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AVANCES EN PRODUCCIÓN ANIMAL EN AMÉRICA LATINA

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FACULTAD DE ZOOTECNIA LA MOLINA

Edited by

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Preface

Advancing modern animal production

2014 Ensminger School – Universidad Nacional Agraria La Molina, Peru

There is a rising demand for livestock products that can be produced in an environmentally and socially responsible way. Food global demand is expected to double during the first half of this century, because of the growing human population. Over the same period, we are experiencing large climate changes which impact on the resources available for food production. As a result, concerns on environmental impacts, animal welfare and food security are emerging as important issues for animal production in developing countries. Advances in animal science are essential for integrating information from new discoveries and techniques into practical knowledge applicable to animal farm and its products. Animal science can make a significant global contribution for resolving conflicts among stakeholder and for translating scientific knowledge into economic and social benefit.

The Ensminger Conference "Advancing Modern Animal Production" is being held at La Molina National Agrarian University, Peru, on November 5-7, 2014, with the aim of bringing together animal scientists, industry representatives, consultants, and students to share and discuss issues directly influencing the future of animal production with focus on swine, poultry and cattle industry. This conference will feature current and future research that will shape the future of Animal Production in Latin America.

This conference will gather invited speakers and attendes from around the world and it will provide an opportunity for delegates to present posters with their latest research. Focal topics of the conference will be Animal Welfare, Genomics, Animal Biotechnology and Nutrition. Key outcomes expected are: (1) the exchange of updated information about emerging topics in animal science; (2) the sharing of visions for future impacts and applications of new technologies on animal production systems. Please enjoy these proceedings.

Carlos A. Gómez
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List of Contributors

Dr. Max Rothschild

Dr. Max Rothschild is a CF Curtiss Distinguished Professor in Agriculture and Life Science and holds the ME Ensminger Chair in International Animal Agriculture. Rothschild received his B.S.at the University of California, Davis in 1974 and his M.S. at the University of Wisconsin in 1975 and Ph.D. in animal breeding from Cornell University in 1978. In 1980 he joined Iowa State University. From 1993 to 2013 Rothschild served as the USDA Pig Genome Mapping Coordinator. In 2007 he was named director of the Center for Integrated Animal Genomics. Rothschild also has been active developing livestock projects in developing countries and serves as associate director for the Center for Sustainable Rural Livelihoods. In 2013, he was named Co-Director of the Global Food Security Consortium, a group of universities and research labs centered at Iowa State University. Rothschild has devoted his research and teaching career to the fields of animal breeding and molecular genetics and he and his lab have discovered a large number of individual genes associated with traits of importance and many of these are used in selection in the commercial pig industry. He is widely recognized as a world leader in the field of pig genetics. He is presently working with the genomics of 7 species including cattle, sheep, goats, pigs, chickens and farm raised shrimp. He has presented numerous invited papers in over 50 countries and has over 350 referred publications, 500 other publications and 12 patents. His many awards include AAAS fellow, USDA Group Honor Award, Rockefeller Prentice award in Animal Breeding and Genetics, the ASAS International Service award, ASAS fellow, two R&D100 awards and he was named Iowa Inventor of the year in 2002.

Dr. Lance Baumgard

Lance is a native of southwest Minnesota who grew up on a swine and row-crop farm. He has a B.S. and M.S. degree from the University of Minnesota and a PhD from Cornell University. He joined the University of Arizona faculty as an Assistant Professor in the Fall of 2001 and was promoted to Associate Professor in Spring of 2007. Baumgard joined Iowa State University in 2009 and is the Norman Jacobson Professor of Nutritional Physiology in the Department of Animal Science. Lance's early research career focused on efforts relating to milk fat synthesis regulation and to the metabolism and energetics of the transition cow. Since 2006, Baumgard's primary research emphasis has been on the metabolic and endocrine consequences of heat stress in both ruminant and monogastric farm animals. Lance and his research team have made a number of novel discoveries with regards to changes in carbohydrate, lipid and protein metabolism during heat stress. These previously unexplained changes in post-absorptive nutrient partitioning reduces milk yield more than what the reduced feed intake and negative energy balance would predict. At Iowa State University, Lance's Environmental Physiology efforts have evaluated how heat stress alters post-absorptive fuel selection in growing pigs and many of the same metabolic changes occurring in ruminants also occur in pigs. His findings primarily explain why heat-stressed pigs accrue more lipid and less protein than energetically predicted. He teaches Principals of Nutrition, and co-teaches Food Processing for Companion Animals at the undergraduate level and teaches Bioenergetics and co-teaches Advanced Ruminant Nutrition at the graduate level. Lance has mentored or co-advised 8 PhD and 15 MS students and all of them are currently successful in a variety of industry and academic positions. Lance has been the PI or co-PI on over $5.0 million in research funding including 4 USDA grants. Lance and his group have published 20 reviews and book chapters, 55 research articles, 140 abstracts, and 62 conference proceedings and for regional meetings concentrating on industry relevant issues. Lance, along with his wife Dr. Aileen Keating and children (Cian and Medbh) live in Ames.

Dr. Dorian Garrick

Dr. Garrick is Professor of Animal Science at Iowa State University (ISU). He got his BS degree from the Massey University, New Zealand in 1981 and his PhD from Cornell University in 1988. Dr. Garrick's current research projects are to improve the accuracy of predicted genetic and phenotypic merit using high-density genomic information. Generally, his work focuses on the portfolio of endeavors that are involved in the design, enhancement, implementation and monitoring of genetic improvement programs. These include aspects of genetics, economics, statistics and biology. Attention is directed at variance component estimation, prediction of breeding values, development of breeding objectives, exploitation of breed/heterosis effects and breeding industry structure, primarily in regard to their application to the national improvement of beef cattle, but other species are also considered.

Dr. Curtis Youngs

Dr. Youngs is a Professor of Animal Science at Iowa State University (ISU). He earned the B.S. and Ph.D. degrees in animal science from the University of Minnesota in 1981 and 1985, respectively. After one year of postdoctoral training in farm animal embryo biotechnologies at Louisiana State University, he served as an assistant professor of animal science at the University of Idaho for three years. He joined the animal science faculty at ISU in 1989, and he holds a teaching/research split appointment. Dr. Youngs teaches lecture and laboratory courses in domestic animal reproduction, as well as a lecture and laboratory course in embryo transfer and related technologies which he developed. Dr. Youngs' primary area of research pertains to factors influencing embryo development and survival in domestic farm animals. He has a special interest in artificial insemination and embryo transfer technologies with a focus on embryo cryopreservation. He is a Fellow of the American Society of Animal

Science, a long-time member of the International Embryo Transfer Society (IETS), and former president of the IETS Foundation.

Dr. Marina (Nina) von Keyserlingk

Dr. Marina (Nina) A.G. von Keyserlingk (B.Sc., M.Sc. Ph.D., Professor) is a Natural Sciences and Engineering Research Council (NSERC) Industrial Research Chair holder in Animal Welfare at The University of British Columbia (UBC) and is recognized internationally for her research on care and housing for cattle. She completed her undergraduate in Agricultural Sciences at UBC, her M.Sc. at the University of Alberta and Ph.D. in Animal Sciences at the University of British Columbia. Following completion of her Ph.D. she worked in agribusiness for 6 years before returning to academia as a post-doctoral fellow in animal behavior and welfare. She was appointed as an Assistant Professor in the Animal Welfare Program in 2002, promoted to Associate Professor in 2006 and achieved the highest rank of Professor in 2010. Dr. von Keyserlingk is an astonishingly productive scientist; having published over 120 refereed research publications during her career as a scientist. She also recently co-authored the definitive scholarly text – The Welfare of Cattle - in her core area of research (Springer Verlag, 2008). She is a much sought after speaker travelling nationally and internationally in excess of 100,000 miles per year. Because of this work as an educator on farm animal care, the impact of her research can now be seen on farms around the world. Dr. von Keyserlingk is the 2012 recipient of he Canadian Animal Industries Award in Extension and Public Service in recognition of her outstanding service to the animal industries of Canada in technology transfer, leadership and education in animal production. She is also the 2013 Recipient of the Elanco Award for Excellence in Dairy Science in recognition of outstanding research in dairy science and 2013 recipient of The Metacam 20 Award for outstanding service in the area of cattle welfare.

Dr. Michel Wattiaux

Originally from a dairy farm, Michel Wattiaux was raised with a strong commitment to making the family farm profitable. After a Bachelor Degree in Agricultural Sciences at the University of Louvain-La-Neuve, Belgium in 1982, Michel got his PhD degree from the University of Wisconsin-Madison in 1985. Michel's research program has focused dairy cattle nutrition and on the environmental impact of dairy production with an emphasis on undesirable gaseous emissions to the atmosphere known to have negative impacts on human heath and balance of natural ecosystems or to contribute to climate change. Specifically, Dr. Wattiaux and his research collaborators have studies the effects of dietary composition on manure ammonia emission and enteric methane emission from dairy cattle. In recent years, Michel has committed himself to a Global-scale research program. He has received USDA International Science and Education (ISE) grants to foster collaboration with Mexican and Canadian partners to develop research tools to evaluate Dairy Agro-ecosystems Sustainability. His meritorious scholarship in teaching and learning and international activities during this period of his career have been recognized by a series of honors and awards. Michel was promoted to Professor in the spring of 2011.

Dr. Anna Johnson

Dr. Johnson is an Associate Professor in the Department of Animal Science at Iowa State University (ISU). Dr. Johnson teaches both undergraduate and graduate level classes and is conducting research in the area of sow productive lifetime, maintenance behaviors in farm animals handling and system designs and their impacts on the transport losses in pigs and pain. In addition, Dr. Johnson provides extension services for state, national and international commodity groups pertaining to welfare related issues. Prior to joining ISU Dr. Johnson held the position of Director of Animal Welfare for the National Pork Board. She developed and implemented Checkoff-funded animal welfare and welfare-related research within the Science & Technology Department. Dr. Johnson was instrumental in the formulation and launch of the Swine Welfare Assurance Program (SWAP). In January 2001, Dr. Johnson earned her Ph.D. in animal welfare from Texas Tech University in Lubbock, Texas, her a M.S. in applied animal behavior and animal welfare from University of Edinburgh in 1997, and her B.S. in animal science from Reading University (1995). Dr. Johnson is active in the International Society for Applied Ethology (ISAE) and the American Society for Animal Scientists (ASAS) she also serves as a member for the Pork Checkoff's Animal Welfare Committee and Sow Longevity Advisory Group.

Dr. Nicholas Gabler

Dr. Gabler is an Associate Professor at Iowa State University (ISU). He got his BS and PhD degree´s from La Trobe University, Australia in 1999 and 2005, respectively. Dr. Gabler's research area is swine nutrition. His research program focusses on ways to improve feed efficiency in swine production. Additionally, Dr. Gabler's progam studies basic and applied aspects of gastrointestinal physiology, and how environmental stresses and disease challenges alter tissue accretion rates and metabolism in livestock.

Dr. Horacio Rostagno

Dr. Rostagno graduated in Agronomy from the Universidad Catolica de Santa Fe in Argentina. He got his MS and PhD degrees from Purdue University in 1970 and 1972, respectively. He is currently Professor at the Federal University of Viçosa, Brazil. He has experience in Animal Science with emphasis in Poultry and Swine Nutrition and Feeding. Dr Rostagno is the editor of the Brazilian Tables for Poultry and Swine – Composition of Feedstuffs and Nutritional Requirements published in 2011.

Dr. Aileen Keating

Dr. Keating received her Bachelors of Science from the National University of Ireland, Galway in Microbiology and her Masters of Science in Biomedical Science from the University of Ulster, Northern Ireland. Her doctoral degree is in Biochemistry from the National University of Ireland, during which she was the holder of a Teagasc-Walsh Fellowship. Dr. Keating joined the faculty in the Department of Animal Science at Iowa State University in 2010, where her research on the role of environmental chemical exposure on ovarian function is continuing. She currently serves as the early career councilor for the Society of Toxicology Reproductive and Developmental Toxicology specialty section. In addition, she serves on the Membership committee for the Society for the Study of Reproduction.

Dr. Abel Ponce de León

El Dr. F. Abel Ponce de León es catedrático principal de la catedra de Genética Molecular en el departamento de Ciencias Animales de la Universidad de Minnesota. También ocupó los cargos de Jefe de Departamento entre los años 1997 al 2006 y de Decano Asociado Ejecutivo (Investigación, Programas de Estudios Graduados y manejo financiero) en el Colegio de Ciencias de la Alimentación, Agricultura y Recursos Naturales de la Universidad de Minnesota entre los años 2006 al 2013. El Dr. Ponce de León es también miembro de los programas de graduados en Ciencias Animales y en Veterinaria Molecular. Él obtuvo el grado de Bachiller e Ingeniero Zootecnista en la Universidad Nacional Agraria “La Molina”, y los grados de M.S. y Ph.D. en Genética en la Universidad de Massachusetts-Amherst, EE.UU. En 1999 fue elegido “Fellow” por la Asociación Americana para el Avance de la Ciencias (AAAS) en reconocimiento a sus contribuciones científicas. El Dr. Ponce de León fue catedrático del Departamento de Veterinaria y Ciencias Animales de la Universidad de Massachusetts entre los años 1986-1997. Trabajo también como consultor internacional para el Instituto Interamericano de Cooperación para la Agricultura (IICA) en Brasil para el convenio IICA/EMBRAPA entre los anos 1981-1984. Los intereses de investigación del Dr. Ponce de León se sitúan en las áreas de 1) genómica estructural y funcional y, 2) reprogramación celular y transgénesis animal. En genómica estructural su trabajo estuvo centrado en la generación de bibliotecas de ADN para cromosomas específicos desarrollados por medio de micro disección física de cromosomas. Esta última tecnología se aplico para generar bibliotecas específicas de cromosomas de bovinos, aves y suinos. La identificación de marcadores genéticos (microsatélites y SNPs) obtenidos de estas bibliotecas ayudaron a generar, para estas especies, mapas genéticos densos. En reprogramación celular, clonación y transgénesis, él participó en la generación de bovinos transgénicos clonados que se obtuvieron en la Universidad de Massachusetts en el año 1998. Su laboratorio desarrollo un sistema de cultivo celular para células primordiales (PGCs) en aves con el objetivo de generar aves quiméricas y transgénicas. Es autor y/o co-autor de más de 100 artículos científicos y capítulos de libros. El Dr. Ponce de León es co-inventor en cinco patentes sobre clonación y sistemas de cultivos celulares.

Organizing Committee

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Introduction

The Ensminger Program in International Agriculture

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Summary
The Ensminger Program is an effort to improve and expand activities in international animal agriculture in the Department of Animal Science and the College of Agriculture and Life Sciences at Iowa State University. The program is funded by an endowment created by Dr. M. E. Ensminger and his wife. This program has several purposes and includes collaboration with international scientists development of schools/conferences in locations around the world, improving training and collaboration of foreign scientists and improved education and opportunities for Iowa State University undergraduates with interests in international animal agriculture. This international event of a school in 2014 in La Molina, Peru is another product of this program.

Resumen
El programa Ensminger en Agricultura Internacional
El Programa Ensminger supone para el Departamento de Ciencia Animal y para la Escuela de Agricultura y Ciencias Biológicas de la Iowa State University un esfuerzo para mejorar y ampliar las actividades en la producción animal a nivel internacional. El programa se financia con un fondo creado por M.E. Ensminger y su esposa. Este programa tiene varias finalidades, entre las que se puede destacar el desarrollo de cursos y conferencias en distintos lugares del mundo en colaboración con científicos internacionales para mejorar la formación y la colaboración entre científicos de todo el mundo. También participa en la mejora educativa de estudiantes de la Iowa State interesados en la producción animal internacional. Esta conferencia internacional del año 2014 en La Molina en Perú es también fruto de este programa.

Organization
The Ensminger program is organized by the M.E. Ensminger Chair with consultation by the Chair of the Department of Animal Science. Dr. Max Rothschild was appointed Ensminger Chair since 2007. The Ensminger Chair works with faculty and students to optimize activities and opportunities in the department and with scientists around the world to develop international schools and conferences.

Activities

Several activities are underway and can be viewed at http://www.ans.iastate.edu/section/Ensminger/. These activities include the following: 1) International Ensminger schools; 2) Ensminger visiting fellows/scientists; 3) Sponsored seminars; 4) Travel and international activities in other countries; 5) Development of a class in international animal agriculture and 6) to continue development and publishing of books formerly published by Dr. Ensminger and coauthors.

Ensminger Schools are designed to be conferences that are offered around the world to help train people of different regions in modern animal agriculture practices. The schools, about 3 every 5 years, consist of 12-14 speakers of international stature and of which many are from Iowa State University. In addition, speakers travel and learn about the culture of the host country. This present Ensminger School will be held in Lima at La Molina National Agrarian University in Peru.

Ensminger visiting scientists are visitors that come and work in the department and seek some assistance from the Ensminger program. To date we have had several visitors from the following countries: Uganda (1); South Africa (1); Italy (1); Korea (1), Spain (1) and the Philippines (3). These people come for several months or up to a couple of years. Projects have included training in molecular biology, quantitative genetics, meat science and animal science. One visitor from Uganda is a graduate student doing a MS and will be working on ideas to develop livestock production in Uganda to help family sustainability.

Seminars include sponsoring experts to come to Iowa State University. The program has supported a series of seminars called "Feeding the World are we making progress" and individual; seminar titles can be seen at http://www.ans.iastate.edu/section/Ensminger/?pg=seminars. These seminars give ISU students, staff and faculty opportunities to meet and hear interesting topics.

Travel and International Activities have included considerable activities in Uganda in the Center for Sustainable Livelihoods and can be seen at http://www.srl.ag.iastate.edu/work/livestock/. Other activities have included speaking in Bulgaria, Canada, China, Costa Rica, Korea, Kenya, Uganda, U.K. and other places. Other faculties have been partly supported to travel with student trips.

International Animal Agriculture course has been taugth in the animal science department at ISU. The course cover differences in animal agriculture around the world as well as topics like sustainability, food production and environmental issues.

Opportunities for the Future

The world is becoming a smaller place. The purpose of this program is to expand those activities and to help faculty and students participate more internationally. All interested parties are encouraged to visit with the Chair to discuss their ideas. Sponsorship of activities both at ISU and international are open to consideration.

1

Reduction of Heat Stress to Improve Production in Cattle

L.H. Baumgard[1], M.K. Abuajamieh[1], S.K. Stoakes[1], and R.P. Rhoads[2]

[1]Iowa State University, [2]Virginia Tech University

Summary

Environmental-induced hyperthermia compromises efficient animal production and jeopardizes animal welfare. Reduced productive output during heat stress was traditionally thought to result from decreased nutrient intake. Our observations challenge this dogma and indicate heat-stressed animals employ novel homeorhetic strategies to direct metabolic and fuel selection priorities independently of nutrient intake or energy balance. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid and protein metabolism independently of reduced feed intake through coordinated changes in fuel supply and utilization by multiple tissues. There may be nutritional, pharmaceutical and managerial options to take advantage of these aforementioned metabolic changes to improve productivity and animal welfare during the warm summer months.

Resumen

Reducción del estrés calórico para mejor la producción en vacunos

La hipertermia inducida por el ambiente compromete la eficiencia de la producción animal y pone en peligro el bienestar animal. La reducción del rendimiento productivo durante el estrés por calor tradicionalmente se pensaba que era resultado de la disminución de la ingesta de nutrientes. Nuestras observaciones cuestionan este dogma e indican que animales estresados por calor emplean estrategias de homeostasis novedosas para dirigir las prioridades metabólicas y de selección energética independientemente de la ingesta de nutrientes o el balance de energía. Por lo tanto, la respuesta al estrés de calor altera marcadamente el metabolismo post-absorción de carbohidratos, lípidos y proteínas, independientemente de la reducción de la ingesta de alimentos a través de cambios coordinados en el suministro energético y la utilización por diversos tejidos. Puede haber opciones nutricionales, farmacéuticas y de manejo para aprovechar estos cambios metabólicos antes

mencionados para mejorar la productividad y el bienestar de los animales durante los meses de verano.

Introduction

The term "stress" is defined in different ways, but is used to describe influences outside of a body system, which can shift the internal mechanisms away from their normal or resting state (Lee, 1965). Therefore, the term heat stress is used to describe the effects of increasing environmental temperature on different physiological systems. This is of interest to the dairy industry because of the detrimental changes (production, metabolic, reproductive) induced by heat stress (West, 2003; Bernabucci et al., 2005).

Heat stress negatively impacts a variety of dairy parameters including milk yield, milk quality and composition, rumen health, growth and reproduction and therefore is a significant financial burden (~$900 million/year for dairy in the U.S.; St. Pierre et al., 2003). Advances in management (i.e. cooling systems; Armstrong, 1994; VanBaale et al., 2005) and nutritional strategies (West, 2003) have alleviated some of the negative impact of heat stress on cattle, but productivity continues to decline during the summer. In the upper Midwest, heat-induced poor reproduction may be the costliest issue. For example, pregnancy rates at the Iowa State University Dairy decreased 19% during the 2010 summer and did not return to spring levels until the middle of December.

Biological Consequences of Heat Stress

The biological mechanism by which heat stress impacts production and reproduction is partly explained by reduced feed intake, but also includes altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements (Collier and Beede, 1985; Collier et al., 2005) resulting in a net decrease in nutrient/energy available for production. This decrease in energy results in a reduction in energy balance (EBAL), and partially explains (reduced gut fill also contributes) why dairy cattle lose significant amounts of body weight when subjected to unabated heat stress (Rhoads et al., 2009; Shwartz et al., 2009; Wheelock et al., 2010).

Reductions in energy intake during heat stress results in a majority of dairy cows entering into negative energy balance (NEBAL), regardless of the stage of lactation. Essentially, the heat-stressed cow enters a bioenergetic state similar (but not to the same extent) to the NEBAL observed in early lactation. The NEBAL associated with the early postpartum period is coupled with increased risk of metabolic disorders and health problems (Goff and Horst, 1997; Drackley, 1999), decreased milk yield and reduced reproductive performance (Lucy et al., 1992; Beam and Butler, 1999; Baumgard et al., 2002; 2006). It is likely that many of the negative effects of heat stress on production, animal health and reproduction indices are mediated by the reduction in EBAL (similar to the transition period). However, it is not clear how much of the reduction in performance (milk yield and reproduction) can be attributed or accounted for by the biological parameters affected by heat stress (i.e. reduced feed intake vs. increased maintenance costs).

Rumen Health:

The heat-stressed cow is prone to rumen acidosis, and many of the lasting effects of warm weather (laminitis, low milk fats etc.) can probably be traced back to a low rumen pH during the summer months. This may be explained by increased respiration rate which results in enhanced carbon dioxide (CO_2) exhalation. In order to be an effective blood pH buffering system, the body needs to maintain 20:1 bicarbonate (HCO_3-) to CO_2 ratio. Due to the hyperventilation induced decrease in blood CO_2, the kidney secretes HCO_3- to maintain this ratio. This reduces the amount of HCO_3- that can be used (via saliva) to buffer and maintain a healthy rumen pH. In addition, the heat-stressed cow ruminates less (because of the reduced feed intake and increased time respiring) and rumination is a key stimulator of saliva production. Furthermore, heat-stressed cows drool and this, coupled with reduced saliva production reduces the amount of buffering agents entering the rumen. Consequently, care should be taken when feeding "hot" rations during the summer months. In addition, fiber quality is important all the time, but it is paramount during the summer as it has some buffering capacity and stimulates saliva production (Baumgard and Rhoads, 2007).

Metabolic Adaptations to Reduced Feed Intake

A prerequisite to understanding the metabolic adaptations which occur with heat stress, is an appreciation of the physiological and metabolic adjustments to thermal-neutral NEBAL (i.e. underfeeding or during the transition period).

Early lactation dairy cattle enter a unique physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and animals typically enter NEBAL (Moore et al., 2005). Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary and tissue derived nutrients towards the mammary gland, and not surprisingly many of these changes are mediated by endogenous somatotropin which naturally increases during periods of NEBAL. One classic response is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. The reduction in insulin action activates adipose lipolysis, leading to the mobilization of non-esterified fatty acids (NEFA; Bauman and Currie, 1980). Increased circulating NEFA are typical in "transitioning" cows and represent (along with NEFA derived ketones) a significant source of energy (and are precursors for milk fat synthesis) for cows in NEBAL. Post-absorptive carbohydrate metabolism is also altered by reduced insulin action during NEBAL which results in reduced glucose uptake by systemic tissues (i.e. muscle and adipose). Reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues are key homeorhetic (an acclimated response vs. an acute/homeostatic response) mechanisms implemented by cows in NEBAL to support lactation. The thermal-neutral cow in NEBAL is metabolically flexible, and can depend upon alternative fuels (NEFA and ketones) to spare glucose (Baumgard and Rhoads, 2013). Glucose can then be utilized by the mammary gland to copiously produce milk (Bauman and Currie, 1980).

Heat Stress and Production Variables

Heat stress reduces feed intake and milk yield in dairy cattle. The decline in nutrient intake has been identified as a major cause of reduced production (Fuquay, 1981; West, 2002; 2003). However, the exact contribution of reduced feed intake to the overall reduced milk yield or average daily gain remains unknown. To evaluate this question in both dairy and beef cattle we have conducted experiments involving a group of thermal neutral pair-fed animals to eliminate the confounding effects of dissimilar nutrient intake. The pair-feeding model is necessary in order to differentiate between the direct and indirect effects of heat stress (mediated by reduced feed intake) on production and metabolism. Utilizing this model has allowed us to determine that the heat-induced decrease in nutrient intake only accounts for approximately 50% of the decrease in milk yield (Figures 1 and 2: Rhoads et al., 2009; Wheelock et al., 2010). The model indicates that direct effects of heat explain ~50-60% of decreased milk synthesis. Therefore, identifying hyperthermia-induced direct changes is likely a prerequisite to develop mitigation strategies to maximize milk yield during the warm summer months.

