conditional residuals of Eq. [2], weighing the least-square means by the quotient between the SE of each mean and the mean of all SE, using the WEIGHT statement in the MIXED procedure of SAS (St-Pierre, 2001; Appendix 2). Data were analyzed using a mixed model, including the fixed effect of sex, and random effects of study, and residual error (Eq. [3]).

$$Y_{ijk} = B^{*}_{0_i} + B_{1_i} EBW_{ijk} + B^{*}_{0_i} S_j + B_{1_i} S_j EBW_{ijk} + e_{ijk}\ , \qquad [3]$$

where, Y_{ijk} is the total body content of Ca, P, Na, K, or Mg expressed in grams for the k^{th} animal of the i^{th} sex in the j^{th} study, B^{*}_{0i} and B_{1i} are the parameters to be estimated for each of the i = 1, 2, 3 sexes, produced by the regression between Y_{ijk} and EBW_{ijk} of studies, S_j is the study effect of the j^{th} study on the intercept and the slope, and e_{ijk} is the residual error. Outliers were removed when the studentized residuals were > |2.5| (St-Pierre, 2007). To fit body Ca, P, Na, K, and Mg, six, five, one, one, and four outliers were removed, respectively, from three different studies (Figueiredo, 2011; Soares, 2013; Santos et al., 2016). The level of significance was $P < 0.10$.

When the sex effect was significant, suggesting that at least two intercepts differed between sexes, three CONTRAST statements were applied to conduct pairwise comparisons of sex. Furthermore, three CONTRAST statements were applied to conduct pairwise comparisons when the interaction between sex and EBW was found to be significant, indicating that at least two slopes differed between sexes (St-Pierre, 2001).

NCa_g, NP_g, NNa_g, NK_g, and NMg_g were estimated by the first derivative of the logarithmized allometric equation (Eq. [2]) with respect to EBW (Eq. [4]).

$$\frac{dY}{dEBW} = B_{1_i} \times 10^{B^{*}{}_{0_i}} \times EBW^{(B_{1_i}-1)}, \qquad [4]$$

where the net macromineral requirements are expressed in grams per kilogram of EBW gained (**EWG**), and with the explicit understanding that either $B^{*}{}_{0i}$ or B_{1i}, or both, can differ between sexes (Appendix 3).

Net Macromineral Requirements for Growth (Degree of Maturity Approach). After applying the same criteria described above to select Eq. [2] as the best model to fit body mineral composition to EBW (i.e., classical approach), we selected the logarithmized allometric equation to fit the body mineral composition with respect to degree of maturity (Eq. [5]).

$$Log_{10}Y_{ijk} = B^{**}{}_{0_i} + B_{1_i} \times Log_{10}(Degree\ of\ maturity)_{ijk}, \qquad [5]$$

where, Y_{ijk} is the value of the dependent variable (total body content of Ca, P, Na, K, and Mg expressed in grams), and degree of maturity is defined as the ratio between EBW and mature EBW (i.e., mature EBW for castrated males, intact males, and females was 34.9, 42.6, and 26.0 kg, respectively; Almeida et al., 2016) for the k^{th} animal of the i^{th} sex in the j^{th} study, and $B^{**}{}_{0i}$, and B_{1i} are parameters to be estimated for each sex. A 'double asterisk' was used in Eq. [5] to show that although $B^{**}{}_{0i}$ is mathematically equivalent to $B^{*}{}_{0i}$ in Eq. [2], their estimates are not statistically equal.

The statistical model and CONTRAST statements used to analyze data, as well as the number of outliers removed to fit body Ca, P, Na, K, and Mg with respect to the degree of maturity, were the same as those described for the classical approach. The level of significance was also declared at $P < 0.10$.

NCa_g, NP_g, NNa_g, NK_g, and NMg_g were estimated by the first derivative of the logarithmized allometric equation (Eq. [5]) with respect to EBW (Eq. [6]).

$$\frac{dY}{d(EBW)} = \frac{B_{1_i} \times 10^{B^{**}{}_{0_i}}}{(mature\ EBW)^{B_{1_i}}} \times EBW^{(B_{1_i}-1)}, \qquad [6]$$

where, the net macromineral requirements are expressed in grams per kilogram of EWG, and with the explicit understanding that mature EBW is constant for each sex, and either $B^{**}{}_{0i}$ or B_{1i}, or both, can differ between sexes (Appendix 3).

Assessing the Net Macrominerals Requirements for Growth Variation. A Monte Carlo-based simulation was used to calculate numerical estimates of the variance and confidence intervals for each EBW for which a net macromineral requirement was calculated. The values of B_0, $\ddot{B}_0$, and B_1, the SE of each parameter in the logarithmized allometric equation, as well as the correlation between B_0 and B_1 (i.e., classical approach) or $\ddot{B}_0$ and B_1 (i.e., degree of maturity approach) were inserted in the algorithm described by Fan et al. (2008), and 10,000 simulated values of net requirements were generated using a multivariate normal distribution.

3. RESULTS

Net Macromineral Requirements for Growth by the Classical Approach

Net Ca, P, Na, and K Requirements. Sex did not affect both parameters, intercept and slope ($P > 0.10$), of the logarithmized allometric equations to estimate the Ca (Fig. 1A), P (Fig. 1B), Na (Fig. 1C), and K (Fig. 1D) in the body of Saanen goats; therefore, allometric equations were generated for each macromineral regardless of sex. Accordingly, equations to estimate NCa_g (Fig. 2A), NP_g (Fig. 2B), NNa_g (Fig. 2C), and NK_g (Fig. 2D) of Saanen goats were proposed. In this regard, NCa_g (g/kg EWG; Fig. 2A) and NP_g (g/kg EWG; Fig. 2A and 2B) remained constant, whereas the NNa_g (g/kg EWG; Fig. 2C) and NK_g (g/kg EWG; Fig. 2D) decreased by 32 and 27%, respectively, when goats grew from 5 to 45 kg BW. Additionally, when the 95% confidence limits of NCa_g, NP_g, NNa_g, and NK_g were evaluated, the uncertainty of NCa_g (Fig 2A) and NP_g (Fig. 2B) increased, whereas the uncertainty of NNa_g (Fig. 2C) and NK_g (Fig. 2D) did not vary, as BW increased from 5 to 45 kg.

When NCa_g, NP_g, NNa_g, and NK_g were presented in grams per kg ADG (mean ± SD), assuming a conversion factor of 1.21 from EBW to BW (Souza et al., 2017), the NCa_g (7.93 ± 0.01 g/kg ADG) and NP_g (6.40 ± 0.01 g/kg ADG) did not vary, whereas the NNa_g (from 1.10 to 0.74 g/kg ADG) and NK_g (1.37 to 1.00 g/kg ADG) decreased, as BW increased from 5 to 45 kg.

When presented in grams per kg ADG, the NMg_g for castrated and intact males increased from 0.378 to 0.407 g/kg ADG, and from 0.412 to 0.475 g/kg ADG, respectively, whereas the NMg_g for females decreased from 0.386 to 0.355 g/kg ADG, as goats grew from 5 to 45 kg BW.

Table 1. Data sources and information of included studies.