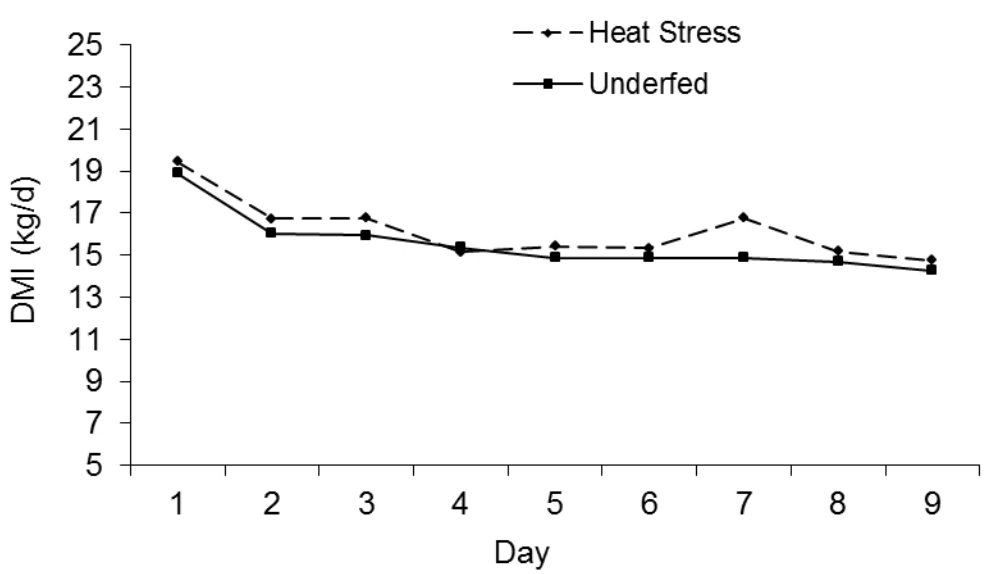

Figure 1. Effects of heat stress and underfeeding (pair-feeding) thermal-neutral lactating Holstein cows on dry matter intake (Rhoads et al., 2009).

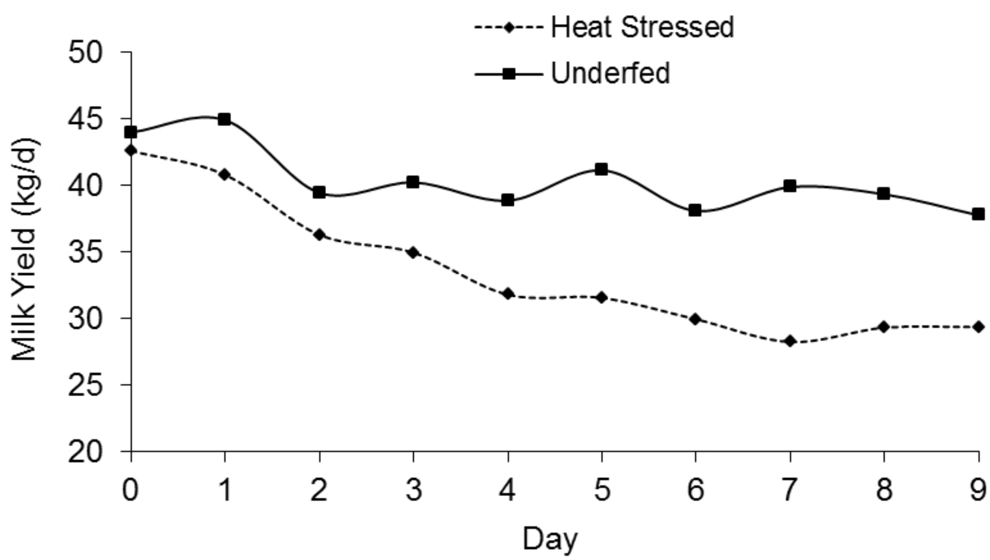

Figure 2. Effects of heat stress and underfeeding (pair-feeding) thermal neutral conditions on milk yield in lactating Holstein cows (Rhoads et al., 2009).

Pre-partum Heat Stress

The effects of heat stress during established lactation are well-characterized (Baumgard et al., 2012; Baumgard and Rhoads, 2013), but the effects of environmental hyperthermia prior to calving on post-parturition production parameters is not as clear. It was demonstrated that heat stress during the "far-off" period reduced gestation length, calf body weight and subsequent milk yield, even in intensely cooled cows following calving (Collier et al., 1982). This has recently been confirmed and results indicate that future milk production is substantially reduced in heat-stressed dry cows (Tao et al., 2012). Interesting, it appears that the heat-induced blunted adipose tissue mobilization "lingers" into lactation and dry cows that were heat-stressed are not able to enlist glucose sparing mechanisms necessary to support maximum milk yield, even though they were intensely cooled after calving (Tao et al., 2012). In addition, future reproductive variables are determinately affected in heat-stressed dry cows (even if they were intensely cooled during lactation; Wiersma and Armstrong 1989). Consequently, actively cooling dry cows should be an important part of a farm's heat stress abatement strategy.

Theoretical Reasons for Altered Metabolism

Well-fed ruminants primarily oxidize acetate (a rumen produced VFA) as a principal energy source. During NEBAL cattle increased their energy dependency on NEFA. However, despite the fact that heat-stressed cows have marked reductions in feed intake and are losing considerable amounts of body weight, they do not mobilize adipose tissue (Rhoads et al., 2009; Wheelock et al., 2010). Therefore, it appears that heat stressed cattle experience altered post-absorptive metabolism compared to thermal neutral counterparts, even though they are in a similar negative energetic state. The unusual lack of NEFA response in heat-stressed cows is probably in part explained by increased circulating insulin levels (O'Brien et al., 2010; Wheelock et al., 2010), as insulin is a potent anti-lipolytic hormone. Increased circulating insulin during heat stress is unusual as malnourished animals are in a catabolic state and experience decreased insulin levels. The increase in insulin action may also explain why heat-stressed animals have increased rates of glucose disposal (Wheelock et al., 2010). Therefore, during heat stress, preventing or blocking adipose mobilization/breakdown and increasing glucose "burning" is presumably a strategy to minimize metabolic heat production (Baumgard and Rhoads, 2007).

The increase in extra-mammary glucose utilization during heat stress creates a nutrient trafficking problem with regards to milk yield. The mammary gland requires glucose to synthesize milk lactose and lactose is the primary osmoregulator, thus determines overall milk volume. However, in an attempt to generate less metabolic heat, the body (presumably skeletal muscle) appears to utilize glucose at an increased rate. Therefore, the mammary gland may not receive adequate amounts of glucose, as a result mammary lactose production and subsequently milk yield is reduced. This may be the primary mechanism which accounts for the additional reductions in milk yield beyond the portion explained by decreased feed intake (Figures 1 and 2).

Heat Stress and Immunity

The metabolic profile of heat-stressed cattle shares some similarities to animals with a stimulated immune system and this is primarily characterized by hyperinsulinemia (Baumgard and Rhoads, 2013). The increased circulating insulin during heat stress is unusual because reduced feed intake, negative energy balance and body weight loss (hallmarks of heat stress) are typically associated with hypoinsulinemia. Incidentally, lactating cows with an activated immune system also have increased circulating insulin concentrations despite reduced feed intake (Waldron et al., 2006). Reasons for the hyperinsulinemia are not clear, but may include lipopolysaccharide (LPS), an endotoxin produced by gram-negative bacteria. We have demonstrated that cattle IV infused with LPS have marked (>50 fold) hyperinsulinemia 2 hours after LPS administration (Rhoads et al., 2009; Figure 3). Interestingly, the severe increase in insulin following LPS injection only causes minor hypoglycemia and this likely means that LPS causes insulin resistance. Heat-stressed rodents, poultry, pigs and humans have increased levels of circulating LPS because of intestinal integrity issues and presumably assume heat-stressed cattle do as well. Insulin's role during the immune response and during heat acclimation is not clear, but proper insulin action is necessary in order to up-regulate heat shock proteins.

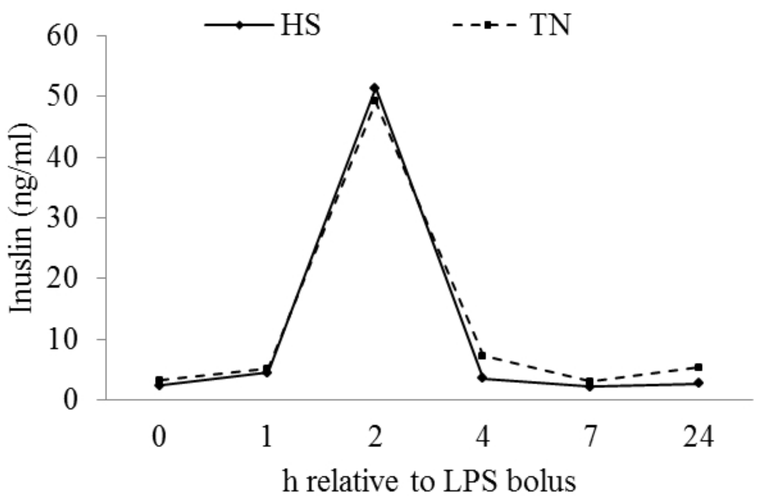

Figure 3. Effects of LPS infusion on blood insulin concentrations in growing Holstein calves either in heat-stressed (HS) or thermal neutral (TN) conditions (Rhoads et al., 2009).

Heat Stress Abatement

Heat abatement strategies are often employed as a means to ameliorate the negative effects of heat stress on production during the warm summer months (Smith et al., 2006). Cooling cows with shade and evaporative cooling with soakers and fans is a relatively cheap strategy to help minimize economic losses during an increased heat load (Collier et al., 2006). However, despite new barn construction and heat abatement systems, milk yield and other production parameters continue to be adversely affected by heat stress (Burgos et al., 2007).

Feedstuffs have varying heat increments (HI), largely due to efficiency of nutrient utilization or digestive end products (VanSoest et al., 1991). Fiber digestion results in a higher heat increment (sum of heat produced from rumen fermentation and nutrient metabolism) than digestion of fat or non-fiber carbohydrates (NFC). The major end product of fiber fermentation (acetate) is utilized less efficiently compared to the major end product of NFC digestion (propionate; Baldwin et al., 1980).

The table below illustrates heat increments of several common feedstuffs. The heat increment value expressed as Kcal/Mcal, net energy lactation (NE_L) was derived for total digestible nutrient (TDN) values of 40-100% and fitted to a multiple linear regression model: $y=a+bx+cx^2$. Where y= Kcal HI/Mcal NE_L and x=TDN solved constants are a= 1350.812, b= -17.1496, and c= 0.091517 (Chandler, 1994).

Table 1. Heat increment of common feed ingredients

Feed Ingredient	DM (%)	NDF % of DM	TDN % of DM	NE_L (Kcal/Kg)	HI/NE_L (Kcal/Mcal)
Haylage	35.0	53.0	59.0	1,326	658
Corn Silage	38.3	48.0	66.1	1,500	617
Grass Hay	88.0	53.0	55.0	1,228	684
Alfalfa Hay	89.9	47.5	60.0	1,350	651
Whole	93.0	49.0	87.0	2,453	386
Corn	87.0	10.0	88.0	2,035	550
SBM, 48%	90.0	14.0	81.0	1,866	562
Palm Oil (FA)	100.0	0.0	170.1	5,676	214
Prill (FA)	100.0	0.0	170.1	6,776	214
Tallow	99.0	0.0	191.3	6,402	214

Adapted from Chandler, 1994

Nutritional Strategies of Heat Stress

There are several nutritional strategies to consider during heat stress. A common strategy is to increase the energy and nutrient density (reduced fiber, increased concentrates and supplemental fat) of the diet as feed intake is markedly decreased during heat stress. In addition to the energy balance concern, reducing the fiber content of the diet is thought to improve the cow's thermal balance and may reduce body temperature. However, increasing ration concentrates should be considered with care as heat-stressed cows are highly prone to rumen acidosis.

Fiber:

Fiber is necessary for proper rumen function; current recommendations state a minimum dietary neutral detergent fiber (NDF) of 25% with the proportion of NDF from roughages equaling 75% of total NDF (NRC, 2001). However, its digestion and metabolism create more heat than compared to concentrates (VanSoest et al., 1991).

One common nutritional strategy involves reducing dietary fiber during an increased heat-load. However, adequate fiber in the diet is essential to maintain rumen health, and high quality forage helps to maintain feed intake. Grant (1997) demonstrated that a roughage NDF value of 60% still provides sufficient fiber for production of fat corrected milk. On the other hand, Kanjanapruthipong and Thaboot (2006) speculated that the minimum dietary NDF of 23% DM and roughage NDF proportion of 55% dietary NDF have sufficient effective NDF for dairy cows in the tropics.

Protein:

Due to reduced feed intake, dietary protein levels may need to be increased during heat stress (West, 1999). Huber et al. (1993) demonstrated that heat-stressed cows fed lower soluble protein levels had increased milk yield and increased dry matter intake (DMI). Huber et al. (1994) showed that heat-stressed cows fed a highly degradable protein diet (65% of crude protein (CP)) had a 6% reduction in DMI and an 11% decrease in milk yield when compared to diets with lower degradable protein (59%) or diets with lower CP (16%). This agrees with recent recommendations which suggest that addition of dietary CP, more specifically rumen un-degradable protein, is not helpful (Arieli et al., 2006). A possible reason why highly degradable protein diets appear to be deleterious during heat stress is that both rumen motility and rate of passage decline. This allows for a longer residence time and thus more extensive protein degradation (Linn, 1997). We have demonstrated that blood urea nitrogen is elevated in heat-stressed cows compared to pair-fed controls (Wheelock et al., 2010), although it is not clear whether this originates from excess rumen ammonia production or from skeletal muscle breakdown. Regardless, excess ammonia needs to be eliminated and this removal has an energy cost (7.2 kcal/g of nitrogen; and thus increases heat production) as it is metabolized to urea and excreted in the urine (Tyrell et al., 1970). How heat stress affects dietary protein requirements is ill-defined and more research is needed in order to generate more appropriate recommendations.

Fat:

Increasing the amount of dietary fat has been a widely accepted strategy within the industry in order to reduce basal metabolic heat production. As stated above, the heat increment of fat is over 50% less than typical forages (Table 1) so it is seemingly a rational decision to supplement additional lipid and reduce fiber content of the diet. However, there are surprisingly few experiments specifically designed to evaluate how supplemental dietary fat affects body temperature indices or even production parameters (Table 2). Most experiments report little or no differences in rectal temperatures (Moody et al., 1967; Knapp and Grummer 1991; Chan et al., 1997; Drackely et al., 2003) and only one paper demonstrated a slight reduction at a specific time of day but not at the other times (Wang et al., 2010). In fact, one report indicated that cows fed additional fat actually had increased in rectal temperatures (Moallem et al., 2010) and these same authors and a recent report (Wang et al., 2010) indicate that additional fat-fed cows had increased respiration rates. A reason why feeding fat does not seemingly improve the thermal balance of heat-stressed cows is difficult to rationalize. It could be that small decreases in a thermal load would be difficult to detect at specific but limited time points, but that these minor changes would accumulate over time into a significant improvement. It would be of interest to evaluate body temperatures in heat-stressed cows fed additional fat utilizing a continuous thermometer system (i.e. HOBOs or eye-button technology).

Additional fat feeding can sometimes decrease DMI in thermal neutral cows (Chillard, 1993) but reduced nutrient intake is typically not observed in heat-stressed cows fed supplemental fat (Moody et al., 1967; Skaar et al., 1989; Knapp and Grummer, 1991; Drackely et al., 2003; Warntjes et al., 2008; Wang et al., 2010). Milk yield responses to additional fat are variable and some authors report no diet effect (Moody et al., 1967; Knapp and Grummer, 1991; Chan et al., 1997; Moallem et al., 2010) while others report an increase in milk yield (Skaar et al., 1989; Drackely et al., 2003; Warntjes et al., 2008; Wang et al., 2010). Similar to body temperature indices and milk yield data, the effects of dietary fat on milk composition during heat stress also vary and no clear consensus has been reached (Table 2). Overall, results from a limited number of experiments vary, but little or no apparent benefit was typically observed when supplemental dietary fat was included. Reasons for the discrepancies are unclear, but could be due to the type of fats used (saturated vs. unsaturated), rate of inclusion, type of "protection" (i.e. calcium salt vs. prill), environmental factors (i.e. severity of heat stress), or other dietary interactions. Regardless, the dairy industry (nutritionists) needs additional controlled experiments (besides theoretical heat calculations) in order to make intelligent ration balancing decisions regarding the inclusion of supplemental fat.

***Ionophores*:**

We propose enhanced extra-mammary tissue glucose utilization may be a key mechanism explaining the decrease in milk yield during heat stress. Two glucose molecules are the substrate for lactose (the primary osmotic regulator of milk yield) synthesis and on a molar basis; lactose is nearly equivalent (95%) to two moles of glucose. Heat-stressed cows secrete about 370 g less lactose (Rhoads et al., 2009) or have approximately twice as much of a decrease in milk lactose yield as pair-fed thermal neutral controls (Wheelock et al., 2010). Therefore, heat-stressed cows in our previous experiments are secreting almost 400 g less glucose/d than thermal neutral counterparts on a similar plane of nutrition. Monensin is a well-described rumen modifier that increases the production of propionate, which is the predominate gluconeogenic precursor in ruminants. The increase in carbon conservation during fermentation is a key mechanism in how monensin increases feed efficiency in growing and lactating ruminants. We have now demonstrated that monensin increases the gluconeogenic rates (on a DMI basis) and utilizing monensin is a key strategy to improve the glucose status of heat-stressed cows (Baumgard et al., 2011).

Water:

Water intake is vital for milk production (milk is ~87% water) but it is also essential for thermal homeostasis. This stresses how important water availability and waterer/tank cleanliness becomes during the summer months. Keeping water tanks clear of feed debris and algae is a simple and cheap strategy to help cows remain cool (Baumgard and Rhoads, 2007)

Dietary Cation-Anion Difference (DCAD):

Having a negative DCAD during the dry period and a positive DCAD during lactation is a good strategy to maintain health and maximize production (Block, 1994). It appears that keeping the DCAD at a healthy lactating level (~+20 to +30 meq/100 g DM) remains a good strategy during the warm summer months (Wildman et al., 2007).

Minerals:

Unlike humans, bovines utilize potassium (K^+) as their primary osmotic regulator of water secretion from sweat glands. As a consequence, K^+ requirements are increased (1.4 to 1.6% of DM) during the summer and this should be adjusted for in the diet. In addition, dietary levels of sodium (Na^+) and magnesium (Mg^+) should be increased as they compete with K^+ for intestinal absorption (West, 2002).

Conclusions

Heat stress negatively impacts economic parameters associated with profitable milk production. Implementing heat stress abatement strategies is crucial to minimize fiscal losses. In addition to physical barn management, nutritional strategies can be implemented to help ameliorate summer-induced losses. Maintaining rumen health is of primary importance as heat-stressed cows are more prone (for a variety of reasons) to rumen acidosis. Another widely held dogma is that supplementing dietary fat is an effective tactic during heat stress and this stems from theoretical calculations indicating that the heat increment of feeding is much lower for lipids (especially compared to roughages). However, a review of the limited literature fails to corroborate the arithmetic heat savings or ultimately demonstrate a consistent effect on production parameters. The dairy industry needs definitive research on whether or not to include supplemental fat during the warm summer months.

Table 2. Effects of supplemental dietary fat on production parameters in

Reference	Fat Type	RT	RR	DMI	FE	MY	MF	MP	Metabolites
1	SFA/UFA	↑	↑	↓	↑	↔	↑	↔	↑NEFA
2	SFA	↓	↔	↔	↑	↑	↑	↑	↓NEFA
3	SFA	NM	NM	↔	↔	↑	↓	↑	NM
4	LCFA	↔	↔	↔	↑	↑	↔	↓	↓NEFA
5	SFA	↔	↔	↔	↔	↔	↔	↔	NM
6	LCFA/Tallow	↔	↔	↔	↔	↔	↔	↔	NM
7	SFA	NM	NM	↔	↔	↑	↔	↔	↔
8	SFA/UFA	↔	↔	↔	↔	↔	↔	↔	↔

NM: Not Measured	RT: Rectal Temperature	1 Moallem et al., 2010
↑: Increase	RR: Respiratory Rate	2 Wang et al., 2010
↓: Decrease	DMI: Dry Matter Intake	3 Warntjes et al., 2008
↔: No Change	FE: Feed Efficiency	4 Drackely et al., 2003
SFA: Saturated Fatty Acids	MY: Milk Yield	5 Chan et al., 1997
UFA: Unsaturated Fatty Acids	MF: Milk Fat	6 Knapp and Grummer, 1991
LCFA: Long-Chain Fatty Acids	MP: Milk Protein	7 Skaar et al., 1989
	NEFA: Non-Esterified Fatty Acids	8 Moody et al.,1967

*Aspects of this manuscript were adapted from the 2013 Ontario Bovine Practitioners Conference proceedings paper (Baumgard et al., 2013).

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2

Genetic and Genomic Improvement in Livestock

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Summary

Peru has a significant cattle population but is a net importer of beef. The efficiency of beef production in Peru could be improved by changing the genetic makeup of the Peruvian herd. In theory, this could be done by crossbreeding, grading up to alternative breeds, or by within breed selection among existing cattle. The opportunities and challenges of some of these options are discussed, including the use of new genomic tools to enhance selection. The most practical option might be to undertake marker-assisted selection using major gene effects that have been identified elsewhere in the world and whose effects have been validated under Peruvian climatic, management and economic circumstances.

Resumen

Selección genómica y mejoramiento genético en vacunos

Perú tiene una población significativa de vacunos de leche, pero es un importador neto de carne de vacuno. La eficiencia de la producción de carne en el Perú podría mejorar, cambiando la estructura genética del rebaño peruano. En teoría, esto podría hacerse mediante el cruzamiento, la absorción de razas alternativas, o mediante la selección de razas entre el ganado existente. Se discuten las oportunidades y desafíos de algunas de estas opciones, incluyendo el uso de nuevas herramientas genómicas para mejorar la selección. La opción más práctica podría ser la de realizar la selección asistida por marcadores usando efectos de genes mayores que han sido identificados en otras partes del mundo y cuyos efectos han sido validados bajo circunstancias climáticas, económicas y de gestión del Perú.

Background

Peruvian livestock includes some 5-6 million cattle, 5-6 million camelids and 2 million goats (FAO, 2014). It is a net importer of beef and dairy products. Argentina and Brazil have ten and forty times greater cattle numbers, respectively. There are many opportunities to increase beef production throughout countries in South America, including the use of improved cattle germplasm more suited to local environments, and the use of improved nutritional regimes that can increase growth rates and reduce ages at harvest. This paper considers the opportunities and challenges for genetic improvement of South American beef cattle.

Genetic improvement

Appropriate choice of breed or breed-crosses, as well as within breed selection can contribute to improved efficiency of beef production. Some particular challenges in Peruvian beef production include some high altitude production systems, some hot and humid climatic conditions, and low forage quality and quantity. Cattle often struggle to perform at high altitude due to their susceptibility to high-altitude or brisket disease (Shirley et al., 2008; Wuletaw et al., 2011), unless they have a history of breeding and selection at altitude such as in the Andes, Northwest Ethiopia, Rocky Mountains or European alps. Cattle selected for high performance in temperate environments struggle to achieve high levels of reproductive performance in hot and humid conditions. In those circumstances, *Bos indicus* cattle or tropically-adapted *Bos taurus* breeds will typically outperform breeds that are more productive in temperate conditions (Burrow et al., 2001). Cross-breeding therefore needs to be approached with caution, and with careful choice of breeds if it is to include anything other than locally-adapted breeds. However, small population sizes and low adoption of breed improvement technologies have frequently limited genetic improvement in locally-adapted breeds. Nevertheless, conventional selection offers promise if appropriately implemented, but perhaps not as much as leveraging information discovered in other breeds and countries using new genomic technologies. This paper reviews some of these opportunities in the context of improving beef cattle in some parts of South America.

Crossbreeding

Outcrossing or outbreeding is the opposite of inbreeding and refers to the mating of animals that are more distantly related than would occur from random mating. An extreme form of outcrossing is crossbreeding whereby animals of different breeds are bred together. In general this can have several advantages, one of which is heterosis or hybrid vigor whereby the first-cross animals outperform the average of the parental breeds. Another advantage is complementarity, which can occur in the absence of heterosis, and reflects weaknesses in one breed being complemented by strengths in the other breed, and vice versa, so that considering all traits the crossbred is more desirable than the contributing purebreds. In practice there is often a problem that natural or artificial selection has been very successful in improving a particular breed so that it is well-adapted to a particular climatic, management or economic circumstance, but no other competitive breeds exist, with the effect that any crossbreeding might result in inferior animals relative to the adapted breed. This is the situation in dairy production using the Holstein breed in some intensive, temperate environments. Another practical complication with crossbreeding is that the first-cross animals may have production superior to the indigenous adapted breed, and thereby immediately recognized as being beneficial in production circumstances with high or low management levels. However, if first-crosses are backcrossed to the foreign breed, the resultant animal with only an average ¼ adapted genome may be too poorly adapted and therefore poor performing, particularly in terms of reproduction, and more so in environments with low management levels (Madalena, 1987). If the first-cross is backcrossed to the indigenous breed, the resultant animals with ¼ foreign breed may not be notably better than the locally-adapted breed. Finally, if the superiority of the first-cross includes a substantial component of heterosis, then second-cross animals may be markedly inferior to the first-cross. This limits the value of crossbreeding unless first-cross animals can be continuously obtained from some other source. Other issues with crossbreeding include the segregation of major gene effects influencing appearance, including coat color, coat color pattern, presence of horns, slick hair coats etc, that may not be desirable in local circumstances. Finally, crossbreeding can be problematic to manage in practice in small herds if it requires maintenance of multiple sire breeds.

In the absence of genotype-environment interaction, and when several breeds are equally competitive as purebreds, crossbred or advanced crosses known as composite cattle are commonplace, as is the circumstance in the US beef industry. In South America where it is common for cattle to be stressed, either through climate (e.g. heat and humidity), altitude, nutritional deprivation (e.g. dry season), or disease, crossbreeding may not be a practical alternative because there may be only one locally adapted breed and it may therefore not be possible to find another breed that is equally competitive. This is the case in humid and tick-infested parts of Brazil for example, where other breeds struggle to compete in overall performance with tropically-adapted tick-tolerant *Bos indicus* cattle such as Nellore.

Furthermore, it is important in the development of crossbreeding programs that the performance of the crosses are well characterized across the entire range of traits that are important from an economic or management viewpoint. In the US, extensive germplasm evaluation (GPE) studies at US-MARC in Clay Center Nebraska (http://www.ars.usda.gov/News/docs.htm?docid=6238) are a good example of thorough research that ensures producers have access to quality information when considering a crossbreeding program in mid Western US production circumstances.

Pedigree-based within-breed selection

Natural and artificial selection within a population can, in successive generations, improve its adaptation to environmental circumstances and increase its productivity. Natural selection influences fitness, whereas artificial selection can influence any heritable trait for which the selection candidates deviate from population average. Natural selection does not require any pedigree or performance testing, whereas artificial selection requires that the selection candidates can be ranked in relation to the attribute under selection.

The efficiency of artificial selection will be influenced by the accuracy of that ranking which we measure as the correlation between true and estimated merit, or its square, which we refer to as reliability. In the case of artificial selection based only on individual phenotype, the efficiency of selection is directly related to the strength of the association between genotype and phenotype, which is reflected in a parameter known as heritability. However, if non-genetic factors such as age at measurement, age of dam, or season of calving influence phenotype, and cannot be recorded and taken into account in the ranking process, then mass selection on individual phenotype will not be as effective as implied by the heritability. If cohort groups, such as animals of the same sex born in the same herd-year-season, comprise no more than a few animals, then reliabilities of prediction can be compromised by non-genetic effects even if recording is of a high standard. This is particularly problematic for categorical traits (e.g. calving score), where no useful information is obtained if all animals in the cohort have the same categorical score.

The reliabilities of predictions can be improved by including pedigree information and performance measured on relatives, particularly for traits that are sex-limited or measured later in life. However, obtaining pedigree information can be problematic in multi-sire pastures, particularly under extensive conditions.

The intensity of selection is determined by the proportion of available candidates that are chosen to be parents of the next generation. In a sufficiently large population, the intensities of selection on each selection pathway (e.g. sires, dams) are insensitive to population size. However, in small populations, the selection intensity may be reduced because the selection proportion has to be large. For example, if the mating ratio is 1 bull to 100 cows, and a herd must use at least two sires, the selection intensity will be compromised if the herd includes less than 200 cows.

Within-breed selection can be more efficient if data from different herds can be pooled together, ideally including all performance recorded animals in a particular environmental circumstance. However, such national improvement programs require various infrastructure, including consistent animal identification system, consistent trait definitions, and database systems to store and access pedigree and performance information. Finally, the analysis of this data to make the most of across-herd and across-generational information involves the development and use of computer software that requires skilled expertise. Collectively, the development of successful national improvement programs can be particularly problematic in countries that do not have experience in such data collection and analysis, or lack a core of motivated breeders with a history of careful pedigree and performance recording along with competent animal management.

Genomic improvement

The distinction between genetic and genomic improvement is that the latter uses molecular features spanning the whole-genome. In beef cattle breeding the molecular features have most commonly comprised 50k or more approximately evenly-spaced single-nucleotide polymorphisms (SNPs). These 50k SNP genotypes are readily available from DNA obtained from hair follicles or other tissues at a cost of US$50-100 per animal depending upon numbers of animals being genotyped. For training and discovery purposes as described below, a minimum of 1,000 genotyped animals are required for most endeavors.

Genomic selection

Genomic selection refers to breeding strategies that use whole-genome information along with performance and perhaps pedigree information to rank selection candidates. Its recent popularity was stimulated by a theoretical paper (Meuwissen et al., 2001) that outlined some analytical approaches about 5 years before Illumina beadchip SNP genotypes (http://www.illumina.com/products/bovine_snp50_whole-genome_genotyping_kits.ilmn) became practically available for large numbers of animals, at prices that were a fraction of the cost of previous microsatellite genotyping strategies that had been used for research. The original Meuwissen et al. (2001) concept involved a two-step process. First was the generation of a prediction equation obtained by statistical analysis of a historical population of animals with SNP genotypes and phenotypes, or SNP genotypes and progeny test predictions of genetic merit. These animals are known as the "training" or discovery population. The analysis involves calculating the genetic merit of all the chromosome fragments that are present in the training population. The number of animals required for this process depends upon the desired accuracy of future predictions, the effective population size of the breed, the heritability of the trait, among other factors (Goddard and Hayes, 2009). Less than 10 animals may be required to map a monogenic trait that has accurate phenotypes, but polygenic traits requires a minimum of thousands of animals in the training population to obtain accurate prediction equations (Goddard and Hayes, 2009). The second step in genomic prediction is to apply the prediction equation to rank new selection candidates. This process will be more accurate if the selection candidates are immediate descendants of animals in the training population. Modern approaches to this problem allow these two steps to be combined in a single analysis that can exploit pedigree, performance and genomic information (Aguilar et al., 2010; Fernando et al., 2014).

Genomic prediction has been widely-adopted throughout the world for dairy cattle improvement, with some training populations now exceeding 100,000 animals. Its adoption has been slower in beef cattle, but many US breed associations now include genomic information in their predictions of merit (Saatchi et al., 2011; 2012; 2013), and training populations now exceed 10,000 animals for each of a number of breeds. The original Illumina 50K beadchip has now been augmented with custom content, and proprietary chips such as the GeneSeek Genomic Profilers (GGP - http://www.neogen.com/Agrigenomics/Beef.html#Seedstock) in low-density (GGP-LD now 30k) and high-density (GGP-HD about 70k), these now being more widely used than the 50k in US beef cattle circumstances. Alternative custom low-density panels are used in dairy and beef cattle in some other countries, particularly in Europe. Many training populations have been genotyped at a mixture of different densities, requiring strategies like imputation (Browning and Browning, 2009) to be applied so that all animals in the training population have (imputed) genotypes of the same density. The major advantages of the GGP-LD is that the genotyping cost is much less than for the 50k, the public content is well distributed along the genome to provide high-accuracy imputation and reliable parentage determination, while the custom content directly interrogates important mutations such as deleterious genes so that one genotyping proves can provide all the information required by most breeders. The major advantage of the GGP-HD is that in addition to the public content from the GGP-LD chip it includes additional markers in regions where the 50k content was inadequate, and fewer markers where 50k content was superfluous.

In contrast to original speculation, it is apparent that continued augmentation of the training population with additional animals with genotypes and phenotypes is required, necessitating continued collection of phenotypic information (Wolc et al., 2011). Genotyping has accordingly provided more accurate prediction of young animals than was previously possible, but has not allowed for investment in collection of phenotypes to be markedly reduced.

To date, the development of beef cattle training populations in South America has lagged North America and Europe. This is likely partly due to the initial investment required in genotyping the training population, and partly because national breed improvement programs that collect and characterize breeding merit have typically involved fewer animals with large numbers of progeny, and a lesser range of trait measurements. Some work has been undertaken with Nellore cattle in Brazil (Lobo et al., 2011; Neves et al., 2014), Hereford cattle in Uruguay and Argentina (Saatchi et al., 2013), and Angus cattle in Argentina (unpublished).

Detection of major gene effects

Genomic prediction computes the genetic merit of every chromosome fragment present in the training population, the chromosome fragments being identified by the SNP genotypes they contain and those flanking SNPs in the immediate vicinity. Genomic prediction assesses the merit of a newly genotyped animal by summing up the genetic merits of all the chromosome fragments that animal appears to have inherited. It is often assumed that complex quantitative traits are determined by a polygenic or infinitesimal model, involving a large number of genes each contributing a small amount to the variation in the trait. It now seems more likely that many polygenic traits include some genomic regions with larger, readily detectable effects, as well as many genomic regions with small effects. Characterizing the effects of various regions in order to find those with largest effects is known as a genome-wide association study (GWAS). These have been applied in a number of beef cattle breeds, across a range of traits and represent widely-used bulls and encompass all those traits routinely measured by breed associations (e.g. birth, weaning, yearling weights, calving ease), or research animals that comprise a specific experiment and specific trait(s) of interest (e.g. feed efficiency, fatty acid profile, or disease resistance). Results to date show a surprisingly high degree of commonality between regions identified with large effects, those regions being identified across disparate breeds, and within breed, across a wide range of traits (Saatchi et al., 2014). Considerable effort including next-generation sequencing of individual sires is now being undertaken with a view to fine map and ultimately determine the causal mutation(s) for these large gene effects. In the absence of knowledge of causal mutations, or tightly linked markers conserved across breeds, results from attempts to train in one breed or population and predict merit in another breed or population have not been very promising (Kachman et al., 2013).

Marker-assisted selection

Marker-assisted selection uses markers to predict or infer the presence of favorable alleles at one or more loci in selection candidates. Prior to application of marker-assisted selection, favorable regions of the genome must be somehow identified, commonly by undertaking genome-wide association studies. Marker-assisted selection is most straightforward if markers in high linkage disequilibrium with the causal mutation have been identified. In that case, the selection can be done across families or breeds. Some early applications of marker-assisted selection required specific characterization of alleles within each family, limiting their utility to populations with extensive pedigree and performance recording (Dekkers, 2004). Marker-assisted selection has now commonly been superseded with genomic selection, typically using genome-wide SNP markers or perhaps just that subset of markers flanking the regions of interest.

Applying marker-assisted selection in countries like Peru could be undertaken to select for regions of interest that have been identified in local studies, but that would require careful pedigree and performance recording in sufficiently large populations prior to implementation. Alternatively, it could be used to select for genomic regions that have been identified in other production circumstances, such as in other breeds or from other countries in South America or elsewhere in the world. The effects of alternate alleles at the genomic region of interest would need to be quantified and validated in the local population prior to their use in selection. There is a recent example of such a finding in a gene known as PLAG1 influencing growth rate (Karim et al., 2011) and fertility (Fortes et al., 2013).

A major gene was coarsely mapped to the region around 24-26 cM on chromosome 14 in Japanese Wagyu cattle. A major gene segregating in F2 Holstein-Friesian Jersey cattle in the same chromosomal region was subsequently fine-mapped and a mutation in *PLAG1* was identified as one of three concordant mutations that could have caused the observed variation (Karim et al., 2011). The same region was discovered to have major effects in a number of *Bos taurus* and *Bos indicus* breeds studied in Australia, and the mutation proposed by Karim et al. (2011) was shown to explain the observed differences (Fortes et al., 2013). The same genomic region was also reported to be segregating pleiotropic effects for a range of traits in a number of US breeds (Saatchi et al., 2014). The Australian data showed that in *Bos indicus* cattle the allele associated with increased growth (14 kg effect on liveweight at feedlot exit) was also associated with delayed puberty (38 days) and extended post-partum anestrus interval (15 days). This gene therefore appears to be segregating in a wide variety of *Bos taurus* and *Bos indicus* breeds, and exhibits the same direction of effects. One breeding strategy would be to use breeding cows with at least one of the allele associated with smaller size, earlier puberty and reduced intercalving interval, and breed these cows to sires that carry one or two copies of the allele associated with delayed puberty and increased weight. Potential sires and replacement heifers would need to be tested to determine their genotype at the *PLAG1* locus.

As more causal mutations are discovered there will be many more examples that should be characterized in cattle breeds used in Peru and some might be applicable for selection in Peruvian circumstances.

Marker-assisted introgression

Introgression is the process of introducing a mutation from another individual into a population in which that mutation does not already exist. For example, it could be used to introduce the polled locus into a horned breed, or the myostatin or double-muscled variant into a normally-muscled breed, or the tropically-adapted slick hair locus into a breed that cannot otherwise tolerate hot and humid conditions. Provided the three alternative genotypes (QQ, Qq and qq) can be cheaply and easily identified from phenotype, the introgression can be done without markers. However, many mutations of interest may be sex-limited, exhibited late in life, or may demonstrate dominant gene action, in which case the introgression will be much more effective if it can be undertaken based on direct interrogation of the introgressed DNA. This can be done by marker-assisted introgression of a large DNA fragment known to contain the mutation of interest, even if the causal mutation has not been characterized. Alternatively, if the mutation is known, the introgression can be more effective if the number of copies of the desirable allele can be directly interrogated. Marker-assisted selection differs from marker-assisted introgression in that marker-assisted selection targets mutations that are already segregating in the population of interest whereas marker-assisted introgression requires initial crossing to introduce the desirable mutation. Once introduced, backcrossing of carriers is followed by selection of those offspring carrying the new mutation with the aim of producing heterozygous animals that only contain genome fragments of the other breed in the immediate region of the new mutation. Such animals can then be interbred to produce homozygotes of this new mutation in the genetic background of the local breed.

Gene editing

Creation of transgenic livestock has historically involved a somewhat primitive process, and has mostly commonly involved introducing traceable modifications that include DNA from other organisms (from *trans* meaning across). In contrast to the situation in plants, there are no examples of transgenic livestock that have markedly increased productivity, and none have been accepted and adopted in local or international markets. There have been a number of transgenic animals produced, principally targeted at the production of pharmaceutical products from milk, such as human lactoferrin in dairy cattle, or α-1-anti-trypsin in dairy sheep. Other transgenic animals have been produced for increasing knowledge of biology (e.g. Australian studies modifying the protein composition of wool to produce stronger woolen yarns), or as models of human disease (e.g. introduction of the Huntingtons disease mutation in sheep).

Gene editing offers a markedly different approach to creating more productive livestock. In its first applications, it is more likely to be used to produce cisgenic individuals (from *cis* meaning same), and the outcome will be animals that do not contain any foreign DNA and their genomes are not detectable as having been modified. Gene editing can now be done using one of three different technologies; ZFN, TALENS, or CRISPR (Gaj et al., 2013). In all three approaches, a particular mutation of interest can be targeted, and the use of natural DNA breakage and repair mechanisms can be used to naturally produce an animal carrying the desired mutation. Likely candidates for mutation would include genes to be disrupted to render them dysfunctional (i.e. knocked out), to be disrupted to reduce their level of expression (i.e. knocked down), or to be modified to mimic an existing mutation already found to desirably influence performance in some other breed of cattle. Such cattle would be functionally indistinguishable from those that could be produced by marker-assisted introgression, but would be produced in a much shorter timeframe and at much less cost.

Conclusions

Selection within or across breeds can be used to improve cattle productivity and profitability. However, these options are not available in all climatic, management and economic circumstances. This is particularly true for animals managed in small herds in stressful environments. New approaches to selection that utilize genomic technologies offer real promise for application in circumstances where funds are available for genotyping and there is already a database comprising historical pedigree and performance records of large cohorts of cattle. In the absence of such a database, there are opportunities for marker-assisted selection for variants discovered in other breeds or populations, provided those variants can be shown to be segregating in local breeds and demonstrating phenotypic differences in local environmental conditions for the trait of interest. If the variant is not segregating, and the local breeds are fixed fro the unfavorable allele, then there is the opportunity for marker-assisted introgression of the alternate allele. In future, gene editing may provide options for directly producing biologically motivated variants of interest for local validation and exploitation to improve cattle production.

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Embryo development and survival in ruminant livestock species

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Summary

Feeding the world's population in the year 2050 will be challenging, and significant improvements in production efficiency will be needed to markedly increase the world's food supply. Any efforts to increase production efficiency in domestic ruminant livestock species will depend on an enhancement in reproductive efficiency. Reproductive efficiency depends on four biological events occurring in a coordinated fashion to result in the production of live offspring. Oocytes must be released from the ovarian follicles of the female, ovulated oocytes must be fertilized by a spermatozoon from the male, the resultant embryo must develop and grow fetal membranes to attach itself to the uterus, and the fetus must develop normally and survive to term. This paper will provide an overview of factors influencing the development and survival of preimplantation embryos from domestic ruminant livestock species.

Resumen

Desarrollo y sobrevivencia embrionaria en rumiantes

La alimentación de la población mundial en el año 2050 será un reto, y una mejora significativa en la eficiencia de producción será necesaria para aumentar notablemente la oferta de alimentos en el mundo. Todos los esfuerzos para aumentar la eficiencia en la producción de especies de rumiantes domésticos dependerán de una mejora en la eficiencia reproductiva. La eficiencia reproductiva depende de cuatro eventos biológicos que ocurren de manera coordinada para dar lugar a la obtención de crías vivas. Los ovocitos deben ser liberados de los folículos ováricos de la hembra, los ovocitos ovulados deben ser fertilizados por un espermatozoide del macho, el

embrión resultante debe desarrollarse y desarrollar membranas fetales para adherirse al útero y el feto debe desarrollarse normalmente y sobrevivir hasta el término de la gestación. Este artículo ofrecerá una visión general de los factores que influyen en el desarrollo y la supervivencia de los embriones de especies de rumiantes domésticos antes de la implantación.

Introduction

Ruminant livestock species are important contributors to the global supply of meat, milk, fiber, and hides. Despite their major contribution to meeting current human needs for food and clothing, a significant increase in production efficiency of ruminant livestock species will be needed to meet the future needs of the world's rapidly growing human population. Some have estimated the global population of humans to reach 9.6 billion by the year 2050 (Searchinger et al., 2014), and the food supply will need to double between now and then in order to avoid widespread famine.

Production efficiency of ruminant livestock species hinges on successful reproduction, and great attention must be given to reproduction if an increase in production efficiency is to be attained. It is easy to understand that females who do not become pregnant will not give birth to offspring which can subsequently be raised for meat, fiber, or hides. Furthermore, females who do not become pregnant will not initiate lactation and cannot, therefore, provide milk for human consumption. Thus, reproductive efficiency is of utmost importance to increasing overall production efficiency.

Successful reproduction is necessary for a species to survive, and it seems logical that there has been strong natural selection pressure exerted on this trait for many centuries. However, successful reproduction is dependent on a series of biological events occurring at a precise time and in a precise order, and any deviation from this expected sequence of events can easily lead to reproductive failure. As livestock farmers move animals from extensively-managed to intensively-managed production settings, and as they deal with growing challenges such as global climate change, the opportunity for reproductive failure is a very real threat to global food and fiber production. The objective of this manuscript is to provide readers with a fundamental understanding of the reproductive process in ruminant livestock species, with a special focus on preimplantation embryonic development and survival.

Four Major Components of Reproduction

Successful reproduction can be defined as the production of live offspring from the mating of a male and female. Matings may occur by natural service or via alternative reproductive approaches such as artificial insemination or embryo transfer. The production of live offspring necessitates that four distinct - but interrelated - biological events occur.

The first of these biological events is the release of a viable oocyte (egg) from the female's ovarian follicle at the time of ovulation. Next, the ovulated egg must be fertilized by a viable spermatozoon from the male. Thirdly, the fertilized egg (also known as a zygote) must grow normally and must develop a functional placenta to enable the conceptus to attach to its mother's uterus. Lastly, the fetus must develop normally *in utero* so that a healthy neonate can be delivered at the time of parturition.

These four biological events - ovulation, fertilization, embryo development, fetal development - must occur sequentially and successfully in order for a viable offspring to be produced. In polytocous species that typically produce more than one offspring per parturition, the *rate* at which these events occur dictates the level of reproductive efficiency. In addition, it should be clearly understood that failure at any one of the four steps in the reproductive process will preclude occurrence of the other subsequent reproductive events and will result in complete reproductive failure.

Ovulation Rate

Ruminant livestock species differ in the expected number of eggs released at the time of ovulation. Some ruminants, such as cattle and alpacas, are classified as monotocous because they typically release only one egg during the period of ovulation and typically give birth to only one offspring per parturition. Other ruminants, such as sheep and goats, are classified as polytocous because they typically release more than one egg during the period of ovulation and give birth to one or more offspring per parturition.

In times of severe stress or negative energy balance (caused by underfeeding or the inability of the female to ingest sufficient dietary nutrients commensurate with her metabolic needs), it is possible for ruminant females to be anovulatory. Such females who do not ovulate obviously will not achieve successful reproduction. In monotocous females who ovulate a single egg, the number of offspring born is expected to be no more than one because the occurrence of monozygotic (identical) twins is quite low in ruminant livestock species (Johansson and Rendel, 1968). In polytocous females, the number of eggs shed at ovulation sets the upper limit to the number of offspring produced during that pregnancy; however, the release of multiple eggs does not guarantee that multiple offspring will be born because fertilization failure, embryo death loss and/or fetal death loss could occur.

Farmers and ranchers have the ability to potentially influence ovulation through management choices they make. In cattle, peri-pubertal females should undergo reproductive tract scoring (Anderson et al, 1991) to ensure that they are not in an anovulatory state. Reproductive tract scoring involves an assessment (via ultrasound or transrectal palpation) of the size and functionality of the ovaries, as well as the size and tone of the uterine horns. Females with a reproductive tract score of 4 or 5 are sexually mature and are ready for breeding. Females with a reproductive tract score of 3 or less, however, should not immediately be used for breeding. Instead, they should be given additional time to sexually mature (if amongst the youngest of the replacement females) or be culled from the breeding population (if amongst the oldest of replacement females). In post-pubertal females, proper nutritional management should occur to ensure that females attain an appropriate pre-breeding body condition score (Edmonson et al., 1989). Under-conditioned females are at risk for anovulation.

In polytocous species such as sheep, several factors are known to influence ovulation rate. Firstly, the breed of female that farmers/ranchers choose to raise can impact ovulation rate. A number of prolific sheep breeds that exhibit high ovulation rates have been identified (e.g., Finnish Landrace, Romanov), yet there are also other sheep breeds (e.g., Rambouillet) that typically do not exhibit multiple ovulations. Secondly, the number of ovulations in sheep is influenced by the age of the breeding females. Ewe lambs have lower average ovulation rate than do yearling ewes, which have lower average ovulation rate than older ewes. Thirdly, the specific time of mating within the biological breeding season can influence ovulation rate. As females make the transition from the non-breeding season to the breeding season, average ovulation rate tends to be low. With each successive estrous cycle, mean ovulation rate will increase until the mid-point of the biological breeding season is reached. Higher ovulation rates can be achieved if farmers simply delay the start of the breeding season until after females have exhibited two or more estrous cycles. Fourthly, proper nutritional management of the breeding females will ensure that females are not anovulatory. Ovulation rate of polytocous females potentially can also be increased through the nutritional management practice known as flushing. Flushing is defined as the feeding of an increased level of dietary nutrients for one estrous cycle prior to expected breeding and continuing for an additional estrous cycle thereafter. Using sheep as an example, flushing would commence 17 days prior to the start of the breeding period and continue each day until 17 days after the start of the breeding season. Flushing seems to work best for females with the genetic potential for high ovulation rate (based on breed and age) but who are below average body condition score (i.e., a body condition score of 2.5 or less - on a scale from 1=thin/emaciated to 5=obese).

Fertilization Rate

Unlike some species such as the pig where fertilization of only a portion of the ovulated eggs is fairly common, fertilization rate in ruminant livestock species tends to be an all-or-nothing trait in naturally cycling females. Either all of the eggs ovulated by a female are fertilized or none of them are fertilized. Overall, fertilization rate in well-managed cattle and sheep is expected to be in excess of 90%.

There are two major factors impacting fertilization rate in ruminants: egg quality and sperm quality. Oocyte quality can be adversely affected by heat stress, exposure to toxicants, and negative energy balance. These topics are being addressed by other speakers in this symposium; thus, subsequent discussion will focus on sperm quality.

Similar to oocytes, quality of spermatozoa can be affected by a variety of environmental factors. Farmers and ranchers should be vigilant about the water provided to their breeding males as this is a common source of toxicants and heavy metals that can impair fertility. Heat stress is also known to cause infertility, this despite a sophisticated system to regulate and maintain temperature of the testes of ruminant males. The temperature regulation system, designed to maintain temperature of the testes 4-6°C lower than the male's core body temperature, consists of the pampiniform plexus, the internal and external cremaster muscles, the tunica dartos muscle, and (in some species) sweat glands in the scrotum. If temperature is not maintained in the target temperature range, normal production of viable spermatozoa will not occur. Fortunately, the spermatogonial stem cells (A_0 spermatogonia) are not particularly sensitive to heat.

Farmers and ranchers can potentially influence fertilization rate by management decisions they make. On the female side, it is important to ensure proper nutritional management of breeding females prior to the start of the breeding season to potentially reduce the incidence of poor oocyte quality due to negative energy balance. Providing breeding females with access to shade, fresh water, and ventilation (if animals are intensively managed) can potentially reduce heat-induced changes in oocyte quality. Maintaining breeding animals in an environment free of heavy metals, environmental estrogens, or other compounds that can be toxic to oocytes can potentially aid in a high rate of fertilization.