Reference	n[1]	Sex	Phase[2]	BW, kg	EBW[3], kg	Age, days
Ferreira (2003)	27	Castrated male	Weaned	20.6 to 35.5	15.6 to 30.3	71 to 187
Figueiredo (2011)	18	Female	Weaned	29.5 to 46.0	20.8 to 40.4	247 to 458
Gomes et al. (2011)	20	Intact Male	Suckling	5.1 to 21.6	4.9 to 17.5	59 to 114
Soares (2013)	12	Castrated male	Weaned	27.8 to 47.4	21.7 to 39.7	260 to 431
	12	Intact Male	Weaned	27.6 to 46.6	21.3 to 39.7	221 to 422
	15	Female	Weaned	27.4 to 44.9	23.1 to 38.2	279 to 426
Santos et al. (2016)	20	Castrated male	Weaned	15.3 to 32.5	13.1 to 26.4	73 to 204
	20	Intact Male	Weaned	15.7 to 34.0	12.8 to 28.3	72 to 242
	18	Female	Weaned	14.8 to 31.7	11.9 to 26.3	160 to 245
Mendonça et al. (2017)	10	Castrated male	Suckling	4.7 to 16.7	3.9 to 13.7	24 to 114
	19	Intact Male	Suckling	4.7 to 16.5	3.9 to 12.8	30 to 83
	18	Female	Suckling	4.6 to 16.3	3.5 to 13.4	32 to 114

[1] Total number of animal records included in each study.

[2] Suckling refers to goats that were fed both milk and solid diet; weaned refers to goats fed only a solid diet.

[3] EBW = Empty BW.

Table 2. Summary statistics of the macromineral body composition of Saanen goats used in this study.

Item	n[1]	Mean	SD	Range
Macrominerals, g/kg EBW[2]				
Calcium				
Castrated male	67	1.02	0.26	0.56 to 2.04
Intact male	69	0.99	0.21	0.42 to 1.55
Female	67	0.99	0.23	0.54 to 1.69
Phosphorus				
Castrated male	68	0.78	0.15	0.42 to 1.24
Intact male	69	0.78	0.12	0.53 to 1.17
Female	67	0.77	0.15	0.41 to 1.05
Sodium				
Castrated male	69	0.12	0.03	0.07 to 0.21
Intact male	70	0.14	0.03	0.09 to 0.21
Female	69	0.13	0.03	0.08 to 0.20
Potassium				
Castrated male	69	0.17	0.05	0.10 to 0.34
Intact male	70	0.19	0.04	0.12 to 0.30
Female	69	0.19	0.05	0.12 to 0.40
Magnesium				
Castrated male	67	0.05	0.02	0.02 to 0.10
Intact male	70	0.05	0.02	0.02 to 0.09
Female	68	0.04	0.01	0.01 to 0.07

[1] Number of data points used for macromineral fitting procedures, after removing outliers.
[2] EBW = empty BW.

Net Mg Requirements. Sex did not affect the intercept (*P* = 0.19); however, it affected the slope (*P* = 0.054) of the logarithmized allometric equations to estimate Mg in the body of Saanen goats. The slopes were similar between castrated and intact males (*P* = 0.11), and between castrated males and females (*P* = 0.48); however, the slopes differed between intact males and females (*P* = 0.018). Thus, an allometric equation was generated for each sex (Fig. 1E), then, equations were proposed to estimate the NMg_g of Saanen goats from 5 to 45 kg BW (Fig. 2E). The NMg_g of castrated and intact males was 8 and 18%, respectively, greater than that of female goats (g/kg EWG; Fig. 2E). The NMg_g of castrated and intact males increased by 8 and 15%, respectively, whereas that of females decreased by 8%, as the BW of goats increased from 5 to 45 kg. When the 95% confidence limits of NMg_g were evaluated, uncertainty of NMg_g for castrated and intact males increased, whereas the uncertainty of NMg_g for females decreased as BW increased from 5 to 45 kg. In addition, the uncertainty of NMg_g for females was lower than that for males (Fig. 2E).

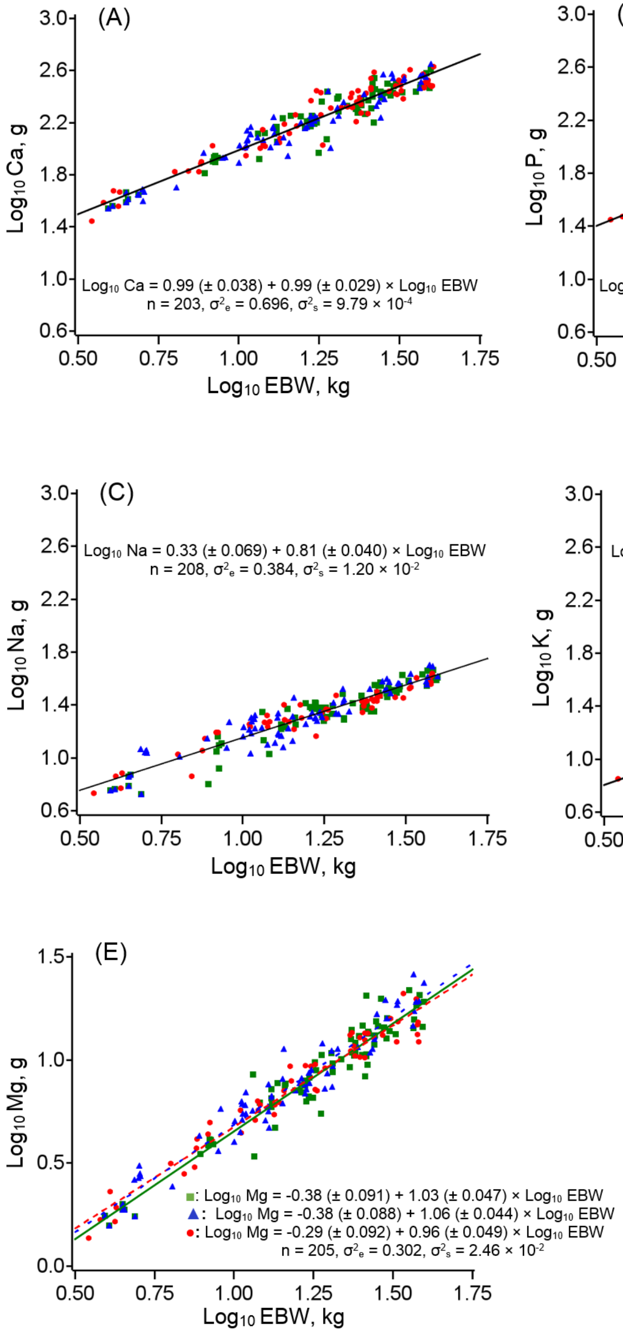

Figure 1. Relationship between Log10 Ca, Log10 P, Log10 Na, Log10 K, and Log10 Mg (g), and Log10 empty BW (EBW) (kg) of growing Saanen goats of different sexes (■ castrated males, ▲ intact males, and ● females). For body Ca, P, Na, and K, the intercepts and slopes did not differ between sexes ($P > 0.10$). For body Mg, the intercepts did not differ between sexes ($P = 0.19$). However, the slopes differed between sexes ($P = 0.054$). The σ_e^2 and σ_s^2 represent the error and study variance, respectively. The observations were adjusted for the study effect.