Farmers and ranchers can potentially avoid problems with poor quality of spermatozoa by providing proper nutritional management of breeding males (to ensure that males do not become over- or under-conditioned), as well as by providing shade, fresh water, and adequate ventilation in order to avoid heat stress. Livestock producers are advised to have a breeding soundness exam (Cehnoweth et al, 1993) performed on any male to be used for breeding to ensure that the male is structurally sound, free of evidence of any infectious disease, and produces an adequate number of morphologically normal spermatozoa with forward progressive motility. If a male fails to pass the initial breeding soundness examination, the exam should be repeated. If an adequate number of morphologically normal spermatozoa with forward progressive motility are not produced after the length of the spermatogenic cycle has passed (47 days for rams and 61 days for bulls [not including epididymal transport time]; Senger, 2012), then it is likely that there has been substantial damage to the seminiferous tubules and infertility will be permanent.

Embryo Survival Rate

Some of the earliest descriptive studies on *in vivo* pre- and post-implantation embryonic development in cattle (Winters et al, 1942) and sheep (Green and Winters, 1945) were performed at the University of Minnesota. These classical studies documented the timing of normal development of a zygote as it progressed from a one-cell fertilized egg in the oviduct to a multi-cellular free-floating embryo in the uterus to a fetus attached to the uterus by the conceptus-derived placenta. These observational data provided the benchmarks from which future studies could ascertain the normalcy of embryonic development.

With the advent of reproductive biotechnologies such as *in vitro* fertilization and embryo transfer, studies on embryonic development shifted from *in vivo* to *in vitro* studies. Early attempts to maintain preimplantation embryos *in vitro* were met with significant challenges. Oocytes fertilized *in vitro* would undergo three or fewer cleavage divisions, and then development arrested. This developmental arrest was called the *in vitro* block to development, and subsequent studies determined the development block occurred due to failure of embryonic genome activation (Meirelles et al, 2004).

Embryonic genome activation, as the name implies, is the time period during embryonic development where active transcription and translation of a substantial number of genes of the embryo is initiated. This phenomenon is also known as the maternal-zygotic transition for control of embryonic development. Messenger RNA (mRNA) and proteins stored in the oocyte at the time of ovulation control embryonic development up until the time of embryonic genome activation, and failure of embryonic genome activation will preclude subsequent embryonic development. Embryonic genome activation can be blocked with DNA synthesis inhibitors such as α-amanitin (Memili and First, 2000) or with, as was the case with early *in* vitro studies, improper biochemical composition of embryo culture medium. The latter scenario clearly documents an important oviductal and/or uterine environmental influence on embryonic development.

There are also other interesting phenomena of preimplantation embryonic development in ruminants. Unlike somatic cells that undergo mitosis to produce daughter cells of the same size as the parent cell, the first several cleavage divisions (mitosis) of the preimplantation embryo result in daughter cells that get progressively smaller with each successive cell division. The biological reason for this phenomenon is to transform the oocyte – which is the largest cell of the female body and possesses 10-fold more mitochondria (the energy-producing cell organelles) than somatic cells- into an entity comprised of normal-sized cells.

As the fertilized egg goes through mitosis (from a 1-cell to a 2-cell to a 4-cell to an 8-cell to a 16-cell to a 32-cell [morula], etc.), the cytoplasm and cell organelles contained therein are partitioned into the daughter cells without significant pre-division synthesis of new cytoplasm and cell organelles. Such a developmental approach places less demand on the embryo during the early part of its development. It also enables the embryo to develop inside the "soft egg shell" known as the zona pellucida which provides physical and immunological protection to the developing embryo. This reduction in blastomere cell size continues until the occurrence of the first major differentiation event during preimplantation embryonic development known as blastulation.

The cells of the preimplantation embryo up until the time of blastulation are called blastomeres. Blastomeres are totipotent, meaning they are biologically capable of developing into a complete conceptus (fetus plus placenta), and they are also genetically identical with respect to nuclear (chromosomal) DNA. At the initiation of blastulation, blastomeres undergo a developmental process known as compaction. During compaction, the blastomeres close down upon one another to form a tight ball of cells. Some blastomeres are allocated to an outer ring of cells that are held closely together via tight cell junctions, whereas other blastomeres are allocated to the inner portion of the embryo. As the embryo emerges from compaction, the first morphological sign of differentiation is evident. The early blastocyst contains two population of differentiated cells (trophectoderm cells and inner cell mass cells), as well as a small fluid-filled cavity called the blastocoele cavity.

In a blastocyst stage embryo, the single cell thick layer of trophectoderm cells completely surrounds the inner cell mass cells. The trophectoderm cells help regulate fluid and nutrient movement in and out of the blastocyst, and later in embryonic development (after the second major differentiation event [gastrulation]) the trophectoderm cells contribute to the formation of the outermost layer of the placenta (the chorion). The inner cell mass cells develop into the fetus proper as well as the remaining layers of the placenta.

Once the conceptus has undergone blastulation, daughter cells produced as a result of mitosis are the same size as their parent cells. As a result, the embryo begins to grow substantially larger and exerts significant mechanical pressure on the zona pellucida – ultimately causing the zona pellucida to thin and rupture. After the blastocyst hatches or escapes from the zona pellucida, it continues to undergo mitosis and becomes considerably larger. At approximately day 12-13 in sheep and day 13-14 in cattle the trophectoderm layer undergoes a period of rapid growth, and during this growth phase the embryo changes from a spherical to an ovoid to a tubular to a filamentous shape. This embryonic metamorphosis occurs concomitantly with gastrulation and precedes maternal recognition of pregnancy (Bazer et al., 1991) and subsequent implantation (Bazer et al., 2011). If the ruminant embryo does not emit the signal for maternal recognition of pregnancy (i.e., interferon-τ), expression of the oxytocin receptor gene will not be inhibited, prostaglandin F2α will be released from the endometrium, the corpus luteum on the ovary will be lysed, blood concentrations of progesterone will lessen, and the embryo will die.

Understanding the normal developmental process of preimplantation embryos is important because it enables a greater understanding of how perturbations in the process can result in abnormal embryonic development and/or embryonic death.

Role of progesterone on embryo survival

Progesterone is a steroid hormone that is produced by the corpus luteum present on the ovary of cyclic females. Progesterone stimulates endometrial glands to produce a nutrient-rich milieu called histotroph. After maternal recognition of pregnancy occurs, blood concentrations of progesterone increase further to provide signals to the endometrium to be receptive to implantation. Progesterone is often called the "hormone of pregnancy" because it is produced at high levels throughout pregnancy, including significant production by the placenta in the latter stages of pregnancy.

There is a growing body of evidence that blood progesterone concentrations in the estrous cycle before conception, as well as the estrous cycle of conception, have a major impact on embryonic development and survival (Diskin et al., 2012; Lonergan, 2013). Although it has been known for quite some time that luteal insufficiency (leading to low concentrations of blood progesterone) in the estrous cycle during which mating occurred can result in embryonic mortality, it is a relatively recent finding that progesterone concentration on the estrous cycle immediately preceding the ovulation influences embryo survival.

A variety of management strategies has been employed in an attempt to increase embryo survival through exogenous hormone treatments that elevate blood progesterone concentrations. These approaches have included administration of human chorionic gonadotropin (hCG) in the post-mating period to induce an additional ovulation and formation of an accessory corpus luteum and administration of supplemental progesterone via an intravaginal device such as a CIDR. Although the administration of progesterone a few days after mating tends to promote embryo elongation, not all studies have shown an impact of pregnancy rate. Unfortunately, studies have led to conflicting results due to differences in timing of treatments, some animals with sufficiently high progesterone levels will not benefit from direct or indirect supplementation with progesterone, and lack of sufficient animal numbers and statistical power in many studies (Lonergan, 2013).

<u>Identification of haplotypes impacting fertility</u>

One of the exciting developments in recent years is the discovery of haplotypes in major breeds of dairy cattle that adversely affect fertility (VanRaden et al., 2011; Fritz et al., 2013). (Haplotypes are a set of single nucleotide polymorphisms [SNPs] found on the same chromosome and inherited together.) These discoveries were made possible through the application of genomic testing of popular AI bulls and their maternal grandsires. Offspring produced from matings of carrier sires with daughters of carrier sires were examined to see if there was an absence of homozygous recessive phenotypes that would be suggestive of an embryonic lethal condition.

Within North America Holsteins, six different haplotypes have been identified: HH0 (also known as brachyspina), HH1, HH2, HH3, HH4, and HH5. These deleterious haplotypes reduce conception rate from 3 to 3.5%. Haplotypes also have been identified in the Jersey and Brown Swiss breeds (VanRaden et al., 2011) that reduce conception rate by 3.7% and 3.4%, respectively.

The specific biological mechanisms through which these haplotypes exert an adverse effect on reproduction have not been elucidated. Nonetheless, dairy cattle producers would be wise to avoid mating a known carrier bull with a known or suspected carrier female. Although one may be tempted to test and eliminate all known carrier females from the breeding herd, such drastic action is not warranted if the mating sire is determined to be free of the undesired haplotype prior to breeding.

Impact of heat stress on embryonic development

In addition to adverse effects of heat stress on oocyte quality, elevated uterine temperatures shortly after the time of insemination can reduce conception rate (Gwazdauskas et al., 1973). It is believed that the elevated core body temperature leads to embryonic death because of alteration in ovarian steroid secretion and altered secretions from the oviduct and uterus. The *in vitro* culture of bovine embryos at elevated temperatures disrupts normal embryonic development (Edwards and Hansen, 1997; Rivera and Hansen, 2001; Sakatani et al., 2004) and can lead to embryonic death.

The thermosensitivity of preimplantation embryos varies as the embryo progresses through development (Hansen, 2013). Zygotes tend to be the most susceptible to heat stress, and sensitivity to heat shock declines as the embryo progresses through the first few cleavage divisions. After embryonic genome activation, the embryo develops the capacity to synthesize heat shock proteins to provide short-term thermal protection; however, this does not appear to be the sole mechanism though which thermotolerance is acquired (Hansen, 2013). At the morula and blastocyst stages of embryonic development, heat stress had marginal impact on development (Edwards and Hansen, 1997; Eberhardt et al, 2009; Sakatani et al., 2012). Evidence for the differential effect of heat stress on *in vivo* developed embryos obtained from superovulated cows also has been reported (Ealy et al., 1993). Blastocyst yield was reduced in cows heat stressed on day 1 after estrus but not in cows heat stressed 3, 5, or 7 days after estrus.

One of the strategies that can potentially be employed by farmers and ranchers to circumvent the reduction in fertility associated with heat stress is to utilize embryo transfer. Embryos can be harvested from donor females during times when heat stress is not occurring, and those embryos may be cryopreserved and stored for subsequent use during periods of heat stress.

Fetal Survival Rate

The last step in the reproductive process is fetal survival. Fortunately, in the absence of pathogenic disease, major trauma or severe malnutrition, fetal survival is expected to be close to 100% in cattle. There is some evidence in sheep, however, that fetal litter size may be inversely correlated with fetal survival rate (Morrical et al, 1994).

Twenty percent of 320 ewes exhibited fetal death loss prior to day 98 of gestation, and the percentage of ewes exhibiting fetal loss was 67%, 56%, 16%, and 2% for ewes with initial fetal litter sizes of 4, 3, 2, and 1, respectively. Although most fetal loss represented a partial loss of the litter, further study will be needed to confirm if this phenomenon is exhibited across multiple years.

Conclusions

Livestock producers face a major challenge in the years ahead to produce enough food to feed the burgeoning human population. Enhancing reproductive efficiency will be vital to achieving increased production efficiency from ruminants. Farmers and ranchers must provide proper nutritional, environmental, and genetic management of animals will be necessary for increased production of animal-derived foods.

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4

Comparative Digestion Strategies and Protein Nutrition of Lactating Dairy Cow, Sheep and Goats

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Summary

As ruminant herbivores cattle, sheep and goats have unique evolutionary adaptions to high fiber low protein diets. However, each species has its unique features and abilities to thrive on stems (high fiber), leaves (high protein), or fruits/seeds (storage carbohydrate) of the plants. For example, cattle have greater fiber fermentation capacity then sheep and goats, and goats are the most selective feeder. A common feature, however, is the ability to conserve nitrogen (N) and recycle it to the gastro-intestinal tract. Recent research, especially with dairy cattle indicated that high milk production can be achieved with levels of crude protein much lower than once thought possible. These advances may contribute to decreasing ration cost (protein supplements are expensive diet ingredients) and reduce the risk of environmental concerns associated with livestock production including air and water pollution and climate change. A better understanding of evolutionary adaptation and feeding behavior of sheep and goats in rangeland condition might offer clues to avoid or alleviate nutritional problems associated with intensive systems. Pasture management and strategies of supplementation are the main factors impacting dairy sheep production. Goats can adapt to either poor pastures or rich and balanced diets. In properly balanced diet, dairy goats can be fed an all concentrate (high in by-product) diet without developing the type of digestive disturbance (rumen acidosis) that would be observed with similar diet fed to sheep or cows.

Resumen

Mejorando la nutrición en vacunos lecheros y pequeños rumiantes

Como rumiantes herbívoros, los vacunos, los ovinos y los caprinos tienen adaptaciones evolutivas únicas a dietas con alto contenido de fibra y bajo contenido de proteína. Sin embargo, cada especie tiene sus características y habilidades únicas para aprovechar mejor los tallos (alto contenido de fibra), hojas (alto valor proteico), o frutos/semillas (carbohidratos de almacenamiento) de las plantas. Por ejemplo, el ganado vacuno tiene una mayor capacidad de fermentación de la fibra que el ovino y el caprino, y el ganado caprino es el más selectivo. Una característica común, sin embargo, es la capacidad de conservar el nitrógeno (N) y reciclarlo en el tracto gastrointestinal. Investigaciones recientes, especialmente en ganado lechero, indicaron que una alta producción de leche se puede conseguir con niveles de proteína cruda mucho más bajos que los que se creyeron posibles. Estos avances pueden contribuir en la disminución del costo de la ración (los suplementos proteicos son los ingredientes con mayor costo en la dieta) y reducir el riesgo de problemas ambientales asociados con la producción ganadera incluyendo la contaminación del aire y agua, y el cambio climático. Una mejor comprensión de la adaptación evolutiva y comportamiento de alimentación de ovinos y caprinos en condiciones de pastoreo podría ofrecer pistas para evitar o aliviar los problemas nutricionales asociados con los sistemas intensivos. El manejo de las pasturas y las estrategias de suplementación son los principales factores que afectan la producción de ovinos lecheros. Los caprinos pueden adaptarse tanto a pastos pobres como a dietas ricas y balanceadas. En dietas correctamente balanceadas, las cabras lecheras se pueden alimentar con sólo concentrado (alto contenido de subproductos) sin desarrollar trastornos digestivos (acidosis ruminal) que se observaría con una dieta similar en ovinos o vacunos.

Introduction

Cattle, sheep and goats, are ruminant herbivores with particular evolutionary adaptions to utilize high fiber, low protein diets. In these proceedings, we have attempted to provide a foundation to understand these adaptations with the premise that emulating the animal's natural behavior and ecology when placed under intensive management would contribute to efficient and economical production systems that minimize the risk of nutrition related disorders and the risk of environmental pollution. Although there is a vast body of literature, efforts to bridge what is known of cattle, sheep and goat nutrition is relatively rare. In doing so, our focus will be mainly on nitrogen (N) utilization.

Evolutionary Adaptations to High Fiber – Low Protein Diets

The digestive tract of animal species has evolved to take advantage to specific feed resources. Herbivores consume herbaceous plant parts including stems, which are rich in fiber and poor in nitrogen compared with leaves, which are comparatively richer in protein and poorer in fiber, or fruits (seeds), which concentrate nutrients in the form of storage carbohydrates (starch), lipids (oilseeds), or proteins, as well as minerals and vitamins. The digestive tract of herbivores tends to have at least one voluminous digestive compartment inhabited by a microbial population that has the enzymes to breakdown fiber (Van Soest, 1994). The fermentation of fibrous carbohydrate (cellulose and hemicellulose) is a process that requires more time than the digestion of simpler carbohydrates (sugars or starch) or proteins that can be digested my mammalian enzymes in the gastric-secreting stomach and the small intestine. Thus one of the functions of the fermentative compartment is to slow down the rate of passage (i.e., increase retention time) to allow sufficient time for the microbial population inhabiting the digestive tract to extract the energy from the fiber.

The fermentative compartment in a horse, a rhinoceros, or an elephant is the caecum (and the colon), which is located after the acid-secreting stomach and the small intestine. Thus these animals are referred to as hindgut fermenters. Other (wild) animals such as kangaroo, sloth, columbus monkey or hippopotamus have a fermentative compartment located before the acid-secreting stomach. These animals, which do not ruminate, are referred to as foregut fermenters. In contrast, cattle, sheep and goat combine pre-gastric fermentation with rumination (chewing their cud) and hindgut fermentation. As ruminating pre-gastric fermenters, cattle, sheep, and goats benefit from: (a) the greatest potential among all animal species to extract energy from fiber (i.e., greater fiber digestibility) and (b) a critical supply of amino acids. Indeed, the protein-rich microbial population that grows in the rumen while fermenting fibrous carbohydrates and other forms of carbohydrates (e.g., starch) will eventually pass though the gastric and intestinal compartments where their digestion will result in amino-acids that will be absorbed by the host.

The main sources of N for microbial protein synthesis are ammonia and secondarily pre-formed amino acids (NRC, 2001). These N sources arise from the degradation of dietary protein and non-protein N (NPN), or the recycling of N in the form or urea, from the body of the host to the fermentative compartments of the gastro-intestinal tract. However, as indicated above, the protein content of the feed resources upon which herbivores rely tend to be low. Thus evolution has provided them with mechanisms to guarantee that microbes in their gastro-intestinal tract will be provided with N needed for their growth. Compared with other animal species, herbivores re-route a substantial amount of urea-N to the digestive tract both with the saliva they mix with the ingested (or ruminated) feeds and through the supply of blood to the rumen and the viscera. Urea, which is synthesized in the liver, is the end-product of N metabolism and is excreted in the urines. There is a strong relationship between blood urea N concentration and rate of urinary N excretion in mammalian species. However, the research of Kohn et al., (2005) showed that the clearance rate of the kidney was lower in herbivores (cattle, sheep, goats and horses) than in pigs or rats, which highlighted the herbivores' ability to salvage N before it is lost from the body.

Another evolutionary mechanism that has allowed herbivores including ruminants to thrive on high fiber diets is body size. The total volume of the digestive tract is directly related to animal size and weight. Compared with shorter and lighter animals the taller and heavier ones have longer gastro-intestinal tract, which in essence provides additional time for fiber digestion as the digesting materials moves through the digestive tract. The nutritional implications of the difference in body weight between sheep and goats on the one hand, and cattle on the other hand, will be explored further below.

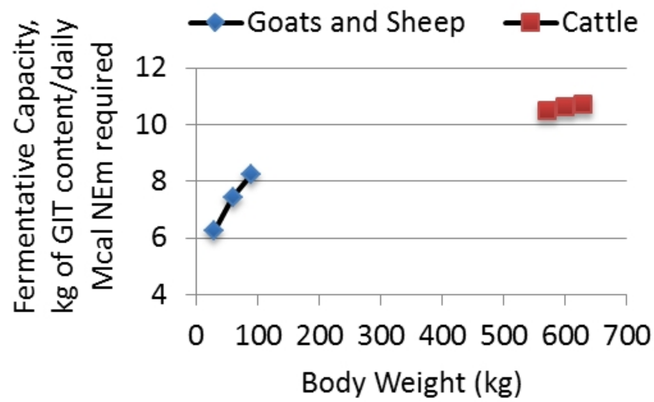
Before leaving the topic of evolutionary adaptation, there is one more key concept that needs to be address in order to shed light on an important implication of pre-gastric fermentation combined with retention of fiber in the rumen and further processing by rumination. As indicated above, these physiological adaptations allow for a great ability to extract energy from fiber, but they impose a major limitation on the amount of feed the ruminant can consumed per day. Fiber is the bulky (low density) part of the plants, and thus, the selective retention of fiber in the rumen creates the potential of a physical fill effect, which will force the animal to slow down or event stop the rate of intake (kg per day) to adjust for the slow clearance rate of fermenting fiber from the rumen (Mertens, 1987). This phenomenon may be at the origin of metabolic disorders observed in early lactating ruminants, such as dairy cattle that have been subjected to intense genetic selection for milk production. High producing cows have extremely high energy requirements in early lactation, but a limited ability to increase dry matter intake due to rumen fill limitation. Consequently the cow may enter a period of negative energy balance during which body reserves must be used to compensate for the lack of dietary energy intake. If not properly managed, this situation may lead to severe ketosis and fatty liver, and low fertility.

Comparative Feeding Behavior and Digestion Strategies in Dairy Cows, Sheep and Goats

As ruminants, cattle, sheep and goats have a number of similar digestive anatomical and physiological features, but also many differences. An obvious difference is adult body weight, which range approximately from 40 to 75 kg in sheep and goats compared with 550 to 680 in beef or dairy cattle. The fact that sheep and goats are approximately 10 times lighter than cattle has profound implications on their ability to process dietary fiber.

Compared with starch or other sources of carbohydrates, the utilization of the fibrous carbohydrate is a process that requires long contact hours between the fiber particles and the microbes that inhabit the gastro-intestinal tract to provide the time needed for enzymatic degradation. The fermentative capacity of the gastro-intestinal tract or its ability to provide sufficient time for fiber fermentation varies linearly with the body weight of an animal. Heavier animal have longer the gastro-intestinal tract and longer retention time. In contrast the amount energy required for maintenance (NEm) is a function of metabolic body weight (body weight to the 0.75 power; $BW^{0.75}$).

Cannas (2004) quoted the work of INRA (Institut National de Recherche Agronomique) French scientists to indicate that in sheep NEm (Mcal/d) = 0.0561 x $BW^{0.75}$, but in cattle NEm (Mcal/d) = 0.070 x $BW^{0.75}$. Thus fermentative capacity can be calculated by dividing the kilos of feeds that the digestive tract can accommodate (estimated as volumetric kilograms of water to fill the gastro-intestinal tract of slaughtered animals) by the calories of energy required for maintenance to obtain an indication of the animal's ability to rely on fiber fermentation to support their daily energy needs. Figure 1 was constructed to illustrate that cattle (heavier animal) have a greater capacity than sheep and goats (lighter animals) to accommodate large amounts of feed in the gastro-intestinal tract in relation to the amount of energy required to insure basic (maintenance) body functions. Thus we can understand now why cattle can thrive on a diet that contains more fiber (plant stems), compared with sheep and goats that may have to be more selective in choosing plant parts that are richer in nutrients (leaves, fruits, seeds) in order to meet their energy requirements.



Although early ruminant nutritionists were hoping to use sheep as an model to study cattle nutrition (because of cost involved in doing the research), the distinction between sheep and cattle nutritional physiology was clearly established when Van Soest (1994) summarized the literature to show that sheep tended to have higher digestion coefficients than cattle when fed high digestibility diets (i.e., diets low in fiber), but cattle tended to have higher digestibility coefficients than sheep when fed diets low in digestibility (i.e., diets high in fiber). More recently Cannas (2004) compared these two species and concluded that compared to cows, sheep:

- have to eat more to satisfy their maintenance requirements; and higher intake results in a higher passage rate and lower fiber (forage) digestibility.
- tend to have more selective feeding pattern;
- are more affected in their intake by the particle size and the fiber content of the forage;
- have to spend more time eating and ruminating each kg of feed;
- tend to have higher digestibility for grain and high-energy diets.

Digestion studies comparing goats, sheep and cattle should be interpreted with caution because of many possible cofounding factors. However the results of Uden and Van Soest (1982) will be used to compare them because they were offered the same timothy grass as the only feed sources in the diet. Data presented in Table 1 summarizes dry matter intake (DMI), digestibility and retention time of forage particles. Although sheep and goats exhibited similar behavior, the greater fermentative capacity of cattle (DMI, g/kg $BW^{0.75}$) allowed them to consume less DM per unit of body weight (DMI, g/kg BW). Furthermore the longer retention time in the rumen (and total gastro-intestinal tract) of cattle was associated with higher neutral detergent fiber (NDF) digestibility compared with sheep or goats. Some authors, however, have argued that with forage low in nitrogen and high in fiber, and not properly supplemented, goats have better digestive efficiency than other ruminants (Tisserand et al., 1991). Greater ability to reduce particle size during chewing in comparison to sheep (Hadjigeorgiou et al., 2003), higher concentration of cellulolytic bacteria in the rumen and higher efficiency of urea recycling from the blood to the rumen may contribute to these advantages.

Table 1. Comparative analysis of intake, digestibility and retention time of forage particles in goat, sheep and Cattle.

Item	Goat (Caprine)	Sheep (Ovine)	Cattle (Bovine)
Body weight (BW), kg	29	30	555
Dry Matter Intake, g/d	700	650	7830
Dry Matter Intake, g/kg BW	24	22	14
Dry Matter Intake, g/kg $BW^{0.75}$	56	51	68
Digestibility of Dry Matter, %	47	47	54
Digestibility of NDF, %	44	44	52
Rumen retention time of forage particles , hr	28	35	47
GIT retention time of forage particles, hr	52	70	79
Rumen/ Gastro-intestinal Tract, %	54	50	59

Source: Uden and Van Soest (1982), cited by Cannas (2004).

[1]: GIT = gastro-intestinal tract.

Recent Research in Lactating Dairy Cow Protein Nutrition and Nitrogen Use Efficiency

Not enough crude protein in dairy cows diets may limit dry matter intake, fiber digestion (i.e., energy yield) and milk production of dairy cows (NRC 2001). On the other hand, excess crude protein may result in both economic and environmental concerns. When expensive protein supplements do not contribute to improving lactation performance, the excess N is lost primarily as urinary urea-N in the manure (Olmos Colmenero and Broderick, 2006). In many parts of the world, manure N has been associated with degradation of water in lakes and rivers, degradation of air quality because of ammonia (NH_3) volatilization to the atmosphere, which contribute also to the emission of nitrous oxide (N_2O), a potent greenhouse gas that contribute to climate change.

Table 2 was constructed to illustrate in part the expected change in dry matter intake and manure production of cows producing milk in the range of 10 to 50 kg/d. The nitrogen balance and nitrogen use efficiency data were obtained assuming that 14 and 17% dietary crude protein diets were provided for milk production of 25 kg/d or less and 30 kg/d or more, respectively. These levels of crude protein should not be interpreted as "requirements", but rather as levels of that are likely to suffice to meet the cow's requirements for amino acids, assuming other dietary nutrients, and especially rumen fermentable carbohydrates are in adequate supply. The important take-home messages in regard to N balance and N use efficiency can be summarized as follows:

1. Efficiency of conversion of intake-N to milk-N ranged from 16 to 35%, which is to say that 65 to 84% of the N consumed by a cow is excreted in manure daily.
2. If at first glance it appears that N use efficiency increases with milk production, it is true only when milk production increases at a fixed level of dietary CP. For example, Table 2 data indicates that in the low production range, a cow can produce twice as much milk (20 vs. 10 kg/d) and milk-N (100 vs. 50 g/d) at a fixed 14% dietary crude protein level simply because as the cow produces more she eats more, and the increase in dry matter intake (16.7 vs. 13.6 kg/d) is sufficient to supply the necessary nutrients for higher milk production.
3. Nitrogen use efficiency is a function of both level of milk production and dietary crude protein concentration. For example, the data in Table 2 indicated a cow producing 25 kg of milk with a diet of 14% CP has essentially the same efficient as a cow producing 40 kg of milk with a diet of 17% CP (31 and 32% conversion rate, respectively).
4. As illustrated by the data for milk production of 25 kg/d obtained with either a 14 or 17% crude protein diet, at a given level of production an increase in dietary crude protein reduces N use efficiency (in this case from 31 to 25%), and increases urinary-N more (52 g/d = 184 - 132) than fecal-N (36 g/d = 188 - 152). Conversely, regardless of level of milk production, reducing dietary crude protein with no change in milk production has the beneficial effects of increasing feed N use efficiency and simultaneously reducing daily excretion of urinary-N.

Given the type of properly balanced diets fed in the Midwest of the United States, milk production is generally not penalized, even in early lactation, when dietary crude protein is approximately 16.5% of the diet (DM basis; Broderick, 2003; Wattiaux and Karg, 2004). Milk production of 25 kg of milk or less can be achieved with less than 14% CP (Olmos Colmenero and Broderick, 2006). In a recent experiment, we collected data with a 128-cow trial in which four diets with crude protein levels of 11.8, 13.1, 14.6 and 16.2% were offered over a 12-week period on late lactation. The average production of fat-and-protein corrected milk was 26.1, 30.0, 31.9 and 32.6 kg/d per cow for the respective dietary levels of crude protein. The production of fat-and-protein corrected milk was not significantly different when feeding the 14.6 versus a 16.2% crude protein diet (Quaassdorff, et al, 2014). Important take-home messages from Table 2 in regard to the relationships among milk production, dry matter intake and manure excretion can be summarized as follows:

1. Cows producing more milk must consume more feed and they produce more manure.
2. Regardless of milk production, cows always produce more manure than milk.
3. As milk production increases, the amount of manure produced per unit of milk produce decreases sharply. For example 4.3 kg of manure is produced per kg of milk when a cow produces 10 kg of milk, but only 1.6 kg of manure is produced per kg of milk when a cow produces 50 kg of milk.
4. Furthermore, the total amount of manure (and manure-N) excreted for a given amount of milk depends on the number of cows needed to produce that amount of milk. For example, 40 kg of milk produced by one cow results in 71.6 kg of manure. In contrast the same amount of milk produced by two cows, each producing 20 kg/d, would result in a total manure production of 105 kg/d (2 x 52.5 kg/d; Table 2).

Sheep Nutrition Under Rangeland Conditions

Rangeland support a substantial proportion of the world's sheep population and play a vital role in supporting low-cost, low-input, wool- and meat production systems (O'Reagain and McMeniman, 2002). These authors defined rangeland as any extensive (unfenced), uncultivated and (or) unfertilized area that supports production of free-ranging herbivores (limited confinement facilities), and they drew attention to the following unique characteristics of rangeland systems:

1. The carrying capacity and animal performance are low compared with more intensive systems (cultivated pastures). Rainfall and soil conditions are oftentimes major limiting factors. Any management inputs must therefore be economical, easy to implement and must have an extremely high probability of substantial return over "investment" cost.
2. Rangelands are characterized by marked spatial and temporal (seasonal and yearly) variability in both forage supply and quality.
3. Plants communities of nearly all rangelands include toxic plants. Toxicity effects may range from subclinical depression in animal performance to causing death.
4. Drinking water is poorly distributed in rangeland and thus may severely limit dry matter intake and animal performance; a problem that is rarely of any concerns in conventional systems of production.
5. Feed resources management of rangeland should be of concern because the loss of vegetation may have irreversible consequences in contrast to conventional system of production in which reseeding and fertilization may help remedy the consequence of poor management (such as overgrazing).

Sheep Nutrition Under Improved Pasture Grazing Conditions

Herbage availability on pasture has an important impact on sheep feeding behavior. Summarizing the Australian literature, Weston (2002) identified three distinct situations. Under high accessibility, such as with sward heights of at least 9 cm (corresponding to approximately 4.8 tons of dry matter per hectare), sheep have no problem to maintain the level of intake required to meet energy requirements by adjusting grazing time (hr/day), bite rate (bites/ min) and bite size (grams organic matter ingested/bite). Under this situation, sheep can demonstrate preferences for legume species (clover) over grasses, in part because of lower resistance of leafy materials to bolus formation and faster clearance rate from the rumen. However as herbage quality and availability declines and bite size decreases, sheep increase time grazing and bite rate to reach the necessary level of intake to meet energy requirements. When sward height is however less than 3 cm, the ability of the sheep to use compensatory mechanism is no longer sufficient to maintain the desired level of intake. Although sheep are capable of grazing for 13-14 hr/day, in harsh environment and sparse pasture, grazing time is often limited to 7-9 hr/day. The author hypothesized that grazing at or near ground level may involve abrasion of the lips and other parts of the mouth, which in turn could have a negative impact on feeding behavior. Furthermore, under arid conditions, the need to travel long distance to water may reduce time available for grazing.

Supplementary Feeding

Both in temperate and rangeland environments, there are times of the year that nutrient demands may not be met by pasture alone given the seasonality of pasture growth. Supplementary feeding may appear as a simple concept, but it actually involves a difficult decision-making process with important implication both in term of biological efficiency of production and financial outcomes. There is a long list of elements to consider including for example, the current physiological state of the animal (body reserves, state of pregnancy, etc.), the amount and nutritive value of the pasture, the availability and cost of supplemental feeds. Table 3 illustrates the impact of energy versus protein supplementation of lactating ewes grazing a perennial ryegrass pasture of 750-850 kg dry matter ha^{-1} as reported in Dove et al. (1985).

Table 3. Influence of "energy" and "protein" supplements on digesta flow, and performance of grazing ewes and their lambs.

Item	Pasture alone	Energy supplement[1]	Protein supplement[2]
Rumen ammonia, *mM*	24.1	16.4	20.1
Flow of dry matter to abomasum, g/d	1065	1288	1340
Flow of crude protein to abomasum, g/d	276	344	431
Flow of microbial crude protein, % of total Flow	93.4	85.1	77.2
Milk production of the ewe, g/d	2048	2133	2846
Weigh change of the ewe to day 80 of lactation, kg	0.0	5.1	-0.9
Lamb weight gain, g/d	254	308	331

Source: Dove et al. (1985), cited by Dove (2002).

[1]: Energy supplement = 600 g/d (air-dry) of sugar beet pulp with molasses (9% crude protein).
[2]: Protein supplement = 600 g/d (air-dry) of a mixture (1:1) of energy supplement and formaldehyde treated soybean meal (48% crude protein).

One of the major issue related to supplementation of free-ranging animal is the substitution between supplement and herbage. Dove (2002) summarized the literature as follows:

1. Substitution is likely to be greater when more pasture is available. With abundant pasture availability (> 4.5 tons dry matter per ha), observed rate of substitution may reach approximately 67%. However a substantial substitution (~ 38%) may occur even when pasture is sparse (< 0.8 tons dry matter per ha) indicating a general disinclination to graze when supplement is freely available.
2. The greater the quality of the pasture the greater the substitution rate. However, energy supplement high in starch may have a depressing effect on fiber fermentation in the rumen leading to an undesirable reduction in daily dry matter intake.
3. The substitution is generally greater when higher-quality supplements are fed compared with lower-quality supplements.
4. The substitution rate may be greater when greater amounts of supplements are fed, but there are conflicting results in the literature. The complexity of understanding the substitution behavior is illustrated with the data of a controlled experiment presented in Table 4. Increasing the restricted amount of supplement increased the substitution rate. However when the supplement

was fed without restriction (ad libitum), the substitution rate was reduced in spite of the fact that intake of hay was reduced by 90%.

5. The substitution rate depends also upon the physiological state of the animal. In general, animals with a greater demand for nutrients (e.g., lactating ewes) will show lower degree of substitution than animals with lower demand of nutrients (e.g., pregnant ewes).
6. The method and frequency of feeding the supplement may influence the rate of substation.

Table 4. Intake of low quality hay and supplements of lambs in controlled feeding situation.

Item	Weight of air-dry supplement offered (g/d)				
	Min.	**200**	**400**	**600**	**Free**
Intake of supplement, g/d	75	176	313	446	1076
Intake of hay, g/d	386	366	271	114	39
Total intake, g/d	461	542	584	560	1115
Substitution rate[1], %	--	20	48	73	35
Reduction in intake of hay, %	--	5	30	71	90
Lamb weight gain, g/d	- 25	- 17	39	54	142

Source: Freer et al. (1988), cited by Dove (2004).

[1]: Increased in intake of supplement divided by decrease in intake of hay relative to Min.

Nutrition of the Lactating Ewes

In the majority of sheep production systems, sheep are kept for meat or wool production and ewes rear their lambs until weaning, at 3 or 4 months of age. During this period, lamb growth is largely determined by milk intake. Early lactation is the period of highest nutrient requirements in the ewe's whole productive cycle and failure to manage the nutritional status of the ewe may impact lamb growth substantially (Treacher and Gaja, 2002). In meat breeds selected for lamb production, yield at lactation peak varies between 2.0 and 4.0 kg/d, with a total three-month lactation yield of 150-200 kg for ewes with twin lambs and from 90-160 kg for ewes with a single lamb.

In a number of Asian and European countries, ewe's milk has been a major source of animal protein in the human diet. Furthermore, in other countries such as France, Spain, Greece, and increasingly in the U.S. (Thomas, 2004) dairy sheep milk is processed in expensive cheese. There are many factors influencing milk production of dairy ewes. Highly selected dairy breed (East Friesian, Assaf) may have lactation performance greater than 1,000 kg collected over more than 200 days of lactations, but production performance lower producing dairy sheep may not be higher than approximately 350-375 kg per lactation (Thomas, personal communication). The slow increase in dry matter intake in early lactation means that ewes are invariably in negative energy balance for a few weeks after lambing. Managing body condition score is thus an important management tool to avoid loss of milk and minimize risk of metabolic disorders.

Pasture and lambing management are critical to sheep production systems. Aside of operational constraints, lambing is usually timed to coincide with the start of the herbage growth so that peak herbage production coincides as much as possible with the period of greatest nutrient requirements of the flock. In many northern-hemisphere temperate pasture regions, spring lambing is constrained the timing of the winter-spring transition. In intensively grazed systems, achieving a particular sward height in concert with fertilization and proper feed supplementation are the basis of a sound management system (Treacher, 1990). In contrast in southern-hemisphere temperate pasture regions, winters are milder but pasture senesce in late spring and there is often a pressing need to minimize supplementary feeding. In this case, lambing in the winter may be more appropriate that lambing in spring (Treacher and Gaja, 2002).

In a recent experiment, Mikolayunas et al., (2008) demonstrated that supplementation of grazing dairy ewes with either a mixture of whole shelled corn and soybean pellet or shelled corn alone resulted in greater milk production compared with un-supplemented ewes. Increased levels of corn supplementation resulted in a positive, linear increase in milk yield and an improvement in pasture protein utilization, as indicated by a decrease in milk urea Nitrogen (MUN) levels. However, similarly to dairy cattle, feeding excess concentrate in the diet of dairy sheep may result in milk fat depression but not milk yield (Goodchild et al. 1999). This problem is likely more common in intensive dairy sheep system where high quality forages are expensive relative to concentrate feed, such as in Mediterranean countries. As in dairy cattle, this problem may be partially corrected with the use of buffer to help maintain rumen pH near neutrality.

The use of rumen undegradable protein sources in the diet of dairy ewes have resulted in variable results. Responses are more likely be positive in early lactation when dry matter intake has not peaked yet, and the ewes are in negative energy balance. The results of Robinson (1983) indicated a milk production response inversely proportional to the estimate protein degradation in the rumen. When ewes were fed a basal diet of hay and barley, urea supplementation resulted in negligible milk production response above the approximately 2.0 kg/d of milk, but supplementation with soybean and groundnut meal (70 g/d; 35-55% ruminal degradability) resulted in an increased milk production from to approximately 2.4 kg/d, and supplementation with fish meal and blood meal (60 g/d; 0-30% ruminal degradability) resulted in an increased milk production from approximately to approximately 2.8 kg/d. In a recent experiment conducted in Wisconsin, Mikolayunas et al., (2009) observed a 14% increase in milk yield, 14% increase in milk fat and 13% increase in protein yield when lactating ewes were fed a diet with 12% rumen degraded protein (RDP) and 6% rumen undegraded protein (RUP) compared with a diet containing 14% RDP and 4% RUP or 12% RDP and 4% RUP. Supplementation of dairy ewes' diet with protected source of lysine and (or) methionine remain inconclusive. In doing so, Bocquier et al., (1994) observed an increased in protein content of milk, but Baldwin et al., (1993) found no response for either the yield of protein or milk protein content.

Adaptations and Feeding Behavior of Goats

Goats are known for their ability to thrive on harsh environments, which would not support other grazing livestock such as cattle and sheep. Part of their adaptation includes the ability to utilize a broad range of herbacious species, shrub and trees, and to select from among them the material with the highest nutritive value. It has been shown that goats traveled longer distance in search of forage compared to sheep in arid conditions, they tend to select more browse than do other domestic ruminants (Narjisse, 1991) and they consume less water per unit of intake compared with sheep on arid lands (Tisserand et al., 1991). In contrast to earlier suggestions, goats are not obligatory browsers or fibrous eaters but they rather tend to be flexible in dietary habits and adjust their behavior to the availability and quality of feed resources. For example goat rely heavily on herbaceous species during the growing season. Work in north Africa has shown that sheep an goats do overlap for the preference for herbaceous species during the spring. However during the dry season while the dietary contribution of grasses to sheep's diet was maintained around 70%, this contribution did not exceed 32% for goats. Goats are highly selective eater, (select specific plants and specific plant parts with high nutritive value) compared with sheep and cattle that are categorized as grass eaters with much less selective in grazing habits (Van Soest, 1994). There are ample evidence that animals of many species, including ruminants, are capable of making choices between different food source that provide a more balanced diet that would be obtained by eating at random (Forbes and Mayes, 2002).

The versatility of goat's feeding behavior seems to be enabled by several anatomical and physiological adaptations. For example their agile and mobile upper lips allow them to graze herbage as short as can the sheep, but also graze plant species with thorns and spine. In addition their tendency to assume bipedal stance provide them with an advantage over other small ruminants to reach higher vegetation layers. In addition goats tolerate a variety of chemical produced by plants to deter grazing ruminants from ingesting them. Examples of such compounds include tannins, alkaloids, and cyanogenic glucosides. For example tannins extracted from oak leaves stimulated rumen microbial activity and nitrogen balance in goats, but inhibited these functions in sheep (Narjisse and El Honsali, 1985). It has been hypothesized that these attribute may be in part the result of higher salivary production compared with sheep (Narjisse, 1991).

In confined environment, goats have demonstrated a particular ability to discriminate feeds according to their palatability. In general goats eat more slowly than sheep because of their very marked selecting behavior. In goats meals are numerous, but they do not last so long. A meal of the goghat fed forages alone at the trough is divided in three phases: a phase of exploration of the feed offered, a phase of intense feed intake and a phase of slower intake during which the goat select the plant fractions to ingest. They select the most nutritive fractions of forages, the leaves more than the stems, the thin stems from then the thick ones, the fractions richest in proteins and poorest in fiber.

Feeding Dairy Goats in Intensive system

Goats can easily adapt to intensive dairy systems. They can tolerate high amounts of concentrate rich in starch but also diets with high amount of forages due to their efficiency in chewing and selecting feeds. In intensive dairy systems, total mixed ration are advantageous to balance nutrient supply and to reduce feed selection. Moreover goats are able to eat and efficiently utilize diets without forages as long as particle size of the ration and its fiber level are carefully balanced (Rapetti and Bava, 2008). Considering the high adaptability to different diets researcher recently tested the hypothesis that the utilization of by-products or concentrates rich in fiber in substitution for forage could be useful for dairy goats. Table 5 shows diet ingredients, chemical composition and milk performance of Saanen goats when fed a grass-based diet, a hay-based diet and a non-forage diet during the mid- lactation (Rapetti et al, 2005). Milk production was similar, but milk fat percentage was lower for the goats fed the non-forage diet compared with grass-based or hay-based diets (Table 5). However, the author indicated that in a second experiment in which dietary lipids were increased in the non-forage diet, no milk fat depression was observed. An interesting data of Table 5 is the significant reduction in MUN, which suggested better N use efficiency when goats were fed the non-forage diet compared with the grass-based or the hay based diets. There are limited information on the benefits of increasing rumen undegraded protein (RUP) in the diets of lactating dairy goats. However Rapetti and Bava (2008) indicated that no benefits had been observed in their own unpublished data in which soybean meal was replaced with treated canola meal or in the work of Lu et al, (1990ab) in which soybean meal was replaced with feather meals or meat and bone meal.

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5

The Scientific Assessment of Animal Welfare in Dairy Cattle

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Summary

Concern about the welfare of dairy cattle is nothing new; producers and veterinarians have always been concerned about the condition of animals in their care and have tried to ensure that they are healthy and well nourished. Although good welfare has traditionally been viewed by farmers and veterinarians to be seen as good health and production there is a growing acceptance that concerns such as pain and distress and the ability to engage in highly motivated behaviors is also of importance. In this proceedings chapter we discuss the concept of animal welfare from three different perspectives: biological functioning, affective state and natural behavior. Drawing largely on the research undertaken by our students we provide examples of how science can help provide solutions to welfare concerns that address each of these concepts. Animal welfare science addresses all three types of concern by identifying problems in production systems and developing solutions to these problems. The best solutions are win-win, improving the lives of cattle and the people that work with them.

Resumen

Aspectos sobre bienestar animal en ganado lechero

La preocupación por el bienestar de las vacas lecheras no es nada nuevo; productores y veterinarios siempre han estado preocupados por el cuidado de los animales y han tratado de asegurarse de que están sanos y bien alimentados. Aunque el bienestar ha sido considerado tradicionalmente por los agricultores y los veterinarios como tener buena salud y producción, hay una creciente aceptación de que malestares como el dolor y la angustia y la capacidad de involucrarse en comportamientos altamente reforzados también son de importancia. En este trabajo se discute el concepto de bienestar animal desde tres perspectivas diferentes: el funcionamiento biológico, el estado afectivo y el comportamiento natural. De la investigación realizada por nuestros alumnos, proporcionamos ejemplos de cómo la ciencia puede ayudar a

proveer soluciones a los aspectos de bienestar que abordan cada uno de estas perspectivas. La ciencia del bienestar animal se dirige a los tres tipos de interés mediante la identificación de los problemas en los sistemas de producción y el desarrollo de soluciones a estos problemas. La mejor solución ganancia-ganancia, mejorando la vida de ganado y de la gente que trabaja con ellos.

Introduction

Concern about the welfare of dairy cattle is nothing new; producers and veterinarians have always been concerned about the condition of animals in their care and have tried to ensure that they are healthy and well nourished (von Keyserlingk et al., 2009). In the tradition of good animal husbandry, good welfare can be seen largely as maintaining production and the absence illness or injury. However, more recent interest in farm animal welfare stems more from concerns about pain or distress that the animals might experience, and concerns that animals are kept under "unnatural" conditions, with limited space and often a limited ability to engage in social interactions and other natural behaviors. Our first objective is to describe a conceptual framework for these different types of animal welfare concern (reviewed in more detail by Fraser, 2008), using examples from dairy production systems. Over the past decade we have seen a tremendous increase in scientific research on the welfare of cattle. Although research alone cannot tell us which types of concerns are most important, it can and has provided solutions to a number of issues. Our second objective is to provide examples of how science can help provide solutions to welfare concerns (these and other examples are reviewed in Rushen et al., 2008).

Animal welfare: a conceptual overview

Animal welfare includes three types if concern: 1) is the animal functioning well (biological functioning), 2) is the animal feeling well (affective state), and 3) is the animal able to live a reasonably natural life (natural living; Fraser et al., 1997). Farm animal care givers are naturally concerned about the first category; addressing issues such as disease, injury, poor growth rates and reproductive problems, issues that are good for the animal and ultimately also vital in terms of the economic viability of the farm enterprise. However, people are also concerned with the affective state of the animal, and focus upon whether the animals are suffering from unpleasant feelings such as pain, fear or hunger. For some people (including many producers and consumers of organic products), a key concern is whether the animal is able to live a relatively natural life (Fraser and Weary, 2004). For example, is the calf kept with the cow and do cows have access to pasture?

These different types of concern about animal welfare can and do overlap. A lactating dairy cow unable to seek shade on a hot day (natural living), will likely feel uncomfortably hot (affective state), and may show signs of hyperthermia and ultimately reduced milk production (biological functioning). In such cases, research directed at any or all the levels can help address the welfare problem. In other cases, overlap may be less obvious or the different concerns may even be in conflict. For example, group housing of dairy calves allows them to engage in natural social interactions, but when poorly managed can lead to increased incidence of certain diseases or aggressive interactions. Different people can thus reach opposite conclusions about the relative advantages of different housing systems by favoring different welfare indicators (see Fraser, 2003 for case study). Clearly the best solutions will be those that address all three concerns, for example, by creating group-housing systems for calves that avoid competition, allow for social contact and maintains healthy calves. In this way, the three types of concerns can be considered as a checklist with researchers working to identify and solve the various welfare issues. Below we review a few examples of recent work showing how science can be used to address dairy cattle welfare issues from the perspective of biological functioning, natural living and affective states.

Biological functioning

Problems in biological functioning, such as disease and injury, are clearly a welfare concern. For example, lameness is now widely regarded as a major welfare problem for dairy cows and in recent years has received considerable attention in the scientific literature. Compounding the problem is that producers find it difficult to identify animals at the early stages of lameness, likely because dairy cows remain stoic unless injuries are relatively severe (Whay et al., 2003).

Current research is developing improved gait scoring system that can be used to identify cows that are becoming lame. Better scoring systems will require improved knowledge of cows' gait, and this can be derived from computer-assisted kinematic techniques that obtain precise measures of gait and how this changes with different types of hoof injuries (Flower et al. 2005). Our group uses a gait scoring system based on several specific gait features (e.g. asymmetric steps, tracking up etc.), and these scores have proven sensitive in identifying cows with sole ulcers (Flower and Weary, 2006), pain reduction following use of local anesthetic (Rushen et al., 2007) or non-steroidal anti-inflammatory drug (Flower et al. 2008), and the advantages of softer walking surfaces for lame cows (Flower et al., 2007). Improved training in lameness detection, can serve to recognize which cows will benefit from treatment, and perhaps more importantly identify management and environmental factors to reduce the risk of cows becoming lame.

Poorly designed and managed facilities cause injuries and increase the risk of health problems including lameness and transition cow disease, arguably two of the most serious welfare challenges facing the dairy industry (see von Keyserlingk et al. 2009). Producers spend millions of dollars building indoor housing for dairy cattle, with the aim of providing a comfortable environment for their animals - one that ensures adequate rest, protection from climatic extremes, and free access to an appropriate, well-balanced diet. Despite these laudable aims, housing systems do not always function well from the perspective of the cow – poorly designed and maintained facilities can cause injuries, increase the risk of disease, and increase competition among herd mates for access to feed and lying space.

Our aim is to provide science based solutions that can facilitate better designs and improvements in management that will prevent some of these problems. Our work has generally evaluated housing systems from the cow's perspective by asking how the housing affects cow health (e.g. by reducing the risk of hock injuries; Barrientos et al., 2013), what housing the cow prefers (Fregonesi et al., 2007; Fregonesi et al., 2009), and how the housing affects behavior (e.g. by reducing competition and increasing feeding time; Huzzey et al. 2006).