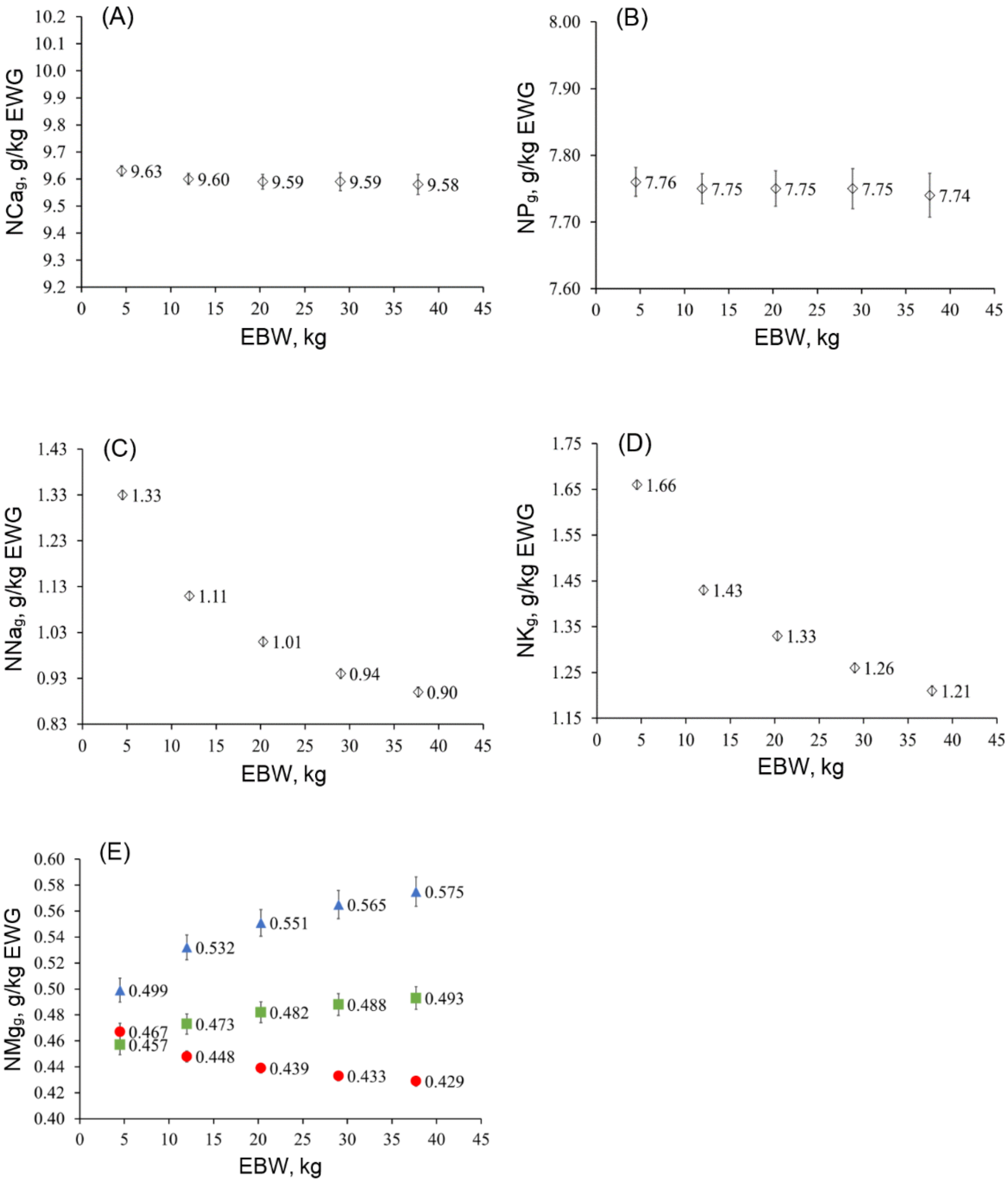

Figure 2. Estimated values of net Ca (NCa_g), P (NP_g), Na (NNa_g), K (NK_g), and Mg (NMg_g) requirements for growth (g/kg empty BW gain - EWG) of growing Saanen goats using the classical approach. EBW = empty BW. Equations used to estimate mineral requirements are as follows: for all sexes (◊): $NCa_g = 9.68 \times EBW^{-0.0031}$; $NP_g = 7.78 \times EBW^{-0.0015}$; $NNa_g = 1.74 \times EBW^{-0.185}$, and $NK_g = 2.07 \times EBW^{-0.149}$. By sex: $NMg_g = 0.431 \times EBW^{0.035}$ (castrated males; 🟩), $NMg_g = 0.448 \times EBW^{0.066}$ (intact males; ▲), and $NMg_g = 0.493 \times EBW^{-0.039}$ (females; ●). The error bars represent the 95% confidence limit of the requirements.

Net Macromineral Requirements for Growth by the Degree of Maturity Approach

Net Ca, P, Na, and K Requirements. Sex affected the intercepts (*P* < 0.01) but not the slopes (*P* > 0.10) of the logarithmized allometric equations to estimate the Ca, P, Na, and K in the body of Saanen goats from 5 to 45 kg BW. Thus, an allometric equation was proposed to estimate the Ca (Fig. 3A), P (Fig. 3B), Na (Fig. 3C), and K (Fig. 3D) for each sex. Correspondingly, equations to estimate the NCa_g (Fig. 4A), NP_g (Fig. 4B), NNa_g (Fig. 4C), and NK_g (Fig. 4D) of Saanen goats were proposed for each sex. The NCa_g and NP_g of intact males were 5 and 2%, respectively, greater than those reported for castrated males and females. Furthermore, the NCa_g (g/kg EWG; Fig. 4A) and NP_g (g/kg EWG; Fig. 4B) remained constant as BW increased from 5 to 45 kg for all sexes. The NNa_g of males was 6% greater than that of females (g/kg EWG; Fig. 4C), and irrespective of sex, NNa_g decreased by approximately 32% as goats grew from 5 to 45 kg BW. The NK_g (g/kg EWG; Fig. 4D) was similar between sexes when the 95% confidence limits were evaluated, and decreased by 26% as goats grew from 5 to 45 kg BW. When the 95% confidence limits of NCa_g, NP_g, NNa_g, and NK_g were evaluated, the uncertainty of NCa_g (Fig. 4A) and NP_g (Fig. 4B) increased, whereas the uncertainty of NNa_g (Fig. 4C) and NK_g (Fig. 4D) did not vary from 5 to 45 kg BW.

When NCa_g, NP_g, NNa_g, and NK_g were presented in grams per kg ADG (mean ± SD), the NCa_g for castrated males (7.77 ± 0.01 g/kg ADG), intact males (8.28 ± 0.01 g/kg ADG), and females (7.90 ± 0.02 g/kg ADG), as well as the NP_g for castrated males (6.39 ± 0.01 g/kg ADG), intact males (6.54 ± 0.01 g/kg ADG), and females (6.38 ± 0.01 g/kg ADG), remained constant, when goats grew from 5 to 45 kg BW. Conversely, the NNa_g for castrated males (from 1.11 to 0.75 g/kg ADG), intact males (1.14 to 0.77 g/kg ADG), and females (1.05 to 0.71 g/kg ADG), as well as the NK_g for castrated males (1.39 to 1.02 g/kg ADG), intact males (1.36 to 0.99 g/kg ADG), and females (1.38 to 1.02 g/kg ADG) decreased, as BW increased from 5 to 45 kg.