Variation in lameness rates can be explained in part by how the facilities are designed and managed, but these factors vary greatly among regions due to differences in tradition, barn builders, and availability of materials such as bedding. This means that the factors associated with lameness also vary among regions. For example, in recent analyses we have found major differences in factors associated with lameness in freestall facilities between the northeastern (NE) – US versus California (Chapinal et al., 2013). In the NE – US, where many farms used mats or mattress with just a little sawdust bedding, the risk of lameness reduced by half for farms using deep bedding and for farms that provided some access to pasture during the dry period. In CA, all farms used deep-bedded stalls (typically with dry manure bedding) and almost all farms provided outdoor access (typically to a well bedded dirt lot). Under these conditions, rates of lameness were much lower than in the NE – US. Rates of lameness were lowest on farms where stalls were kept clean (i.e. not contaminated with feces) and on farms that used rubber in the alley to the milking parlour.

Unlike lameness, hock lesions are obvious to anyone who cares to look. Indeed, it is pretty hard to avoid noticing hock lesions when you are standing at hock level in the milking parlour. But even though we can see these lesions they remain common on many farms. Again, we found that prevalence varied among regions, from 42% in British Columbia, to 56% in California, to 81% in NE – US (von Keyserlingk et al., 2012). And again, the good news is that within each region some farms had very low rates suggesting that others could learn from these most successful producers.

One of the greatest challenges is to translate science into practice. Our recent work on benchmarking lameness shows promise as a possible vehicle to promote the adoption of best practices that result in improved dairy cattle welfare (von Keyserlingk et al., 2012; Chapinal et al., in press). In summary, across regions, farms that use well-maintained, deep-bedded stalls have lower risk of lameness and lower rates of hock injuries. Benchmarking programs that provide famers the relevant data from their farms and other farms in their region can motivate farmers to change practices resulting in improved welfare. Farmers can use this data, together with the recommendations described here and elsewhere, to develop formulate tailor-made solutions to problems with lameness and leg injuries.

Affective state

Measures of biological functioning, like disease and growth, can normally be characterized scientifically with little disagreement. The same cannot always be said for measures of how animals feel. Developing validated measures of animal affect remains one of the most interesting and challenging problems in animal welfare science. Painful procedures remain part of the everyday business of dairy farming, but new scientific studies are showing ways that this pain can be reduced or avoided. Considerable research has shown that all methods of dehorning and disbudding cause pain to calves (reviewed by Stafford and Mellor, 2005) but recent research has also shown that hot iron dehorning an result in negative judgment bias argued to reflect low mood in calves (Neave et al., 2013; Daros et al., 2014).

It is now also becoming clear that use of local anesthetic alone does not fully mitigate this pain. For example, local anesthetic does not provide adequate post-operative pain relief. Lidocaine is effective for 2 to 3 h after administration and treated calves actually experience higher plasma cortisol levels than untreated animals after the local anesthetic loses its effectiveness (Stafford and Mellor, 2005). However, the use of non-steroidal anti-inflammatory drugs, in addition to a local anesthetic, can keep plasma cortisol and behavioral responses close to baseline levels in the hours that follow disbudding and dehorning. A second consideration is that animals respond to both the pain of the procedure and to the physical restraint. Calves dehorned using a local anesthetic still require restraint, and calves must also be restrained while the local anesthetic is administered. The use of a sedative (such as xylazine) can essentially eliminate calf responses to the administration of the local anesthetic and the need for physical restraint during the administration of the local anesthetic and during dehorning (Grøndahl-Nielsen et al., 1999). Thus a combination of sedative, local anesthetic and a non-steroidal anti-inflammatory drug reduces the response to pain during dehorning and in the hours that follow. Unfortunately, such a combination of treatments may not be practical for farmers and may itself have drawbacks for the animal. For example, an effective local block requires repeated injections and additional restraint.

One common alternative to hot-iron dehorning is using caustic paste to cause a chemical burn. This method of dehorning is still painful for the calves (Morisse et al., 1995), but the pain appears easier to control. Calves treated only with the sedative xylazine showed no immediate response to application of the paste, and little response in the hours that followed (Vickers et al., 2005). Moreover, caustic paste dehorning combined with a sedative actually resulted in less pain to calves than dehorning with a hot iron combined with both a sedative and a local anesthetic. This example shows how methods of pain treatment can be developed that are effective and practical for use on farm.

In this section we have focused on pain, in part because the science is clear but also because there is considerable social consensus regarding the ethics of intentionally causing (or failing to prevent) pain to animals. However, we urge readers not to focus only on pain; other affective states may be equally or more important to many cattle, including negative states like fear associated with poor handling practices and facilities and perhaps also positive affect associated by cows suckling their calf or grazing on pasture. The ability to perform these types of natural behavior are also considered important in their own right, as we turn to in the next section.

Natural living

For some, the natural living criteria may seem clear – simply allowing animals to live as naturally as possible. We see this approach as naïve; some natural conditions such as exposure to climatic extremes, disease, parasite infections and predator attacks cannot be seen as good for the animals. Thus the welfare benefits of providing more natural living must be assessed through the lens of the first two criteria. We use the example of more natural feeding systems for calves to illustrate how research can be used to determine if access to more natural environments also provides benefits to the animals in terms of biological functioning and affective state.

Traditionally calves are fed milk twice daily at 10% body weight, but calves often fail to gain weight during the first weeks of life (Hammon et al. 2002). When provided the opportunity, calves consume considerably more than 10% of their body weight (de Passillé and Rushen, 2006). Calves grow much more rapidly when allowed to suckle from the dam (Flower and Weary, 2003), but this biological functioning benefit does to not require keeping the cow and calf together. Simply feeding more milk allows for much higher weight gains, better feed conversion, and reduced age at first breeding (Jasper and Weary 2002; Diaz et al. 2001; Shamay et al., 2005). A better understanding of the calf's natural behavior and preferences, and how allowing this behavior this can benefit calf growth, is helping to revolutionized calf feeding practices.

The milk feeding practices also affect calf hunger. Calves vocalize when hungry and this vocal response, even in the first days after separation from the cow, can be much reduced or eliminated by providing more milk or colostrum (Thomas et al., 2001). Calves that are fed restricted amounts of milk from an automated calf feeder typically visit the feeder more than 20 times a day even when they only receive milk on 2 of these visits. Increasing the milk ration much reduces the frequency of these 'non-nutritive' visits (Jensen 2006; De Paula Vieira et al. 2008). This reduction benefits the other calves using the feeder by reducing feeder occupancy and competition for feeder access. Thus allowing more natural feeding behavior reduces hunger and in this case also improves the efficiency of the feeding system facilitating group housing of calves.

The benefits in terms of improved growth and reduced hunger can be achieved by proving the calves more milk. Nipple feeding is clearly more natural but does this provide other benefits for the calf or the producer? Calves allowed to suck on a teat during or after a meal show higher concentrations of cholecystokinin and insulin (de

Passillé et al., 1993) and a greater degree of relaxation after the meal (Veissier et al., 2002). Group-housed milk-fed calves will sometimes suck each other (i.e. cross sucking), but this cross-sucking can be much reduced or eliminated if calves consume their milk ration via free access to a teat (de Passillé, 2001), likely because the sucking behavior per se, rather than the ingestion of milk, is responsible for reducing sucking motivation (de Passillé, 2001). Thus nipple feeding also facilitates group housing, saving labor for producers (Kung et al., 2001) and perhaps providing other benefits to the calves.

For the past decades, common wisdom among North American dairy experts was that calves should be housed individually, in separate pens or hutches. This practice was considered to maximize performance and minimize the risk of disease. Individual housing also helps avoid behavioural problems such as competition and cross-sucking.

The new calf-feeding methods described above work well for individually housed calves, but also facilitate group housing. Group housing provides more space for calves and allows for social interactions. For the past decades, common wisdom among North American dairy experts was that calves should be housed individually, in separate pens or hutches. This practice was considered to maximize performance and minimize the risk of disease. Individual housing also helps avoid behavioural problems such as competition and cross-sucking.

The new calf-feeding methods described above work well for individually housed calves, but also facilitate group housing. Group housing provides more space for calves and allows for social interactions. Research and practical experience show that group rearing of calves can result in considerable benefits through reduced labour requirements for cleaning pens and feeding. Calves are social animals that need exercise and keeping dairy calves in groups may provide a number of advantages to both producers and their calves. Successful adoption of group housing will mean avoiding problems such as increased disease and competition. Recent research provides some insights into how these risks can be minimized.

We evaluated the behaviour and growth rates of calves housed in pairs versus individually (Chua et al., 2002); calves gained weight steadily regardless of treatments. Interestingly, during the week of weaning (approximately 5 weeks of age), pair-housed calves continued to gain weight normally but the individually housed calves experienced a slight growth check. There were no differences between groups in the amounts of milk, starter or hay consumed, or in the incidence of scouring or other diseases. Aggressive behaviour and cross-sucking were almost never observed (less than 0.2% of time).

In a more recent study, De Paula Vieira et al. (2010) found that calves housed in pairs vocalized less during weaning than did individually housed calves. The results of this study also illustrated some longer-term costs to housing calves individually. When all calves were eventually introduced to a group pen after weaning calves that had previously been single housed took on average 50 h to begin feeding, in comparison to just 9 h for the pair-reared calves. Calves are also able to learn a simple colour discrimination task, and then re-learn the task when the colour treatments were reversed. However, despite the speed of learning for the simple discrimination task being similar for individually housed and pair-housed calves, the pair-housed calves are able to adapt more easily when the training stimuli are reversed. Together, the results of these studies suggest that individual housing of dairy calves can result in measurable learning deficits. Social housing for calves may result in animals that are more flexible in their responses to changes in management and housing (de Paula Vieira et al., 2012; Gillard, et al., 2013).

Successful group rearing requires appropriate management, including feeding method and group size. Large epidemiological surveys of U.S. and Swedish dairy farms found increased mortality and disease on farms keeping calves in large groups (more than 7 or 8) (Losinger and Heinricks, 1997). Thus, small groups are likely a better alternative than large ones.

Calf immunity and the design and management of the housing systems, such as its cleanliness and ventilation, likely affect disease susceptibility more than group housing per se. Our work shows that housing young dairy calves in small groups is viable in terms of calf health, performance and behaviour. New research is now required on management strategies that will help prevent disease. For now, we encourage producers to consider keeping a closed herd (i.e. no new animals entering the herd), keeping groups small and physically separated from one another (e.g. in super hutches), and managing group pens in an all-in-all-out basis.

Calves in groups sometimes compete with pen mates. In one experiment using a simple teat-feeding system, we found that group-housed calves can displace one another from the milk teat many times each day if there are not enough teats (von Keyserlingk et al., 2004). However, giving each calf access to its own teat greatly reduced these displacements. This improved access to teats resulted in longer feeding times and increased milk intakes.

Other research has focused on how computerized feeding stations can be managed to reduce competition between calves. Increasing the daily milk allowance for calves from 5 to 8 litters per day reduced by half the number of times calves visited the feeder, reducing occupancy time and displacements from the feeder, and improving the efficient use of this equipment (Jensen and Holm, 2003; de Paula Vieira et al. 2008; Sweeney et al., 2010). Our research shows that young calves can be introduced into a group with little disruption when they are trained to feed from the computerized feeding station prior to the introduction (O'Driscoll et al., 2006). Although the calves visited the feeder less frequently on the day of mixing, they were able to compensate by increasing both the duration and amount consumed per meal, and established their pre-mixing feeding pattern after just one day.

Conclusions

Many in the dairy industry may have assumed that animal welfare concerns can be met by working to ensure good health and productivity for the cows and calves in their care. We have argued above that good biological functioning is a necessary component of welfare, but this focus alone is not sufficient; affective states like pain or hunger, and concerns about naturalness are also important. Animal welfare science addresses all three types of concern by identifying problems in production systems and developing solutions to these problems. The best solutions are win-win, improving the lives of cattle and the people that work with them.

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6
On-farm animal behavior and welfare

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Summary

Animal welfare is an all-encompassing term that aims to quantify how an animal is functioning, feeling and if the animal can live a “relatively” natural life. Farm animal welfare standards and expectations differ greatly between regions of the world. The European Union has a plethora of law that covers housing systems, care and management practices. The United States are somewhat in-between, with two federal laws, and a few states outlawing certain housing practices. On the opposite end of this spectrum, developing countries have little to no on-farm animal welfare standards. However, farm animal welfare as it relates to the multilateral trade policy framework has been the subject of global discussion. Recently, private standards and specifications as it relates to on farm animal welfare and effects on international trade in animal products has further stimulated interest in consistency from the World Trade Organization. It is not a case of “if” but “when” farm animal welfare trade disagreements will occur, and farmers, scientists, funding agencies and policy makers must continue to work together so that on-farm animal welfare and global trade in animal protein can continue in harmony.

Resumen

Comportamiento y bienestar animal

El bienestar animal es un término global que tiene como objetivo cuantificar cómo un animal está funcionando, sintiendo y si el animal puede vivir "relativamente" una vida natural. Estándares y expectativas de bienestar de los animales de granja difieren mucho entre las regiones del mundo. La Unión Europea tiene una plétora de leyes que cubre los sistemas de alojamiento, cuidado y prácticas de manejo. Los Estados Unidos están en un término medio, con dos leyes federales, y con algunos estados que prohíben ciertas prácticas de alojamiento. En el extremo opuesto de este espectro, los países en desarrollo tienen poco o ninguna norma de bienestar animal en las explotaciones. Sin embargo, el bienestar animal y su relación con el marco multilateral de la política comercial ha sido tema de debate mundial. Recientemente, las normas privadas y especificaciones, así como su relación con el bienestar de los animales de granja y los efectos sobre el comercio internacional de productos de origen animal han estimulado, aún más, el interés por su consistencia en la Organización Mundial del Comercio. No es un caso de "si" sino de "cuándo" se producirán desacuerdos comerciales de bienestar de los animales de granja, así ganaderos, científicos, instituciones financieras y los responsables de la elaboración de políticas deben seguir trabajando juntos para que el

bienestar de los animales de granja y el comercio mundial de proteína animal puedan continuar en armonía.

Poultry and swine behavior and welfare challenges

Poultry and swine behavior and welfare continues to be under scrutiny (Johnson, 2008). Housing for both species has been a major challenge, specifically conventional cages for the laying hen and gestation stalls for swine (McGlone et al 2004; Bas-Rodenburg et al 2005). Predominate criticisms for the conventional cages has been the lack- and quality of space (Mench and Blatchford, 2014), no perches (Donaldson et al 2012), no nest boxes (Riber and Nielsen, 2013) and no ability to dust bath (Appleby et al 1993). Another criticism towards this housing has been levied at the inability of a hen to escape during aggressive interactions (Shimmura et al 2010). Conversely criticisms to the gestation stall include the inability of the sow to form social relationships (Pitman-Elmore et al 2011), lack of space (Li and Gonyou, 2007), detrimental effects on bone and muscle strength (Marchant and Broom, 1996) and the lack of biologically relevant environmental enrichment (Elmore et al 2012). More recently, concerns have centered on how much pain an animal experiences, how to manage pain and euthanasia techniques. Pain can be inflicted during routine procedures, for example beak trimming in the hen (Gentle, 2011), or castration, tail docking and teeth re-sectioning in the piglet (Hanssen et al 2011; Sutherland et al 2012). Classical work by Danbury and others (2000) concluded that severely lame broilers will self-medicated with a non-steroidal anti-inflammatory drug, indicating that animals do perceive pain and will seek pain relief. However, in the U.S. science-based guidance for the industry on optimal housing, management and treatment of lame birds and pigs is deficient. In addition, there are no approved drug treatments for lame swine analgesia, and the identification and validation of robust, repeatable pain measurements is fundamental for the development of effective analgesic drug regimens and management strategies (AVMA; 2010; FDA, 2010). At Iowa State University work on the lame sow (Karriker et al 2013), pain management (Pairis-Garcia et al 2013; Pairis-Garcia et al 2014) and the use of objective tools (Tapper et al 2013; Mohling et al. 2014a,b), will provide scientific data to help the FDA in the area of pain management. As regards euthanasia, the most predominant U.S. method for non-viable piglet euthanasia (less than 5 kg [12 lb.]) is manually applied blunt force trauma (Ma-BFT). Manually applied blunt force trauma is one of several euthanasia techniques considered acceptable or conditionally acceptable by the most recently published guidelines from the American Veterinary Medical Association (AVMA). However, Ma-BFT is being criticized on the basis of aesthetics, impacts on those performing the procedure, and the ability to produce humane euthanasia consistency. Swine producers, veterinarians and animal scientists generally agree that euthanasia is the best choice for low viability piglets, especially when there is suffering due to injury or illness but research on humane options for this classification of pig is needed. Work at Iowa State University has been addressing euthanasia of the low viable piglet (Sadler et al 2014a) and the use of gas (Sadler et al 2014b). This data will be critical as the AVMA and U.S. swine industry update their recommendations and policies.

Poultry and swine welfare: Functioning, feeling and a natural life

Animal welfare is an all-encompassing term that aims to quantify how an animal is functioning, feeling and if the animal can live a "relatively" natural life (Fraser et al 1997). Traditionally, producers, veterinarians and animal scientists have studied, measured and addressed challenges that fall into the functioning category. Using the Pork Quality Assurance (PQA®) Program® as an example, questions that are used during on-farm assessment include "*is the body condition score (BCS) for healthy animals in the breeding herd 1% or less BCS 1*?" or "*are pigs euthanized in a timely manner*?" However, consumers, scientists and groups with an interest in farm animal agriculture and particularly animal welfare, are also concerned with how an animal is feeling or coping in agricultural systems. In the presence of humans, farm animals can display behaviors/postures that have been frequently labeled as fear or fear responses, and these negative affective states are of particular importance to measure and manage. For example, in the Welfare Quality Assessment Program (2009) using sows as an example, the assessors are asked to "*classify social behaviour (positive [rooting, sniffing, licking etc] and negative [aggression]) or other (resting)*." This secondary category has been more challenging to make sure that data is collected in a uniform way, over a variety of housing systems, and that the interpretation is correct. Finally, for some entities, the ability of the animal to live a relatively natural life is extremely important. Global markets have animal produce that relates to natural living. These are marketed as "niche", "organic" and or "free-range". In the U.S. Wholefoods have a 5-step animal welfare rating standards. In their chickens raised for meats standards it is noted that "*all chickens from 4-weeks of age must be given continuous access to the outdoors during daylight hours if climatic conditions do not pose welfare risk"* These three areas of animal welfare do overlap for example, a free ranging hen unable to find a shelter from a cold wind would likely feel cold and show signs of shivering (feeling) and perhaps reduced egg production (functioning). Conversely, grouped housed gestation sows at mixing will engage in intense aggression until the hierarchy for that pen has been established, but a static pen of gestating sows whose hierarchy has been established can live in relative harmony. There are discrepancies in the use of functioning, feeling and animals living a relatively naturally life in farm animal welfare educational, assessment, third party auditing and law at the global scale. For example, the PQA Plus and the swine third party audit in the U.S. do not measure and report a human-animal interaction test, but the Welfare Quality Assurance Program from the European Union (EU) does (Welfare Quality, 2009; NPB, 2013; 2014). Hence differing weights of importance have been placed into these three areas of welfare that in turn reflect the outcomes measured within a countries farm animal welfare programs. This makes hypothetically using animal welfare as a trade barrier more complicated.

Animal behavior, welfare and trade

Animal-welfare legislation in Europe and in several U.S. states has outlawed the use of particular housing systems. Currently, such legislative efforts may have only a limited effect on farm animal welfare, so long as consumers continue to demand, and are supplied with, products imported from other nations or states lacking similar farm animal-welfare laws. International trade represents a special challenge for farm animal-welfare legislation. As the European Commission noted, *"Animal welfare standards, notably those concerning farm animal welfare, could be undermined if there is no way of ensuring that agricultural and food products produced to domestic animal welfare standards are not simply replaced by imports produced to lower standards*" (European Commission, 2002). An example of this came through the United Kingdom passing a ban on sow gestation stall and tether use in 1999, yet the rest of the European Union did not ban these practices until 2013. Consequently, the United Kingdom pork costs increased and imports of fresh and frozen pork products increased by 77%. In 2005, more than half of all pork products in British supermarkets were imported, and more than two-thirds of these imports were produced using systems illegal in the United Kingdom (British Pig Innovation, 2006). The European Union has not yet attempted to restrict imports from countries that do not meet its farm-animal welfare legislation standards. However, if such an attempt was made other non-European countries would likely submit formal complaints. Trade disagreements can be made through the multilateral trade policy framework which comprises of the General Agreement on Tariffs and Trade 1947 (GATT), the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) and the Agreement on Technical Barriers to Trade (TBT). These Agreements are a set of rules that must be respected by the World Trade Organization (WTO) and they should not arbitrarily or unjustifiably discriminate between countries where identical or similar conditions prevail (WTO, 2014a). Animal welfare as it relates to these WTO rules has been the subject of global discussion. Recently, private standards and specifications as it relates to on farm animal welfare and the effect this has had on international trade in animal products has further stimulated interest in the question of WTO consistency (Kahn and Varas, 2014). Protecting compliant producers from unfair competition with non-compliant producers will depend upon allowing one or more of the following: animal-welfare considerations in Article III or Article XX of GATT, international standards, labeling, tariffs, and Green Box provisions. Article III of GATT states that imported products should be treated no less favorably than "*like products*" of domestic origin (GATT, 1947). Disagreement has focused on the interpretation of "*like products*" which, in past WTO disputes, have been understood as "*directly competitive or substitutable products*" (Raj and Gantz, 2005). For instance, the WTO may not permit a nation to restrict imports of cage eggs while it allows production of cage-free eggs, which are physically identical. However, because consumers concerned about animal welfare do not view such products as substitutable, there may be room for differentiating products according to process and production methods (PPM). No GATT rule explicitly forbids PPM distinctions, and the criteria for what constitutes "*like products*" continues to evolve in WTO case law (WTO, 2014b). If the WTO Council establishes an interpretive rule accepting PPM distinctions, the European Union could restrict imports that do not comply with its domestic regulations.

The Agreement most likely to be used for such dispute settlements would be the Agreement on the Application of SPS Measures that sets out the basic rules for food safety and animal and plant health standards. These are as follows, to;

- Protect human or animal life from risks arising from additives, contaminants, toxins or disease-causing organisms in their food;
- Protect human life from plant- or animal-carried diseases;
- Protect animal or plant life from pests, diseases, or disease-causing organisms;
- Prevent or limit other damage to a country from the entry, establishment or spread of pests.

The SPS Agreement also recognizes the World Organization for Animal Health (OIE; 2014), the International Plant Protection Convention and the Codex Alimentarius Commission as the reference international standard- setting organizations animal health and zoonotic diseases, plant health and food safety (Kahn and Varas, 2014). Animal health is clearly linked to better animal welfare. However, extensive livestock production systems which have been advocated as providing superior animal welfare (Wholefoods, 2014) may have increased health challenges through predation, exposure to pathogens, temperature extremes and other environmental contaminants (Quintern and Sundrum, 2006; Kijlstra et al 2009). Developing countries may view the European Union's animal welfare proposals as disguised protectionism. However, developing countries do have strengths as regards on farm animal welfare, (1) it is seen as a value-adding attribute for some exporting developing countries (Bowles et al 2005) (2) farms tend to be smaller and not considered concentrated-animal-farming-operations (CAFO) and (3) developing countries are still in the early stages of creating welfare assurances that can be built in a flexible manner to meet the market specifications. These assurances can be created through law and legislation, educational, assessment- and third party auditing programs (Grandin 2007; Hemsworth et al 2009; Johnson 2008).

U.S. law and legislation

Food animal production government regulations are a common practice for European Union livestock and poultry producers. The transition had both controversy and economic cost. In contrast, U.S. livestock and poultry producers have been relatively free of mandatory animal welfare standards that address the way food animals can be housed until recently (Swanson, 2008). The first legislation involving food animal welfare was the Humane Methods of Slaughter Act (1958) which set forth to *"to establish the use of humane methods of slaughter of livestock as a policy of the United States, and for other purposes."* The Act covers animal ante-mortem handling and the slaughter process itself. It requires animals be made insensible to pain by "*a single blow or gunshot or an electrical, chemical or other means that is rapid and effective*" prior to being shackled, hoisted or cut. The humane slaughter act requires that cattle, calves, horses, mules, sheep, swine, and other livestock be stunned before slaughter. The nation's humane slaughter laws currently do not cover poultry. The second federal regulation, called "The 28-hour Law", originally passed in 1873 (49 U.S.C. 80502, last amended in 1994; 2005 to include road), notes that many animal transport vehicle types "*may not confine animals in a vehicle or vessel for more than 28 consecutive hours without unloading the animals for feeding, water, and rest.*" In the past decade however, there has been a monumental shift in state law that mandates how animals can be housed. For example, the following states have outlawed the use of individual stalls for the gestation sow; FL, AZ, OR, CA, CO, ME, MI and OH (Johnson, 2008).