Net Mg Requirements. Sex affected both the intercept (*P* < 0.01) and the slope (*P* = 0.054) of the logarithmized allometric equations to estimate Mg in the body of Saanen goats; therefore, an allometric equation was proposed for each sex (Fig. 3E). Accordingly, the equations used to estimate the NMg_g of Saanen goats (Fig. 4E) revealed that the NMg_g of castrated and intact males was 7 and 17%, respectively, greater than that of female goats (g/kg EWG; Fig. 4E). Moreover, the NMg_g of castrated and intact males increased by 8 and 16%, respectively, whereas that of females decreased by 7%, as goats grew from 5 to 45 kg BW. Likewise, when the 95% confidence limits of NMg_g were evaluated, the uncertainty of NMg_g (Fig. 4E) for male and female Saanen goats increased as BW increased from 5 to 45 kg.

When presented in grams per kg ADG, the NMg_g for castrated and intact males increased from 0.379 to 0.410 g/kg ADG, and from 0.413 to 0.478 g/kg ADG, respectively, whereas the NMg_g for females decreased from 0.388 to 0.361 g/kg ADG, as BW increased from 5 to 45 kg.

4. DISCUSSION

The goal of the current study was to investigate the effects of sex on the macromineral requirements of Saanen goats from 5 to 45 kg BW, with or without consideration for the degree of maturity. Our results indicated that without considering the degree of maturity on the macromineral requirement estimation, sex did not affect the net macromineral requirements for growth, except by NMg_g. Conversely, sex influenced all macromineral requirements for growth when the degree of maturity was considered.

Energy and nutrient contents in the body of different ruminant species have been defined using EBW as a predictor, because it is considered a precise index (Owens et al., 1995). However, using just EBW as a predictor does not take into consideration differences in mature weight, and consequently, in patterns of growth and body composition (Tedeschi et al., 2004; Almeida et al., 2016), where the mineral concentrations in animal tissues may vary with maturity stage (Suttle, 2010). Thus, the degree of maturity should yield more adequate predictions of mineral composition than the use of EBW alone.

The total mineral content in the body varies considerably in ruminants (Almeida et al., 2016; Marcondes et al., 2016). Therefore, we suggest that the degree of maturity maybe used as a predictor of body mineral composition in order to reduce the variation in body composition and consequently, in macromineral requirements among goats, allowing differences across sexes in NCa_g, NP_g, NNa_g, NK_g, and NMg_g to be elucidated.

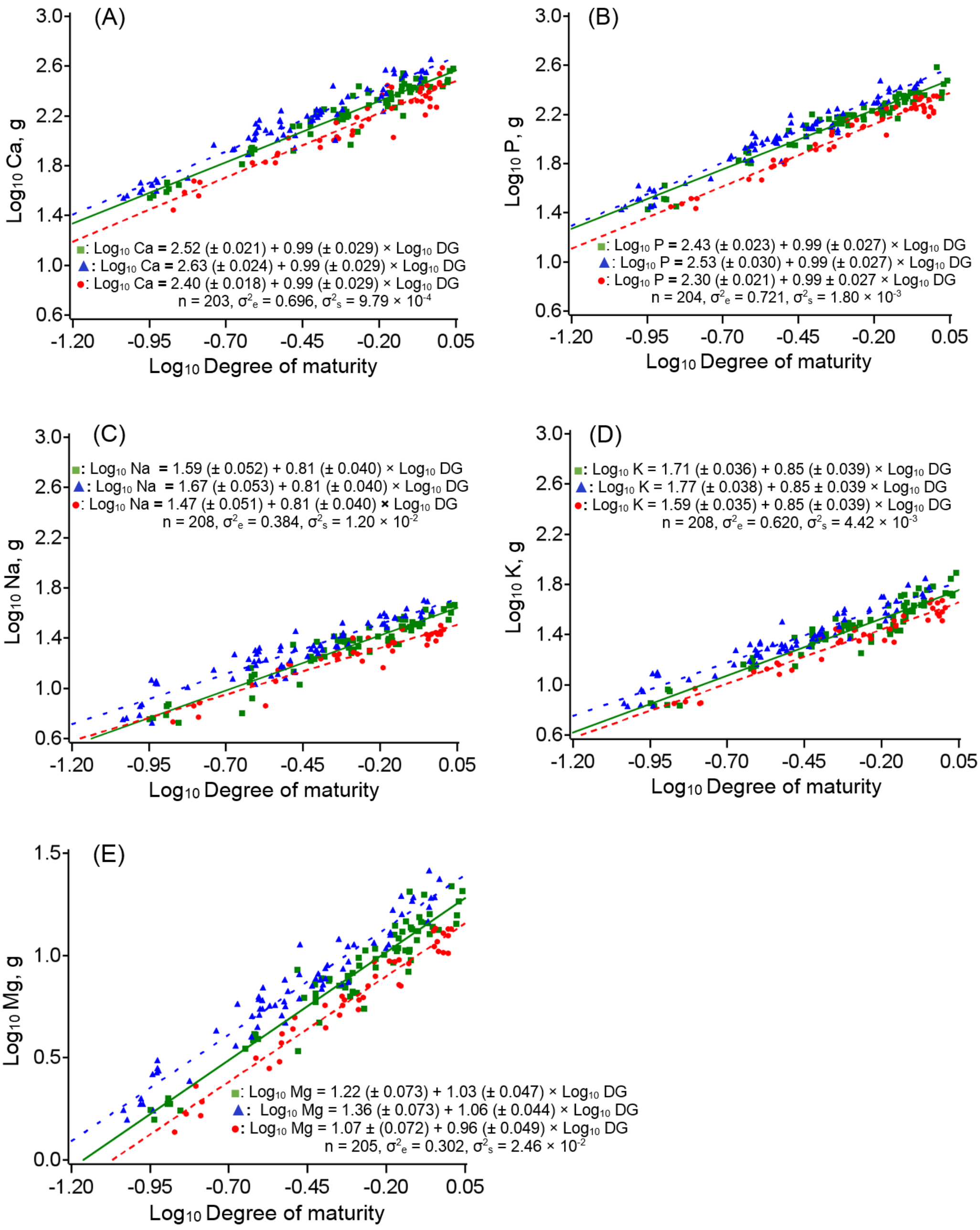

Figure 3. Relationship between Log_{10} Ca, Log_{10} P, Log_{10} Na, Log_{10} K, and Log_{10} Mg (g), and Log_{10} degree of maturity (i.e., degree of maturity = DG) of growing Saanen goats of different sexes (■ castrated males, ▲ intact males, and ● females). For body Ca, P, Na, and K, the intercepts differed between sexes (*P* = 0.054), but not the slopes (*P* > 0.10). For body Mg, both the intercepts (*P* < 0.01) and slopes (*P* = 0.054) differed between sexes. σ_e^2 and σ_s^2 represent the error and study variance, respectively. The observations were adjusted for the study effect.