Third party auditing

Third party auditing programs provide an additional verification to the customer and consumer that products for human consumption are cared for following program specifications (Johnson, 2008). In the U.S., Canada, Australia, and New Zealand, approximately 90% of large beef and pork slaughter plants are audited by major customers (Grandin, 2007). Recently in the U.S. the National Pork Board released an industry on-farm third party auditing program. The program will use the existing Pork Quality Assurance® Plus (PQA Plus®) program as its foundation and expand on it to serve as a common audit platform for the pork industry. The overarching goal of the common audit process is to provide consumers greater assurance of the care taken by farmers and pork processors to improve animal care and food safety. The common platform seeks to create and standardize a common process that will: (1) meet individual company and customer needs, (2) focus on outcome-based criteria that measure animal welfare, (3) provide clarity to producers with regard to audit standards and expectations, (4) minimize duplication and prevent over-sampling and (5) ensure greater integrity of the audit process through consistent application. The new common audit framework has several key components, including a new audit tool, requirements for auditor training and biosecurity and a platform that will allow audit results to be shared to prevent duplicative audits. The audit tool is currently being beta-tested on farms across the U.S. (NPB, 2014).

Assessment programs

Animal welfare assessment programs provide customers and consumers with assurance that the food products they purchase and consume are derived from production systems where the animals are monitored and evaluated according to an organizations published standards. These programs focus to ensure transparency, creditability, and accountability for the methodologies utilized in managing food producing animals (Johnson, 2008). On-farm welfare assessment involves the practical evaluation of animal state, defined as health, performance, physiological, behavioral, and cognitive functions under commercial farm conditions. On-farm welfare assessment measures can be broadly divided into animal- and resource-based. Resource based measures are usually indirect measurements of animal welfare, for example production measures such as feed/gain ratio, body weight gain, and space allowance (AMI, 2010; NCC, 2014; NMPF, 2013; NPB, 2013; NTF, 2012) and the environment i.e. penning integrity, feed and water structures. Animal based resources are considered more direct measurements of animal welfare, and include body condition scoring (NCBA, 2010; NMPF, 2013; NPB, 2013), hygiene scoring (NMPF, 2013), slips and falls (AMI, 2010; NCBA, 2010; NPB 2013), broken and dislocated wings, broken legs (NCC, 2014), gait/lameness scores, (NCC, 2014; NMPF, 2013; NPB, 2013) hoof and hock lesions (NMPF, 2013; NPB, 2013), and vocalizations (AMI, 2010; NCBA, 2010).

Numerous animal-based measures can be collected, for example body condition score, lameness severity, abscesses, wounds, and lesions. Wounds and lesions are collated and reported because when farm animals are socially housed, behavioral challenges can occur, that in turn can affect individual welfare. One example of a behavioral challenge in socially housed animals is aggression. The term "aggression" is a very broad category, that can result from a variety of causal factors; i.e. aggression around the time of reproduction (Ewing et al 1999), parent to off-spring (Ahlström et al 2002; Marchant-Forde, 2002; Harris et al., 2003) and social which can include fighting within newly formed- or established groups (Anderson et al 1999; Arey 1999; Gabor et al 1999; Marchant-Forde and Marchant-Forde, 2006). Factors that affect the level, intensity and frequency of social aggressive interactions are numerous, can include, but are not limited to, the quality and quantity of space, number of resources per pig (i.e. feeders and drinkers), placement of resources, number of animals in a group and how the structure of the group has been formed. A more challenging animal-based measure in assurance programs is the animal-human relationship (Hemsworth and Barnett 1991). Previous animal-human paradigm tests have included the open field- (Mormède et al 1984), novel object approach-, and human approach tests (Hemsworth et al 1996; Rushen et al 1999; Grandin 2007; Pairis et al 2009; Colpoys et al 2014). Fangman and colleagues (2010) coined the term "*willingness-to-approach*" as a positive alternative to fear that describes nursery pig approach behavior elicited by a human observer in the home pen. Regardless of the animal-human paradigm test used, the methodology must be repeatable, objective, meaningful, and fast. The person conducting the test must be able to interpret the findings correctly and hence understand the animal species' normal behavioral patterns within a specific environment (Wailblinger et al 2006). Finally, this on-farm animal-human paradigm test should provide consistent results regardless of the environment that the animals are housed in, size of enclosure or global region of the world. Forkman and co-workers (2007) have suggested that the first animal response to a novel- or unfamiliar object is the most accurate. If digital techniques can be utilized to capture an image of animals within a pen at a given time point, then postural classification and precise proximity from the human observer could be determined, and hence provide a more objective and repeatable result for animal-human paradigm tests on-farm. Work at Iowa State University has tried to provide information on this animal-human interaction during on-farm welfare assessments. A nursery-pen image capturing device was developed (Figure 1) and we were able to compare a human observer and the digital image for nursery pig-human interaction measures (Figure 2).

Figure 1: Nursery-pen image capturing device (Weimer et al 2012)

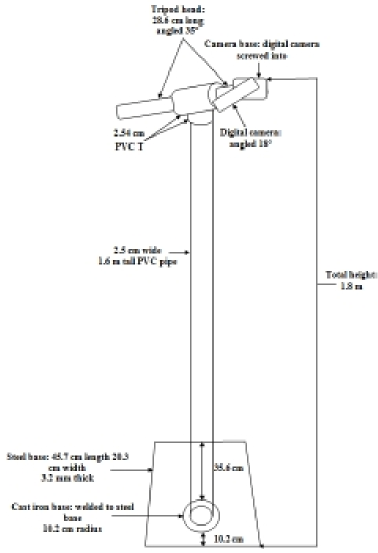

Figure 2: Swine postures and behaviors that were collected digitally to determine the animal-human relationships during an assessment (Weimer, 2012)

Findings from this work noted that there were no differences in pigs classified as Touch, Orientated and Not Orientated to the human in the pen between methodologies. We concluded that this animal-human based methodology showed promise for inclusion into on-farm welfare assessment programs. In addition this test has indicated robustness (Weimer, 2012), regardless of farm size (family vs. corporate) and geographical location (developed vs. developing countries). This is an important finding for swine producers because if this animal-based measure was required in a country's welfare standards, it may allow them continued or new access to local, regional and global markets.

Conclusions

Assuring on-farm animal behavior and welfare will continue to be interwoven in future discussions as it relates to national and global trade. These discussions must include a variety of stakeholders involved in animal production. It is vital that countries are developing and/or improving their farm animal welfare options that can assure other countries that humane care is forefront in daily business decision making. Developing countries must be at these discussions to present their views and concerns and to help shape the future. Creation of farm animal welfare options must not be cost prohibitive, content should be based on sound science, the measures must be meaningful and the results and processes be transparently communicated. It is not a case of "if" but "when" farm animal welfare trade disagreements will occur, and therefore, farmers, scientists, funding agencies and policy makers must continue to work together so that animal welfare and global trade can continue in harmony.

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7
Nutritional ways to maximize feed efficiency and performance

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Summary

In recent years, much attention has been given to feed efficiency (FE) in swine and poultry production due to the rising costs of feed and other inputs. The main biological factors that contribute to differences in FE include physical activity, feed intake patterns and behaviour, environmental (climate, nutritional and immunological) stress, nutrient and energy digestibility and efficiency of utilization, composition of gain and metabolism. In other words, both the maintenance and growth components of nutrient and energy utilization can be involved. Strategies to improve growth and FE of poultry and swine can be achieved with the use of feed additives that regulate and improve gastrointestinal function, health and integrity. With the recent volatility of traditional feed ingredients such as corn, wheat, soybean meal and canola meal, the swine and poultry industries have moved towards alternative cost-effective feed ingredients such as cereal co-products from biofuel and milling industries. However, the ability of mongastric species to fully utilize and capture the nutrients and energy out of these cereal co-product feed stuffs is limited by their gastrointestinal tract endogenous enzyme production and anatomy. To compensate for this, over the past decade there has been an increase in the use of exogenous enzymes (EE) in diet formulation to facilitate improved energy and nutrient utilization. Additionally, the use of pre- and probiotics, organic acids, mycotoxin binders and other functional ingredients have been used to promote gastrointestinal health and function. This paper will cover the use of conventional and alternative feed stuffs in swine and poultry nutrition. In particular, the contributions of bioactive nutrients and exogenous enzyme (EE) usage in monogastric diets that facilitate intestinal health and function will be discussed.

Resumen

Manejo nutricional para maximizar la eficiencia alimentaria y la performance productiva

En los últimos años, se ha prestado mucha atención a la eficiencia alimenticia (EA) en la producción de cerdos y aves de corral debido al incremento de costos de los

alimentos y otros insumos. Los principales factores biológicos que contribuyen a las diferencias en la EA incluyen la actividad física, los patrones y comportamientos de consumo, el estrés ambiental (climatológico, nutricional e inmunológico), la digestibilidad y la eficiencia de utilización de nutrientes y energía, la composición de la ganancia y el metabolismo. En otras palabras, tanto los componentes de mantenimiento y crecimiento en la utilización de nutrientes y energía pueden estar involucrados. Estrategias para mejorar el crecimiento y la EA de las aves de corral y cerdos se pueden lograr con el uso de aditivos que regulan y mejoran la función, la salud y la integridad gastrointestinal. Con la reciente volatilidad de los insumos tradicionales como el maíz, el trigo, la harina de soya y la harina de canola, las industrias de porcinos y aves de corral se han movido hacia el uso de insumos alternativos rentables tales como subproductos de cereales de las industrias bioenergéticas y de molienda. Sin embargo, la capacidad de las especies monogástricas para utilizar plenamente y capturar los nutrientes y la energía de estos subproductos de cereales está limitada por su anatomía y por la producción de enzimas endógenas del tracto gastrointestinal. Para compensar esto, en la última década se ha producido un aumento en el uso de enzimas exógenas (EE) en la formulación de dietas para facilitar una mejor utilización de nutrientes y energía. Adicionalmente, los pre y probióticos, ácidos orgánicos, secuestradores de micotoxinas y otros ingredientes funcionales se han utilizado para promover la salud y la función gastrointestinal. En este trabajo se discutirá el uso de insumos tradicionales y alternativos en la nutrición porcina y de aves de corral. En particular, se discutirán los aportes del uso de nutrientes bioactivos y enzimas exógenas (EE) en las dietas de monogástricos que facilitan la salud y la función intestinal.

Biological factors driving feed efficiency

Together with growth rates (average daily gain; ADG), days to market and mortality, feed efficiency (FE) is considered one of the most important parameters in swine and poultry production to assess. As such, improving FE is a major objective in swine and poultry production due to the rising costs of feed and the need to enhance overall production efficiency and profitability. Feed efficiency is not a directly measureable trait; however, it is typically used to describe variation in weight gain with respect to feed input. The traditional measurements of FE include the ratios body weight gain:feed intake (G:F) or feed intake:body weight gain (feed conversion ratio, FCR). Due to the economic importance of FE and the increase pressure for selection of lean carcasses (swine) and faster growth rates (swine and poultry), FCR trends have decreased over the last few decades due to genetic selection and improved nutrition and environmental management strategies.

While is it known that the key factors contributing to FE differences are similar across different breeds and species due to selection for similar genetic parameters and production traits, the underlying physiological and molecular mechanisms are poorly described. However, the main biological factors that may contribute to differences in FE have been partially quantified in poultry (Luiting, 1990), pigs (Barea et al., 2010), and beef cattle (Richardson and Herd, 2004). These key factors have been summarized (Figure 1) and include physical activity, feed intake patterns and behaviour, stress, body composition, nutrient digestibility, protein turnover, and metabolism (Richardson and Herd, 2004; Herd and Arthur, 2009). Biologically, although all these factors contribute to the variation associated with FE, dietary strategies to improve animal health, nutrient and energy digestibility have had some success. With the advances in biotechnology, exogenous feed enzymes, mycotoxin binders and gut modifiers have shown some promise in aiding in improving poultry and swine FE.

Intestinal health and function

Significantly contributing to the production efficiencies of poultry and swine is the maintenance and improvement in gastrointestinal tract (GIT) health. Therefore, the use of dietary ingredients to improved monogastric GIT functions and health is not a new concept. The lumen of the GIT is considered a space outside the body because of its continuity with the external environment. It has the arduous task of absorbing the nutrients that are essential for the organism while preventing the absorption of substances that are not needed and harmful to the system. The GIT primarily serves two important functions: 1) Selectively absorbing nutrients, vitamins, minerals and water from the lumen; and 2) Forming a physical barrier between the luminal contents and systemic circulation. A single layer of intestinal epithelial cells which line the intestine selectively absorbs most of the nutrients needed through active and passive processes with the help of specific transport or carrier proteins. For example, glucose and fructose are absorbed through Na-dependent glucose transporter 1 and glucose transporter 5, respectively. Water is absorbed through aquaporin proteins or via paracellular processes, and amino acids and di- and tripeptides are absorbed through numerous transporter proteins located on the apical and basolateral membranes. The epithelial

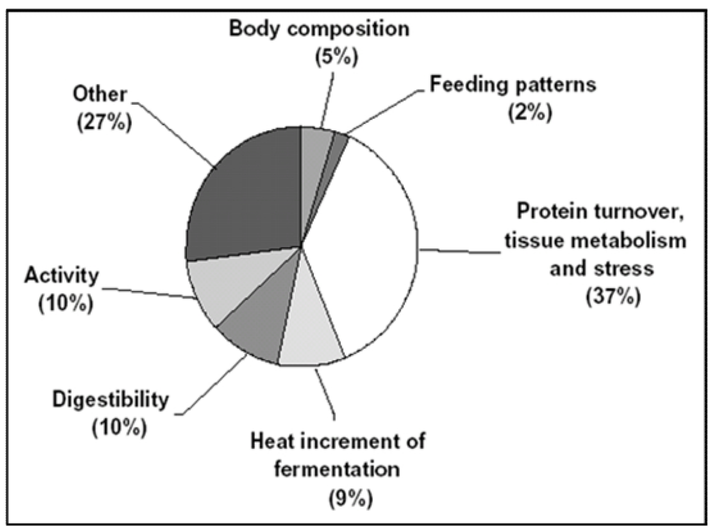

Figure 1. Contributions of biological mechanisms to variation in residual feed intake divergently selected cattle (Richardson and Herd, 2004).

or intestinal integrity is therefore critical for maintaining a physical barrier between the intestinal lumen and the body. This is dependent largely on the junction complexes connecting enterocytes together and is achieved via a well-organized intercellular array of tight junctions, adhesion junctions, and desmosomes surrounding the apical region of epithelial cells.

Changes in intestinal function and integrity can be detrimental to swine and poultry health and efficiency. An overzealous immune response due to these changes can antagonize key production parameters, egg and lean tissue accretion (Escobar et al., 2002; Klasing, 2007, 2009; Mani et al., 2012). In poultry and pigs, nutrition is a critical component of maintaining and responding to changes in the intestinal epithelium. In pigs, specific amino acids (arginine, glutamine, glutamate and threonine), long chain n-6 and n-3 polyunsaturated fatty acids and glucose have been shown to elicit positive benefits in the growth and repair of the intestinal epithelium in ischemia-reperfusion injury, prenatal stress and rotavirus challenge models (Rhoads et al., 2007; Rhoads and Wu, 2009; Jacobi and Odle, 2012). Glutamine is a non-essential amino acid and is important for rapidly dividing cells, especially immune and epithelial cells of the intestine. In several studies, glutamine has been shown to reduce intestinal injury, as well as stimulate cell turnover via increased cell proliferation and decreased apoptosis (Rhoads and Wu, 2009; Swaid et al., 2013). A dipeptide derivative of glutamine, alanyl-glutamine has also been shown to increase proliferation and migration while reducing apoptosis (Rodrigues et al., 2013). Other amino acids, including arginine and leucine may be beneficial (Rhoads and Wu, 2009). These amino acids, along with glutamine are prototype amino acid signals. Glutamine activates the mitogen-activated protein kinase (MAPK) pathway and increases heat shock protein synthesis while arginine and leucine activate the mammalian target of rapamycin (mTOR) pathway to promote synthesis of new proteins (Rhoads and Wu, 2009). Several growth factors such as transforming growth factor beta (TGF-β), and insulin-like growth factor 1 (IGF-1) are stimulators of cell proliferation, migration, intestinal restitution and decreased paracellular permeability (Blikslager et al., 2007).

Probiotics have also shown promise in the area of intestinal restitution. Lactobacillus and Bifidobacteria species, as well as yeast help preserve intestinal integrity (Rao and Samak, 2013). Glucagon like peptide-2 (GLP-2) is also capable of stimulating wound repair/restitution in the intestine (Bulut et al., 2008). In addition to probiotics and amino acids, medium-chain triglycerides, short chain fatty acids and long-chain polyunsaturated fatty acids are also essential components in providing energy and maintaining intestinal growth and development. Of particular interest is the n-6 polyunsaturated fatty acid, arachidonic acid, and the prostanoid orchestrate recovery of paracellular resistance within restituting epithelium (Blikslager et al., 1997; Blikslager et al., 1999; Jacobi et al., 2012). Omega-3 fatty acids such as docosahexaenoic and eicosapentaenoic acids have also been shown to modulate intestinal barrier function and integrity in pigs (Gabler et al., 2007; Gabler et al., 2009; Liu et al., 2012).

Exogenous enzymes (EE)

Volatility of traditional feed ingredients such as corn, wheat, soybean meal and canola meal, as forced the swine and poultry industries to seek alternative cost-effective feed ingredients such as cereal co-products from biofuel and milling industries. However, the ability of mongastric species to fully utilize and capture the nutrients and energy out of these cereal co-product feed stuffs is limited by their gastrointestinal tract endogenous enzyme production and anatomy. To compensate for this, over the past decade there has been an increase in the use of exogenous enzymes (EE) in diet formulation to facilitate improved energy and nutrient utilization. Most exogenous enzymes (EE) additives have been derived from microbial (bacteria or fungal) fermentation processes. The classic example for monogastric species has be the rampant adoption of phytase, which makes up 60% of the EE world market, with non-phytases marking up the rest (carbohydrases ~30% and proteases ~10%) (Adeola and Cowieson, 2011). The proposed mode of action for the use of EE in improving productivity has been classified into four categories (Kiarie et al., 2013):

1. Improved hydrolysis of feedstuffs that are not sufficiently degraded by the animal's own enzymes. Thus, increased luminal degradation of anti-nutritional factors that are present in feed ingredients, thus making their constituents more available.
2. Degradation of cell wall polysaccharides, thereby preventing the nutrient-encapsulating effect these polysaccharides have and thus improving availability of starches, fat, amino acids and minerals.
3. Improving the solubilisation of insoluble compounds such as non-starch polysaccharides for more effective hindgut fermentation and energy utilisation
4. Complementation of the enzymes (for example, amylase, protease, lipase) produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate

Altogether, these EE may not directly confer a health benefit; however, the resulting oligosaccharides from enzymatic digestion may be beneficial as prebiotics for commensal microflora and high nutrient and energy digestibilty (Kiarie et al., 2013). Furthermore, Kiarie's review reported that feed enzymes and their actions might help alleviate animals under challenge model conditions while increasing feed intake and gain.

Phytase

Traditionally, producers have added costly phosphorus rich ingredients to the diet to meet the animal's requirements for this mineral. As phosphorus is an expensive nutrient in diets, and excess phosphorus excretion has environmental concerns, phytases (myo-inositol hexakisphospahte phosphohydrolases) has widely been adopted in swine and poultry nutrition. As the majority of phosphorus in plant feed stuff fed to swine and poultry is bound in mixed salts of phytic acids, this EE catalyzes the stepwise hydrolysis of phytic acid (myo-inositol hexahydrogen phosphate; phytate). The phytic acid is largely unavailable to swine and poultry without EE derived dephosphorylation. Therefore, the addition of phytase to diets has been shown to increase the availability of phosphorus, thus reducing the amount of added phosphorus needed in diet formulation. Phytases can be characterized as 3- or 6-phytases, depending on where they begin dephosphorylating the inositol ring of phytate. Optiphos™ and Natuphos™ are 3-phytases, while Phyzyme™, Ronozyme™ and Quantum™ are 6-phystases. Advances in phytase technologies are making these EE more thermal stable, resistant to endogenous proteolytic enzymes in the GIT and pH optimal. Typically, phytase can be added to the diet at 500 FTU/kg and this is believed to release about 0.10% phosphorus in corn based diets. However, this will vary depending on the phytase source and feed substrate.

Carbohydrases

With the increased inclusion rates of fiber and the reduction of starch in diets due to the use of co-products, specific carbohydrases that breakdown fiber are being adopted. In particular, xylanase, β-glucanase, β-mannanase, α-galactosidase and pectinase are of major interest to poultry and swine nutrition. These are all non-starch polysaccharides (NSP) or fiber degrading enzymes that aid in the partial hydrolysis of NSP, decrease digesta viscosity, rupturing of NSP-containing cell walls, and thus increasing the digestibility and availability of nutrients to monogastric diets. EE manufacturers design enzymes and enzyme cocktail blends to work on specific diets and to match the fiber type found in the diet. Collectively, these carbohydrases are most effective in poultry and young swine diets. Their use in grow-finisher pig diets has had limited success.

Exogenous enzymes and nutrient and energy digestibility

Digestibility of a feedstuff depends mostly on the general composition (proteins, lipids, and carbohydrates) of the feed. However, digestibility can be affected through the action of protease inhibitors within feed, such as glycinin and β-conglycinin in soybeans, or due to the presence of non-starch polysaccharides (NSP) that have limited digestibility in monogastric species due to the lack of endogenous GIT enzymes. Non-starch polysaccharides and other fiber components have minimal digestion in the small intestine and are passed on to the large intestine. The cecum and proximal colon are the primary sites for fiber fermentation in the large intestine. Hindgut fermentation produces volatile fatty acids (VFAs), which serve as intermediates for gluconeogenesis, lipogenesis, or the TCA cycle in intestinal cells and throughout the body. Butyrate has also been shown to positively influence cell proliferation and differentiation, and secretion of antimicrobial peptides, e.g., defensins (Sunkara et al., 2011). Additionally, non-starch polysaccharides can also serve as prebiotics, i.e., a food source for healthy commensal bacteria in the intestines (Liu et al., 2010). Non-starch polysaccharides such as chitin, chitosan, and β-glucans have been noted for their role in intestinal health. However, they appear to have high functionality in poultry verses swine nutrition.

Of particular interest to nutritionists is the contribution of nutrient and energy digestibility to changes in FE in poultry and swine. One of the single largest factors affecting FE is energy intake. The amount of energy intake and the energy utilization thereafter, contributes to changes in metabolizable energy (ME) that could potentially point to differences in digestion, absorption, and utilization of energy for maintenance and production. Digestible energy in swine corrects for energy that is not absorbed by the pig and is excreted in feces. While ME in poultry and swine goes even further as it adjusts DE for the loss of energy from voided urine and gases (Patience, 2012). Lastly, net energy (NE) adjusts ME for loss of energy due to heat production leaving energy available for maintenance and growth or production purposes (Figure 2). Exogenous enzymes supplementation in poultry and nursery pig diets has been shown to increase energy and nutrient availability. This increased energy availability allows for the removal of high cost dietary energy without negative consequences on performance. Numerous studies have also shown the EE supplementation improve energy utilization and performance in poultry (Fuente et al., 1998; Zanella et al., 1999; Rutherfurd et al., 2007).

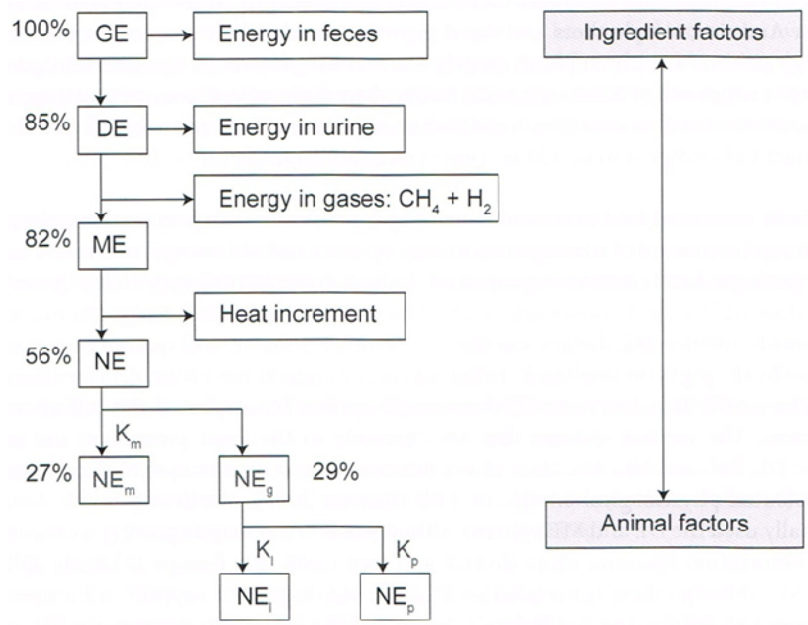

Figure 2. Traditional view of energy systems using a highly digestible diet typical of that used in North American in the late 20th century. Adapted from Ewan, (2001); Oresanya, (2005).

Prebiotics and probiotics

A comprehensive review by Roberfroid et al. (2010) reported prebiotics having mixed effects, but overall prebiotics do modulate the gut microbiome and promote growth of *Bifidobacteria* spp. (lactic acid producing bacteria), a beneficial species. Probiotics have been the next natural choice as growth promoters as they are microorganisms that are commensal to hosts, outcompete pathogenic organisms for nutrients, and/or produce antimicrobials thus suppressing them, stimulate the immune system, and have favorable interaction with the host microflora. However, a disadvantage to probiotic usage is the challenge of an organism colonizing the gut, requiring continual supplementation to maintain the effect. Most probiotic species chosen for experimentation are those belonging to the lactic acid producing bacteria. These organisms are regarded for their ability to produce lactic acid from numerous substrates, which can inhibit pathogenic bacteria. There are numerous studies working to characterize probiotics and their effects on health and growth performance. Wang et al. (2012) reported higher weight gain and feed efficiency in pigs fed a *Lactobacillus* probiotic when compared with control and antibiotic treated pigs. Many other studies have been summarized already (Cho et al., 2011) with a common consensus, that probiotics fed at a concentration of 10^6 CFU/g have been able to improve growth rates and feed efficiency in pigs of different ages. Probiotics may also increase digestibility of nutrients due to production of catabolic enzymes. Work by Giang et al. (2010) showed an increase in apparent ileal digestibility and apparent total tract digestibility in weaned pigs 14 days post-weaning using multiple probiotic strains. As the livestock industry will likely have to move away from antibiotics in the future, it will be necessary for probiotics to be developed to help cover the gap.