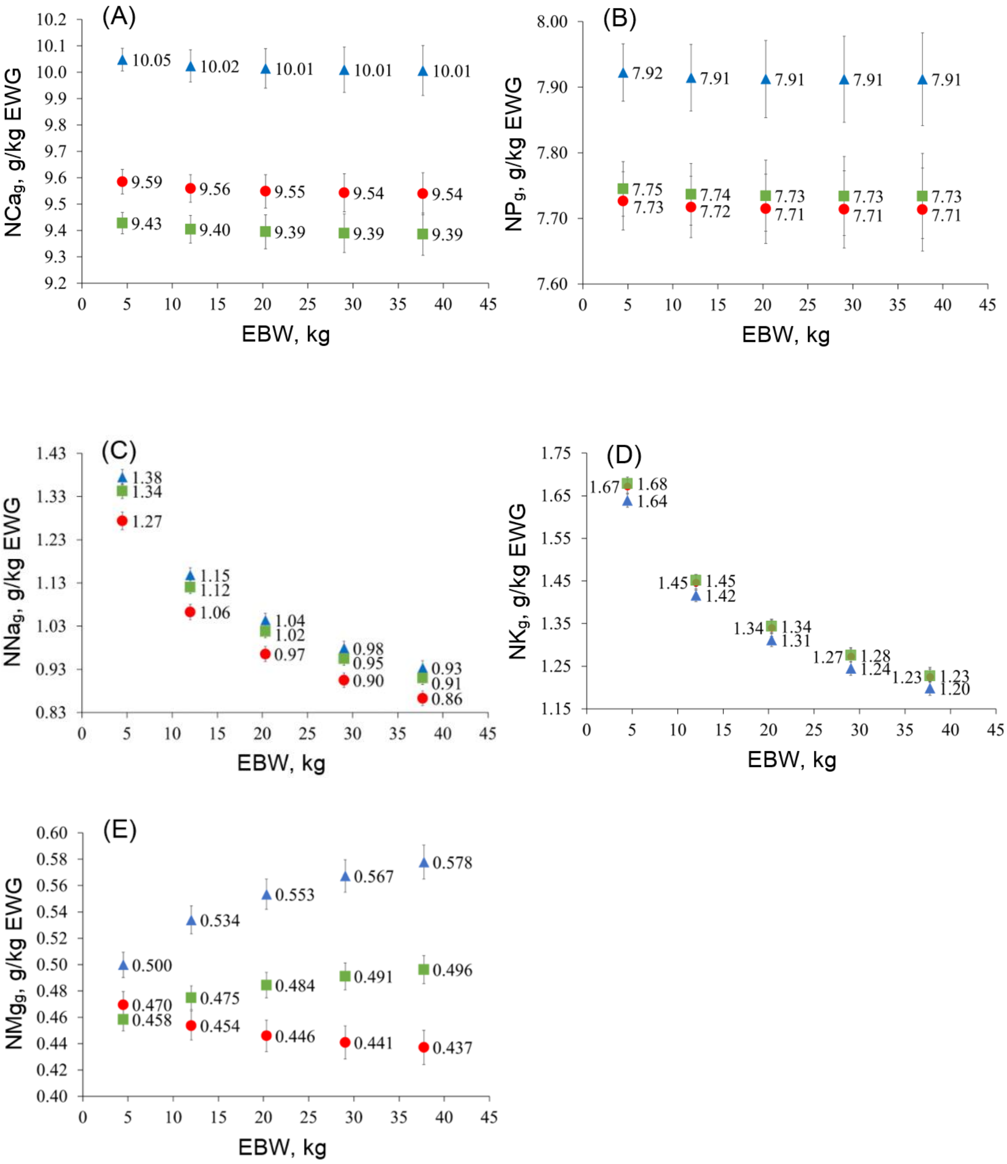

Figure 4. Estimated values of net Ca (NCa_g), P (NP_g), Na (NNa_g), K (NK_g), and Mg (NMg_g) requirements for growth (g/kg empty BW gain - EWG) of castrated male (■), intact male (▲), and female (●) growing Saanen goats using the degree of maturity approach. EBW = empty BW. Equations used to estimate mineral requirements are as follows: for castrated males: $NCa_g = 9.47 \times EBW^{-0.0031}$, $NP_g = 7.77 \times EBW^{-0.0015}$, $NNa_g = 1.76 \times EBW^{-0.185}$, $NK_g = 2.10 \times EBW^{-0.149}$, and $NMg_g = 0.428 \times EBW^{0.0351}$; for intact males: $NCa_g = 10.10 \times EBW^{-0.0031}$, $NP_g = 7.94 \times EBW^{-0.0015}$, $NNa_g = 1.81 \times EBW^{-0.185}$, $NK_g = 2.05 \times EBW^{-0.149}$, and $NMg_g = 0.449 \times EBW^{0.066}$; and for females: $NCa_g = 9.64 \times EBW^{-0.0031}$, $NP_g = 7.74 \times EBW^{-0.0015}$, $NNa_g = 1.67 \times EBW^{-0.185}$, $NK_g = 2.09 \times EBW^{-0.149}$, and $NMg_g = 0.494 \times EBW^{-0.039}$. The error bars represent the 95% confidence limit of the requirements.

Growth hormone (GH) regulates skeletal growth (Cannata et al., 2010), modulating Ca and P dynamics through its action on parathyroid, calcitriol, and calcitonin hormones (Breier, 1999; Bouillon et al., 2004). Moreover, GH release is regulated by the action of multiple hormones, including serum testosterone (Webster, 1986). In this sense, differences in serum testosterone levels across sexes may explain why NCa_g and NP_g in intact male Saanen goats (5 to 45 kg BW) were greater than those of castrated males and females when the degree of maturity approach was applied. Considering that we used growing goats, and that 99% of Ca and 80% of P are stored in the bone (Suttle, 2010), we suggest that body Ca and P were mainly directed toward bone growth (Lawrence et al., 2012). Consequently, intact males had greater NCa_g and NP_g than castrated males and females, probably due to differences in skeletal growth, which is expected in Saanen goats because castrated males and females reach maturity earlier than intact males (Almeida et al., 2016).

We propose that the NCa_g and NP_g did not vary from 5 to 45 kg BW, because the Ca and P accretion rates were constant over this BW range. Our findings are in accordance with those of the ARC (1980), who reported a non-significant change in the Ca and P accretion rates of lambs during their growing phase. Additionally, Almeida et al. (2016) reported that minerals in the fat-free EBW did not show a stable asymptote from 5 to 45 kg BW.

Confident limits for NCa_g and NP_g increased with the increase in EBW. This may be because, as the animal grows, spongy tissue is replaced by compact tissue, and this compact bone is mostly filled with blood vessels, nerves and fat deposits, enhancing the heterogeneity of bone composition (Zobrinsky, 1969). Furthermore, the confident limits observed for NCa_g and NP_g differed between sexes when the degree of maturity was considered. The importance of steroids increases with advancing growth phase, thus modulating bone development (McCarthy et al., 2000), which may differentiate the dynamics of Ca and P deposition between sexes in compact tissue in bones.

NCa_g estimated by the classical approach (7.9 g Ca/kg ADG) was 16% lower than the NRC (2007) recommendation (9.4 g Ca/kg ADG), yet the NP_g estimated by the classical approach (6.4 g P/kg ADG) was similar to the NRC (2007) recommendation (6.5 g P/kg ADG). However, when the degree of maturity approach was considered in the mineral requirement estimations, the NCa_g of castrated males (7.8 g Ca/kg ADG), intact males (8.3 g Ca/kg ADG), and females (7.9 g Ca/kg ADG) was 17, 12, and 16% lower than the NRC (2007) recommendation, respectively. Likewise, the NP_g of castrated males (6.4 g P/kg ADG), intact males (6.5 g P/kg ADG), and females (6.4 g P/kg ADG) was similar to the NRC (2007) recommendation. Differences in NCa_g and NP_g between sexes, and the use of the degree of maturity approach, were not taken into consideration in the mineral NRC (2007) guidelines.

Sodium and K are mainly located into body fluids as Na^+ and K^+ ions (Suttle, 2010). There is an inversely proportional relationship between water and fat in the animal's body, whereby the fat content increases and the water content decreases as the animal grows. Therefore, changes in body water contents may explain the decrease in total Na and K in the body of Saanen goats. We also observed that the NNa_g of males was greater than that of females, considering the degree of maturity. Aldosterone regulates electrolytic balance, enhancing Na reabsorption by the small intestine and kidneys, and resulting in a greater Na body composition (Schrier, 2006; Squires, 2010). Previous studies have shown that male rats have higher aldosterone levels than female rats, which is associated with the effect of androgens (Kienitz and Quinkler, 2008; Spyroglou et al., 2012). Therefore, we hypothesize that aldostenone acts similarly in goats, resulting in a greater Na body composition, and consequently, greater Na requirements in males than in females. Contrary to an effect on NNa_g, we observed no sex effect on NK_g as the EBW increased. Despite Na and K being metabolically linked to aldosterone action, K is mainly located within the cells (Suttle, 2010). Changes in aldosterone concentration have a greater impact on extracellular K, because this hormone plays a critical role in maintaining cell membrane resting potential (Palmer, 2015). Therefore, this differential effect of aldosterone on extra- and intracellular K contents may explain the absence of a sex effect on the NK_g.

We observed no variation in the uncertainty of NNa_g and NK_g between sexes, or with the increase in BW. This may indicate that NNa_g and NK_g could be estimated more accurately than other macromineral requirements in Saanen goats; this information may aid the design of more efficient strategies for optimizing mineral formulation programs. Additionally, the evaluation of confidence limits was decisive to define that sex did not affect the NK_g using the degree of maturity approach, despite equations suggesting that the K contents of castrated males and females were numerically greater than those of intact males. This suggests that evaluation of uncertainty enables a more adequate recommendation of macromineral requirements to be made. Therefore, the uncertainty in mineral requirements should be included in prediction models, using stochastic programming techniques to formulate diets (St-Pierre and Harvey, 1986; Yoder et al., 2014).

Our estimates of NNa_g (from 1.1 g to 0.74 g Na/kg ADG), determined by the classical approach, were slightly lower than that reported by NRC (2007) for goats (1.1 g Na/kg ADG), regardless of sex. When the degree of maturity was considered in the calculations, the NNa_g of castrated males (from 1.1 g to 0.75 g Na/kg ADG), intact males (from 1.1 g to 0.77 g Na/kg ADG), and females (from 1.1 g to 0.71 g Na/kg ADG) was also lower than the NRC (2007) recommendation. Although the NRC (2007) recommendation is included in the 95% confidence interval of NNa_g reported herein, we found that the NNa_g is not constant even when varying BW. Therefore, developmental stage must be considered for Na recommendations in Saanen goats.

Our estimates of NK_g (from 1.4 g to 1.0 g K/kg ADG) using the classical approach were lower than that reported by the NRC (2007) for goats (1.8 g K/kg ADG), regardless of sex. When the degree of maturity was considered in the calculations, the NK_g (from 1.4 g to 1.0 g K/kg ADG) was also lower than the NRC (2007) recommendation. This suggests that special care must be taken when making K recommendations because imbalances in K concentration in the diet may impair Mg absorption in the rumen due to the antagonistic K:Mg relationship (Suttle, 2010).

Approximately 65% of the total body Mg is contained in bones (Suttle, 2010), 15% in muscle, 15% in other soft tissues, and 5% in extracellular fluids (Mayland, 1988). The Mg in muscle and other soft tissues activates more than 300 enzymes (Wacker, 1980), such as those involved in ATP-dependent membrane transport (e.g., the Na^+/K^+ ion pump), and ATP production (e.g., pyruvate oxidation, oxidative phosphorylation, phosphate transferences, and β-oxidation of the fatty acids), which are important processes affecting muscle activity and growth (Ammerman and Goodrich, 1983; Shils, 1997). With both approaches used in our study, we found that the NMg_g of males was greater and increased as goats grew from 5 to 45 kg BW. Conversely, the NMg_g of females was lower and decreased, as goats grew from 5 to 45 kg BW. Males deposit more protein in their body than females at the same BW (Lawrence et al., 2012); therefore, we suggest, that the differences in NMg_g observed between sexes, and their variability across BW, could be associated with the effect of sex on muscular tissue deposition, whereby males may demand more ATP and consequently, more Mg for muscular activity than females. Hence, the greater total body protein of males may increase their energetic demands, increasing the catabolic rates for ATP production, and consequently enhancing the NMg_g, considering the role of Mg in those processes.

In the present study, we observed that at BW exceeding 15 kg, NMg_g differed between sexes. This may be expected considering that sex steroids (i.e., testosterone and estrogens) in combination with the central effects of IGF-1 production on tissue growth and metabolism, regulate the differences in body composition between sexes during puberty (Lawrence et al., 2012).

With both approaches, we found that estimates of NMg_g in intact male Saanen goats (from 0.41 to 0.47 g Mg/kg ADG) were greater than the NRC (2007) recommendation (0.40 g Mg/kg ADG), whereas those of castrated males (from 0.38 to 0.41 g Mg/kg ADG) and females (from 0.39 to 0.36 g Mg/kg ADG) were slightly lower than the NRC (2007) recommendation. The NRC (2007) reports a unique NMg_g value for sheep and goats, which may be inaccurate, because mineral metabolism and body composition differ between these two species (Meschy, 2000; Sheridan et al., 2003; Wilkens et al., 2014). Therefore, beyond the effects of ruminant specie, the effects of sex must be considered for Mg recommendations in Saanen goats.

To our knowledge, this is the first meta-analysis to evaluate the effects of sex considering the degree of maturity on the macromineral requirements for growth of Saanen goats. The findings of this study indicate that considering the degree of maturity in goat highlights differences across sexes in the macromineral requirements for growth. Additionally, the uncertainty in macromineral requirements is not accounted for the current feeding systems, in which requirements are reported as mean values. The use of mean values may result in inaccurate recommendations, hampering mineral requirements evaluation across different scenarios, and negatively affecting the animal’s performance. However, modern stochastic programming techniques may help to incorporate the uncertainty in the estimation of nutritional requirements, as we have developed in the current study.

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CHAPTER 4 - IMPLICATIONS

Minerals play important roles in animal metabolism and physiology, contributing to assure animal production and health. Thus, our study focused on evaluating sex effects on net macromineral requirements as well as mineral utilization in growing Saanen goats.

Regarding sex effect on net macromineral requirements for maintenance, as well as the tissue macromineral retention efficiency of Saanen goats, we found that sex does not affect the Ca, P, and K requirements, but it influences the Mg requirements. Moreover, we found that sex does not influences Ca, P, and Mg retention efficiency in the body, but it affects K retention efficiency. Furthermore, we found that Ca, P, Mg, and K retention efficiency may be predicted from average daily gain, regardless of sex. These results suggest that sex may influence the Mg and K role in biochemical processes associated to the maintenance of tissues in the animal. Therefore, we encourage to develop future studies aiming to identify these biochemical processes, which can further optimize the accuracy of mineral requirements values, as well as the mineral utilization by the animal.

We found that comparative slaughter method (CST) produced lower estimates of the net macromineral requirements for maintenance than those produced using minimum endogenous losses method (MEL). Moreover, standard error (SE) of Ca requirements for maintenance estimated using MEL were lower than those estimated using CST, whereas SE of Mg and K requirements for maintenance estimated using MEL were greater than those estimated using CST. Furthermore, we found that sex affected the net Mg requirements for maintenance estimated using CST, but it did not affect them when estimated using MEL. It suggests that the estimation method could affect both the mineral requirement values as well as the response of factors that may affect them. Hence, our data remark the need to conduct additional studies comparing the macromineral requirement estimation methods, evaluating their selectivity, sensibility, detection and quantification limits, precision, sturdiness, and applicability, to get more accurate values of mineral requirements for maintenance, as well as responses of factors that potentially may affect them.

Regarding sex effect on the net macromineral requirements for growth, we found that differences between sexes were highlighted when the maturity stage of the goat was used as a predictor of its mineral body composition. It suggests that considering maturity degree of the goat, enhanced the statistical power of detection of differences between sexes in mineral requirements for growth, with the respective increase of their accuracy. Hence, the present study encourages the development of future studies evaluating potential factors that affects mineral requirements for growth of goats, but involving the maturity degree on the calculations. It also brings the necessity of future studies estimating the weight at maturity of males and female goats of different breeds.

Other novel contribution of our study was the evaluation of confidence limits of mineral requirements for growth. It is known that if all experimental measurements are subject to error, then the uncertainty must be reported. However, mineral requirements for growth in previous studies had not been reported with their uncertainty measure. In our study, we used the Monte Carlo method to get estimates of the confidence limits of mineral requirement estimated from our equations. Indeed, the evaluation of the confidence limit of mineral requirements was decisive to detect differences between sexes in mineral requirements for growth. Hence, we encourage to use the methodology proposed here in future studies of nutritional requirements, which may bring the development of more efficient formulation programs.

Studies conducted exploring sex effect on mineral requirements are scarce, and the inference range is limited to these studies. On the other hand, the equations presented here were fitted from a meta-analysis combining data from multiple studies, and setting the study as random effect, which extend their predictive range. It suggests that the models built here, may be used to make accurate predictions of macromineral requirements of Saanen goats of different sexes under different practical scenarios.

Animal production has a huge compromise in the present century: to meet a great part of the nutritional demands of a growing worldwide population. It implies that more efficient animal production strategies need to be developed. It is known that between 70 and 90% of the total animal production cost, is due to feeding costs. Likewise, nutrient wastes of animal production have negative consequence for the environment. Therefore, the design of nutritional strategies directed to increase the nutrient utilization efficiency by the animal, are strictly necessary to enhance the competitiveness of animal production in the worldwide market.

In the case of minerals, excessive inorganic nutrients in the diet may result in an enhancing of their excretion via feces and urine, with the consequent pathologic problems for the animal and economic losses for the producer. In addition, it has been suggested that certain minerals may be extremely negative for the environmental systems when excreted in excess, as the case of P, which has been associated to the enhancing of the eutrophication in aquatic systems.

Therefore, accurate definition of mineral requirements may significantly contribute into the optimization of mineral formulation programs, leading to a decreasing of mineral excretion as well as to an improving of animal performance, with the respective economic and environmental benefits. Hence, the results derived here, may be associated with future strategies directed to optimize the life cycle of products or services derived of goat production systems, which is the central focus of sustainable management. Thus, in accordance with sustainability principles, the enhancing of mineral utilization by the animal, may increase the socio-eco-efficiency of the goat production systems.

APPENDIX

Appendix 1: Chapter 2 – SAS Inputs

Initial linear regression analyses to fit mineral retention to mineral intake (i.e., comparative slaughter technique) as well as to fit mineral excretion to mineral intake (i.e., minimum endogenous losses method) were conducted using the MIXED procedure of SAS (SAS Inst. Inc., Carry, NC). From the Studentized residual plot of macromineral retentions or excretions, we observed that the assumption of homoscedasticity variance was not suited for representing the variability observed in the Ca, P, and Mg retention, and for representing the variability observed in the Ca excretion. To solve this, we modeled the error variance using the NLMIXED procedure of SAS, testing the following mathematical functions proposed to accommodate the heteroscedasticity of the afore mentioned variables:

$$\sigma_e^2 = \sigma^2, \quad [1]$$

$$\sigma_e^2 = (\sigma_0^2)\times\exp(\mathrm{c}\times\mathrm{independent\ variable}), \quad [2]$$

$$\sigma_e^2 = (\sigma^2)\times\mu^{2\varphi}, \quad [3]$$

where σ^2 is the homogeneous residual variance (Eq. [1]). The exponential variance (Eq. [2]) contains the starting residual variance (σ_0^2) which increases exponentially at a rate c proportional to mineral intake. Equation [3] represents the residual variance ($\sigma_e^2 = \sigma^2$), scaled by a power (φ) function of the expected mean, μ. The SAS code used in these analyses, is presented below:

```
/* Macromineral requirements for maintenance of Saanen goats*/

/*Importing dataset*/

DATA <database name>;
INPUT <variables>;
DATALINES;
<data>;
RUN;

*Creating a numerical category for each sex;

DATA <database name>;
SET <database name>;
IF sex = 'M' THEN DO; k1=1; k2=0; k3=0;  END;
IF sex = 'F' THEN DO; k1=0; k2=1; k3=0;  END;
IF sex = 'C' THEN DO; k1=0; k2=0; k3=1;  END;
RUN;
```

```
/*Running the meta-analysis*/

PROC NLMIXED DATA=<dataset name> Tech=newrap HESS MSING=1E-15 maxiter=500;

*lock or unlock the parameter, according to the error variance model
selected. After that, insert adequate numerical parameters;
PARMS   a1=<parameter>  b1=<parameter>
        a2=<parameter>  b2=<parameter>
        a3=<parameter>  b3=<parameter>
        c=<parameter>
        psi=<parameter> to <parameter> by <parameter>
        s2u1=<parameter> sigma=<parameter>;

A = a1*k1+a2*k2+a3*k3;
B = b1*k1+b2*k2+b3*k3;
BOUNDS  sigma>0;
BOUNDS  s2u1>0;
mu = (A) + (B+u1)*(mineral_intake); *independent variable

*keep unlock, only one error variance model;
v = sigma**2;
v = sigma**2 * exp(c * mineral_intake); *independent variable;
v = sigma**2 * mu**(2*psi);
model mineral_retention ~ normal (mu, v); *dependent variable;
RANDOM u1~ NORMAL(0,s2u1) SUBJECT=study;
predict mu out=P;

*Comparison of slopes and intercepts between sexes;
CONTRAST 'Ma-Fa' a1-a2;
CONTRAST 'Mb-Fb' b1-b2;
CONTRAST 'Ma-Ca' a1-a3;
CONTRAST 'Mb-Cb' b1-b3;
CONTRAST 'Ca-Fa' a3-a2;
CONTRAST 'Cb-Fb' b3-b2;

*Estimation of a common intercept and slope for three sexes;
ESTIMATE 'A all' (a1 + a2 + a3)/3;
ESTIMATE 'B all' (b1 + b2 + b3)/3;

*Estimation of one slope and intercept for each sex;
ESTIMATE 'A INTACTS'   a1;
ESTIMATE 'B INTACTS'   b1;
ESTIMATE 'A FEMALES'   a2;
ESTIMATE 'B FEMALES'   b2;
ESTIMATE 'A CASTRATED' a3;
ESTIMATE 'B CASTRATED' b3;
RUN;
```

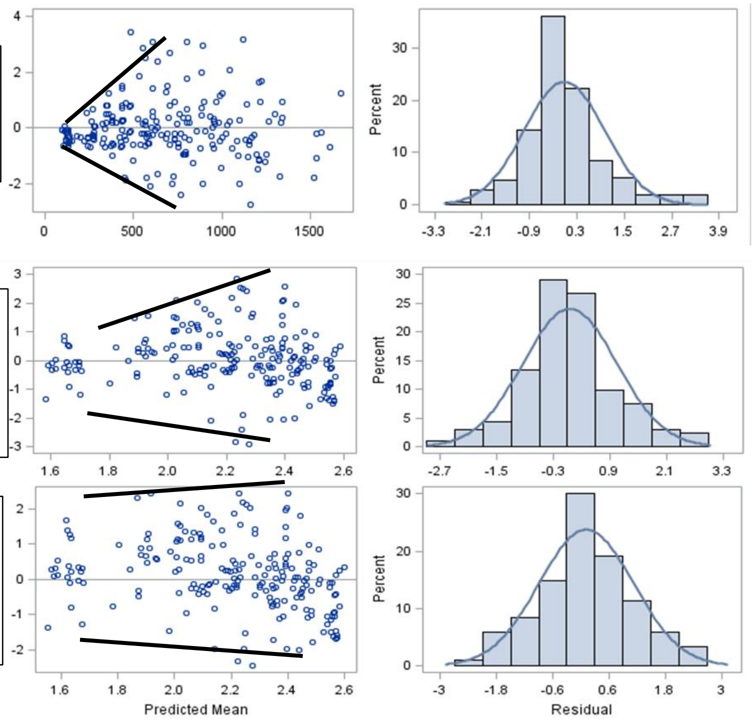
Appendix 2: Chapter 3 – Criteria for selecting the logarithmized form of the allometric equation to estimate mineral body composition from empty body weight and degree of maturity

This appendix aims to illustrate, with an example (i.e., body Ca fitting to empty body weight), the graphical criteria for selecting the linear form of the allometric equation, and the impact of the WEIGHT Statement on the Studentized residuals. The detailed SAS program used to obtain this output is provided in Appendix 2. Dark lines describe the studentized residual pattern.

The SAS System

The Mixed Procedure

Studentized residuals for Ca and Log$_{10}$Ca



Non-linear form of the allometric equation

Linear form of the allometric equation without the WEIGHT statement

Linear form of the allometric equation with the WEIGHT statement

Appendix 3: Chapter 3 – SAS Inputs

The logarithm of the total body Ca, P, Na, K, or Mg was regressed to the logarithm of EBW (i.e., classical approach), or the logarithm of degree of maturity to estimate equations for macromineral body composition. Parameters of the equations were estimated using the MIXED procedure of SAS, defining the study as a RANDOM effect. The WEIGHT statement was applied to attend the criteria of homogeneity of variance. When the sex effect was found to be significant (*P* < 0.10), indicating a different intercept and/or slope for at least one sex, three CONTRAST statements for intercept and/or slope were used to conduct all three pairwise comparisons of sex. The SAS code used in this analysis is presented below:

```
/* Macromineral requirements of Saanen goats*/

/*Importing dataset*/

DATA <database name>;
INPUT <variables>;
DATALINES;
<data>;
RUN;

/* calculating degree of maturity (DG) and transforming data using Log */
DATA <database name>;
SET <database name>;
IF sex= 'C' THEN DG = (EBW/34.9); *34.9 kg is the mature weight of
castrated males according to Almeida et al.,(2016);
IF sex= 'F' THEN DG = (EBW/26.0); *26.0 kg is the mature weight of females
according to Almeida et al.,(2016);
IF sex= 'I' THEN DG = (EBW/42.6); *42.6 kg is the mature weight of intact
males according to Almeida et al.,(2016);
LogEBW = Log10(EBW);
LogM = Log10(DG);
run;

/*Note: if you want to use the LogDG as independent variable (i.e., degree
of maturity weight approach), you must replace the word LogEBW by LogDG in
the following statements*/

/*Running the meta-analysis*/

PROC MIXED DATA=<dataset name>;
CLASS study sex;
MODEL <nutrient content> = LogEBW|sex /RESIDUAL INFLUENCE;
WEIGHT <correction factor>; *see St-Pierre, (2001) for more details.
However, if your data has homogeneous variance, you can disable this
program line;
LSMEANS sex/AT logEBW=0;
ESTIMATE 'b0 for sex=C' Intercept 1 sex 1;
ESTIMATE 'b0 for sex=F' Intercept 1 sex 0 1;
```

```
ESTIMATE 'b0 for sex=I' Intercept 1 sex 0 0 1;
ESTIMATE 'b1 for sex=C' LogEBW 1 sex*LogEBW 1;
ESTIMATE 'b1 for sex=F' LogEBW 1 sex*LogEBW 0 1;
ESTIMATE 'b1 for sex=I' LogEBW 1 sex*LogEBW 0 0 1;
CONTRAST 'C vs F' sex -1 1 0;
CONTRAST 'C vs I' sex -1 0 1;
CONTRAST 'F vs I' sex  0 -1 1;
CONTRAST 'C vs F' sex*LogEBW -1 1 0;
CONTRAST 'C vs I' sex*LogEBW -1 0 1;
CONTRAST 'F vs I' sex*LogEBW 0 -1 1;
RANDOM Study/SOLUTION;
RUN;
```