Organic acids

Organic acids such as formic, acetic, propionic, butyric, citric and lactic acids are being used to promote GIT health and integrity in poultry and swine nutrition. Many of these organic acids are also available as sodium, potassium or calcium salts which are oderless and easier to handle in feed manufacturing. Fed in blends or individually, these acids prevent high moisture feed from molding and have antibacterial properties that may improve animal performance and disease resistance (Ricke, 2003). Dietary humic acid, a naturally occurring decomposed organic constituent of soil and lignite, has been shown to increase ADG and FE in young pigs (Ji et al., 2006; Wang et al., 2008). In broilers, humic acid has been shown to decrease blood heterophil counts and the heterophil:lymphocyte ratio (Rath et al., 2006). While in rats, orally administration of potassium humate has been shown to decrease carrageenan-induced paw edema (Naude et al., 2010) and leonardite humate attenuates the magnitude of the delayed-type hypersensitivity response (vanRensburg et al., 2007). Mechanistically, dietary addition of humic acid appears to directly suppresses the activation of the inflammatory nuclear factor-kappa pathway by *E. coli* lipopolysaccharide (Gau et al., 2000) and have antimicrobial properties. Another dietary additive that regulates performance and inflammation is the short chain fatty acid, butyric acid. Recently, Lu et al. (2012) reported that butyrate supplementation to gestating sows and piglets enhanced post-weaning growth performance, which was suggested to be mediated by increased substrate oxidation. Additionally, dietary butyrate has been shown to reduce liver steatosis and inflammation in animals (Mattace Raso et al., 2013) and suppresses the inflammatory response in numerous cell types (Weber and Kerr, 2006; Ohira et al., 2013).

Betaine

Betaine is a small naturally occurring N, N, N-trimethylglycine derivative of the amino acid glycine that serves as an osmolyte and methyl donor to protect cells against osmotic and temperature stresses (Petronini et al., 1992; Petronini et al., 1993). Over the last few decades, betaine has been commonly used in swine and poultry diets to improve energy utilization and lean tissue accretion, with modest improvements in growth performance, feed intake and carcass yields under normal and stress conditions (Dunshea and Walton, 1995; Odle et al., 2000; Partridge and Greimann, 2002; Zulkifli et al., 2004; Dunshea et al., 2013; Sakomura et al., 2013). Dietary betaine supplementation may be beneficial to the intestinal epithelium (Kettunen et al., 2001) due to its osmolyte function, maintaining villi integrity and maintaining nutrient digestibility and absorption (Eklund et al., 2005).

Mycotoxin binders

Mycotoxins are toxic metabolites produced by fungi are natural contaminants of food stuffs (Table 1) which can cause harmful effects to both humans as well as animals. It is estimated that approximately 25% of world's agricultural commodities are contaminated with mycotoxins. Global losses of foodstuffs due to mycotoxins are in the range of 1000 million tonnes per year. USA and Canada incur approximately $5 billion in losses annually because of the impact of mycotoxins on the feed and livestock industries (FAO, 2001). Even though more than four hundred mycotoxins are known, six are considered important to the feed industry because of the propensity of the moulds to grow in the grains used for feed production. These include aflatoxin, deoxynivalenol (DON or vomitoxin), ochratoxin, zearalenone (Zen), fumonisin and T-2 toxin. Among the six mycotoxins, aflatoxin is the most toxic and most studied one. Ingestion of the mycotoxin affects health and reduces growth and production performance and consumption of the meat and milk contaminated with mycotoxins leads to adverse health effects on humans. Overall, mycotoxin contamination leads to severe economic losses to the producers and health risks to the consumers. It is imperative that contamination with mycotoxin needs to be prevented at each step of processing and storing of feed and absorption of mycotoxin from the intestine should be blocked to stop the harmful effects to animals and humans.

Table 1. Commonly found crop mycotoxins and their cautionary levels

Mycotoxin	Crop	Cautionary levels (ppm)
Aflatoxin	corn, peanuts, cottonseed	0.02
Ochratoxin	barley, oilseed crops	0.2
Deoxynivalenol	corn, wheat, barley, rye, oats	1.0
T-2	wheat, barley	0.5
Zearalenone	corn, wheat, barley, rye	0.5
Fumonisin	corn	5.0

Moisture, temperature and availability of oxygen are the major factors which influence the fungal infestation of foods and grains. Fungi themselves may not be toxic but the toxins produced by fungi known as mycotoxins are natural contaminants of food stuffs. Even though more than three hundred mycotoxins are known, six are considered important to the swine industry because of the propensity of the moulds to grow in the grains used for swine feed production. Fungal infestation of the grains and the subsequent toxin contamination could have three significant consequences. First, the fungal infestation reduces the nutrient content of the feed stuff. Second, ingestion of the toxins by poultry and pigs can negatively effects health and production performance.

Aflatoxin which is produced by Aspergillus sps contaminates mainly corn, peanuts and cotton seed. There are four types of aflatoxins, B1, B2, G1 and G2. They generally cause reduced feed intake and weight gain, liver damage, thymic atrophy and reduced immunity in pigs and poultry. Ochratoxin is produced by Aspergillus and penicillium fungal species. Ochratoxin A contaminates corn, barley, wheat, oats and oilseeds. It is nephrotoxic, teratogenic and hepatotoxic (Wood, 1992) and depresses growth rates and egg production in poultry.

Fusarium species produce two different types of toxins. The non-trichothecene toxins including deoxynivalenol (DON), T-2 toxin and fumonisin, and the mycosestrogens like zearalenone (Zen) and zearalenol toxins. Deoxynivalenol (DON) is formed by several species of the fungal genus Fusarium. Wheat and maize are the two major feed stuffs which get contaminated by DON frequently. The primary effect of the presence of DON in the feed is reduced feed intake which directly correlates with reduced weight gain and it inhibits protein synthesis in swine (Dänicke et al., 2006). Interestingly, poultry are reasonably resistant to DON. Fumonisin is produced by Fusarium *verticillioides* and F. *proliferatum*. Both these fungi mainly contaminate maize. F. *verticilloides* produces Fumonisin B_1 (FB_1) which is the most prevalent mycotoxin. In general, fumonisins alter sphingolipid biosynthesis, induce hepatotoxicity and elevate serum cholesterol concentration. Zearalenone is also produced by Fusarium sps. and is commonly found in oats, barley, wheat and sorghum. Because of its estrogenic activity, it causes hyper estrogenism and affects the reproductive health leading to infertility in swine (Diekman and Green, 1992; Wood, 1992). However, it poses a relatively low risk to poultry production. Fumonisin toxicity in swine causes injury to pulmonary, hepatic, cardiovascular and immune systems. Further, sphingolipid metabolism is altered and growth rate and carcass composition is affected. High dose exposure to fumonisin toxin particularly to FB1 causes a species specific pulmonary edema, abortion and cardiovascular changes. It alters intestinal epithelial cell composition and blocks its proliferation (Haschek et al., 2001; Bouhet et al., 2004). DON consumption in swine causes growth depression, loss of appetite and injury to the gastrointestinal tract (Dillenburger et al., 2001) and DON causes reduced protein synthesis in kindneys, spleen and intestine of pigs (Dänicke et al., 2006). Zearalenone because of its estrogenic effects causes tumefaction of the vulva, prolapses of the vagina and rectum and enlargement of the mammary glands leading to reproductive failure. The prepubertal gilt is most sensitive to Zen toxicity. In the case of cycling animals, zearalenone causes conception failure, pseudo pregnancy and abortion (Rainey et al., 1990). Consumption of T-2 toxin leads to reduction in performance in pigs and poultry (Rafai et al., 1995; Rafai et al., 2000). Apart from the individual effects when two or more mycotoxins are ingested together it leads to potentiation of their effect by synergestic action which could severely affect the health and performance of the pigs (Harvey et al., 1991).

Aflatoxin (B1, B2, G1 and G2) causes reduced feed intake and weight gain in poultry and swine. Contaminated feed reduces egg production and ducks are relatively more susceptible than broilers. It affects the immune system rendering the animals more susceptible for other diseases. In swine, it can be transferred to the fetus during pregnancy or through milk to the suckling pigs (Coffey et al., 1990; Silvotti et al., 1997). Ochratoxin depresses growth rates and causes and egg production, causes abnormal feathering and mouth lesions. Ochratoxin targets the kidney, other organs like liver, intestines, spleen, lymphoid tissue and leukocytes at higher doses. At higher doses it causes reduced growth performance.

The recommended dietary mycotoxin concentrations for swine and poultry are in the ppm or ppb (Table 2). However, if a producer is feeding contaminated grains, then the absorption of mycotoxins from the GIT can be prevented by the use of dietary binders (Kabak et al., 2006; Thieu et al., 2008). Mycotoxin binders are non-nutritional adsorbents which reduces the bio-availability of mycotoxins. Common binders include cationic materials and clays such as hydrated sodium calcium aluminosilicate (HSCAS), zeolites, bentonites, koalinite, and diatomaceous earth. Additionally, activated charcoal, humic acid, yeast cell walls, peptidoglycans and polyvinylpyrrolidone compounds can be utilized to attrach and bind luminal mycotoxins. HSCAS appear to be very effective adsorbent of aflatoxin. However, bentonites are commonly used for binding most mycotoxins.

Table 2. Recommended maximum dietary mycotoxin concentration

	Deoxynivalenol (ppm)	Zearalenone (ppm)	Aflatoxin (ppb)
Pig breeding herd	1.0	2.0	100
Young-grower pig	1.0	2.0	20
Finishing Pig	1.0	3.0	200
Poultry	5.0	0.5	1.0-2.5

Conclusions

Improving feed efficiency and growth rates in poultry and swine production is a major gaol for competitive and sustainable production. However, as producers move towards more alternative cost-effective feed ingredients such as cereal co-products from biofuel and milling industries, monogastric digestive efficiencies may decline as the complex carbohydrate and anti-nutritional components of the diet increases. As such, improving nutrient and energy digestibility through the use of EE and other dietary additives is a way producers can overcome this dilemma. Reductions in intestinal health and integrity also reduce production efficiencies. Therefore the use of pre- and probiotics, mycotoxin binders and organic acids provide exogenous dietary strategies to maximize health and productivity. However, the collective efficacy of these various exogenous dietary strategies discussed generally appear to work better in poultry verses swine production.

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8
El futuro de la utilización de aminoácidos industriales en la producción de aves

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Summary
Feed formulation based on the ideal protein concept optimizes the amino acids (protein) utilization efficiency, and decreases nitrogen (N) excretion. As dietary protein is reduced, a deficiency of some amino acids (AAs) occurs, and therefore, supplementation with industrial amino acids becomes necessary to achieve the requirements for optimal broiler growth performance. Here, we highlight the importance of industrial amino acid supplementation in broiler diets, and their effect on production parameters. It is concluded that updating the ideal protein is necessary to keep up with changes in performance, as well as with common health challenges. The order of limiting AAs in broiler diets changes with age and type of diet. The recommended essential N to total N ratio (Ne:Nt) is 50% or less. More research should be developed to better understand the relationship between essential and nonessential AAs in broiler diets with reduced protein levels. The addition of industrial AAs to broiler diets can be increased significantly, depending on economic viability.

Resumen
La formulación de raciones con base en el concepto de proteína ideal optimiza la eficiencia de la utilización de los aminoácidos (proteína) e disminuye la excreción de nitrógeno (N). Conforme es reducida la proteína dietética, algunos aminoácidos (AAs) esenciales pasan a ser deficientes, siendo necesaria la suplementación de aminoácidos industriales hasta alcanzar el requerimiento para óptimo desempeño de las aves. En este trabajo es resaltada la importancia de la suplementación de aminoácidos industriales en las raciones y su influencia sobre los parámetros productivos en pollos de engorde. Así, podemos concluir que la actualización y la utilización de la proteína ideal es necesaria para acompañar los cambios del mercado consumidos y el desafío sanitario de las aves. El orden de los aminoácidos limitantes en las dietas para pollos de engorde varía con la edad y el tipo de dieta. La relación de N esencial : N total recomendada es 50% o menor. Nuevas investigaciones deben ser realizadas con pollos de engorde para mejorar el conocimiento de la relación entre AAs esenciales y no esenciales en dietas con reducción proteica. El nivel de AAs industriales en las dietas de pollos de engorde puede ser aumentado significativamente dependiendo de la viabilidad económica.

Introducción

La avicultura es una actividad que se ha desarrollado especialmente en las últimas décadas debido principalmente a la acción conjunta entre la genética, nutrición, sanidad y manejo. El avance de la nutrición está asociado al conocimiento del valor nutricional de los ingredientes y los requerimientos nutricionales de los animales en las diferentes fases productivas. La alimentación de las aves representa aproximadamente 70% del costo total de producción, siendo necesario el continuo desarrollo de investigaciones relacionadas al establecimiento de niveles nutricionales óptimos que promuevan el máximo desempeño productivo del animal a un menor costo.

En la actualidad, aparte de los aminoácidos normalmente adicionados en las raciones (Met, Lis y Thr), existen otros aminoácidos industriales que de acuerdo al precio de los ingredientes pueden ser utilizados en las raciones avícolas, como Val, Arg, Trp, Gli, Gln y Glu; que han estimulado la realización de investigaciones para actualizar los niveles nutricionales recomendados para optimo desempeño y mejor rendimiento de cortes nobles.

En los últimos años, la formulación de dietas, con base en el concepto de proteína ideal, ha adquirido mayor importancia, pues determina el balance exacto de los aminoácidos sin deficiencias o excesos, reduciendo así el nivel de proteína cruda en la dieta. Conforme es reducida la proteína dietética, algunos aminoácidos pasan a ser deficientes siendo necesaria la suplementación de aminoácidos industriales hasta alcanzar el requerimiento para optimo desempeño de las aves (Wu, 2013).

El objetivo de este trabajo es resaltar la importancia de la utilización de aminoácidos industriales en las raciones para pollos de engorde, los factores que pueden influenciar el nivel recomendado de los aminoácidos y el efecto de la relación N esencial : N total (Ne:Nt) sobre el desempeño de las aves.

Aminoácidos

Los aminoácidos (AAs) son las unidades estructurales de las proteínas. En el organismo existen más de 100.000 tipos de proteínas que son constituidas por la combinación de apenas 20 AAs los cuales son llamados proteicos (Ala, Arg, Asp, Asn, Cis, Phe, Gli, Glu, Gln, His, Ile, Leu, Lis, Met, Pro, Ser, Tir, Thr, Trp y Val) (Wu, 2013).

Algunos aminoácidos proteicos no son sintetizados por el animal para mantener adecuado balance de N, y deben ser proporcionados en la dieta, estos son llamados AAs esenciales. Los AAs que pueden ser sintetizados por el animal para mantener el balance de N son llamados no esenciales. Sin embargo, en ciertas condiciones de desafío sanitario o estrés algunos AAs no esenciales se vuelven esenciales para mantener el equilibrio metabólico del animal, estos son llamados AAs condicionalmente esenciales o funcionales (Blachier et al., 2013).

Proteína Ideal

En la actualidad es posible formular dietas para pollos de engorde con bajo nivel proteico y con la suplementación de varios aminoácidos industriales. Para usar este procedimiento, es necesario utilizar el concepto de proteína ideal para atender con precisión los requerimientos nutricionales de los aminoácidos y obtener óptimo desempeño zootécnico y económico.
Existen varios trabajos en la literatura mostrando valores de proteína ideal para aves utilizando experimentos dosis respuesta. Las recomendaciones de Rostagno et al (2011), publicadas en las Tablas Brasileñas de 2011, sobre proteína ideal fueran determinadas utilizando resultados de experimentos de dosis respuesta los cuales determinan la relación ideal con base en la respuesta en el desempeño de los animales alimentados con dietas conteniendo niveles crecientes del AA en estudio.
Sin embargo en pesquisas con cerdos determinaron la mejor relación entre los AAs esenciales partiendo del principio de que los cambios en la retención de N por la remoción de un AA esencial puede ser usado para formular una ración testigo con AAs dietéticos donde todos sean igualmente limitantes.
Otra metodología usada para determinar la mejor relación entre los AAs esenciales es la retención de N utilizada con cerdos por Wang e Fuller (1989). Recientemente fueron publicados dos trabajos, con pollos de engorde, donde fue aplicado el método de balance de N también llamado de método Gottingen (Dorigan et al, 2013 y Wecke y Liebert, 2013). En la Tabla 1 son mostradas las relaciones AA digestible:Lis digestible (dig) recomendadas por estos autores y por Rostagno et al 2011). Es posible concluir que salvo pequeñas discrepancias los niveles recomendados en las tres publicaciones son similares, especialmente cuando son considerados los principales AAs: Met+Cis, Thr, Trp, Arg y Val.

Tabla 1. Valores de proteína ideal recomendada por diferentes autores. Lis Dig = 100

AA Digestible	Tab. Bras. 2011	Wecke & Liebert 2013	Dorigan et al., 2013
Fase	Ini / Cre	Ini / Cre	Ini / Cre
Met + Cis, %	72 / 73	--	73 / 71
Treonina, %	65 / 65	60 / 62	66 / 64
Triptofano, %	17 / 18	19 / 17	17 / 17
Arginina, %	108 / 108	105 / 105	108 / 105
Valina, %	77 / 78	63 / 79	77 / 76
Isoleucina, %	67 / 68	55 / 65	67 / 67
Leucina, %	107 / 108	--	107 / 107
Glic + Ser, %	147 / 134	--	140 / 135
Histidina, %	37 / 37	--	36 / 35
Fenil + Tiros, %	115 / 115	--	115 / 114

En una revisión hecha por Prymot et al. (2010) y utilizando los resultados de 21 publicaciones que evaluaron las relaciones Val:Lis dig (14 referencias) y Ile:Lis digestible (7 referencias), permitieron concluir que la mejor relación Val:Lis dig para ganancia de peso y eficiencia alimentar en pollos de engorde fue de 80% y para Ile:Lis dig fue de 67%.

Factores que pueden Alterar la Relación AA:Lis Dig

- Modelo Usado para Estimar la Relación y el Parámetro Evaluado

Existen varios factores que pueden influenciar la respuesta de los animales al nivel del AA en la dieta y alterar la relación AA:Lis dig, entre ellos se puede destacar el ambiente, clima y genética. Otro factor que puede influenciar la relación recomendada es el modelo estadístico utilizado para procesar los datos experimentales. El investigador tiene que conocer los diferentes modelos, saber acerca de sus ventajas y las limitaciones optando por el (los) que mejor se ajuste a los datos obtenidos (Sakomura e Rostagno, 2007).

Euclydes e Rostagno (2001) compararon diferentes metodologías Cuadrática, 95% Cuadrática, Linear Response Plateau (LRP o Broken Line), Cuadrática con Plateau y Exponencial utilizando los datos medios de 5 experimentos realizados en la Universidad Federal de Viçosa, en que se estimaron los requerimientos de Lis dig para pollos de engorde. Para conversión alimenticia, los autores observaron una diferencia de 8,7% entre la menor (LRP) y la mayor (Cuadrática) estimativa.

Según Leclerq (1998) algunas respuestas biológicas son próximas al modelo LRP, pero otras son claramente curvilíneas, por tanto, para muchos AAs el modelo LRP (Broken Line) subestima el requerimiento cuando es comparado a otros modelos curvilíneos y económicos.

Un procedimiento similar fue realizado por Rostagno et al. (2014) con el objetivo de determinar la mejor relación Val:Lis dig para pollos de engorde de 22 a 42 días, usando los resultados medios de 11 experimentos, publicados entre 2004 y 2013. Los autores utilizaron diferentes modelos matemáticos y concluyeron que, con base en los datos de ganancia de peso y eficiencia alimenticia, el valor promedio para Val:Lis dig fue 79,7%. Sin embargo, para eficiencia alimenticia la estimativa varió entre 83,5 y 88,4% para el modelo LRP y Cuadrática respectivamente. Para ganancia de peso la diferencia entre el modelo LRP y el Cuadrático fue 11,4% (Tabla 2, Figura 1 y 2). Otro factor a llevar en consideración en el momento de interpretar los datos es el parámetro evaluado, que puede ser de desempeño o rendimiento de pechuga.

Tabla 2. Relaciones Val:Lis digestible (%) obtenidas aplicando diferentes modelos estadísticos para pollos de engorde de 22 a 42 días de edad (Rostagno et al. 2014)

Val:Lis dig (%)	Cuadrática	95% Cuadrática	LRP	Cuadrática + Plateau	Promedio	
		11 Experimentos				
Ganancia Peso	81,3	77,2	72,0	75,9	76,6	79,7
Eficiencia Alimentar	88,4	83,9	83,5	75,1	82,7	

LRP: Linear Response Plateau o Broken Line

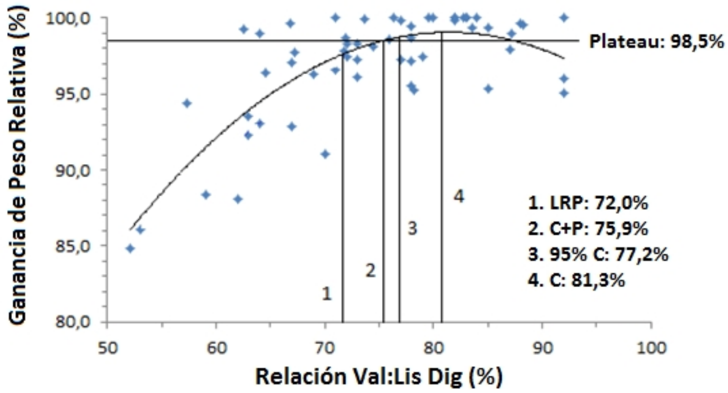

Figura 1. Relación Val:Lis dig (%) para ganancia de peso relativa (%) en pollos de engorde de 22 a 42 días evaluados por diferentes modelos estadísticos (C, LRP, C+P y 95% C)

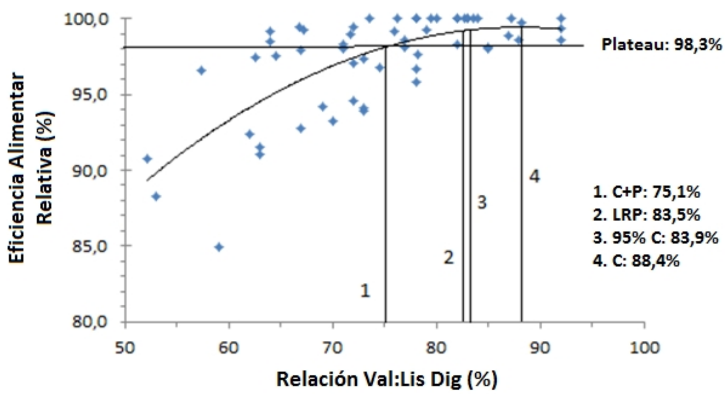

Figura 2. Relación Val:Lis dig (%) para eficiencia alimenticia relativa (%) en pollos de engorde de 22 a 42 días por diferentes modelos estadísticos (C, LRP, C+P y 95% C).

Los datos mostrados en la Tabla 2 permiten concluir que para el parámetro eficiencia alimenticia la relación Val:Lis dig recomendada (82,7%) es mayor que para ganancia de peso (76,6%). La literatura muestra resultados contradictorios cuando son comparadas las recomendaciones de AAs para optima conversión alimenticia versus rendimiento de pechuga en pollos de engorde, sin embargo existe consenso de que el nivel del AA para una mejor conversión y rendimiento de pechuga es mayor que para ganancia de peso (Rostagno et al, 2007).

La Met es el primer AA limitante para pollos de engorde alimentados con raciones a base de maíz y harina de soja, siendo destacada su participación en la síntesis de proteína y precursora de Cis, así como donadora de grupos metil (Wu, 2013).

En este contexto Pessoa (2013) determino la mejor respuesta de las aves al suministro de diferentes relaciones Met+Cis/Lis dig en pollos de engorde. El autor observo que el aumento en la relación Met+Cis/Lis dig. en las fases de 1-10; 10-21 y 21-42 días, 77-78-73 y 77-78-78 en pollos de engorde de 1 a 42 días mejoro el peso del file de la pechuga en +2,8 e +4,5%, respectivamente.

No fue observada diferencia estadística ($P<0,999$) en los parámetros de desempeño de las aves, lo que sugiere que el requerimiento para optimo desempeño (ganancia y conversión) es menor que para rendimiento de pechuga (tabla 3).

Tabla 3. Efecto de la Relación Met+Cis dig. : Lis. dig. Sobre el Desempeño y Rendimiento de Pechuga de Pollos de Engorde (1 – 42 días) (Pessoa, 2013)

Relación Met+Cis/Lis (%)	Desempeño		Filé de Pechuga	
	Ganancia, g	Conversión Alimenticia	Peso, g	Rendimiento, %
72-72-73*	2812	1,703	657,8 b	23,16
72-78-73	2801	1,704	657,7 b	22,98
77-72-73	2795	1,706	656,2 b	22,78
77-78-73	2814	1,700	675,8 ab	23,54
77-78-78	2856	1,689	687,1 a	23,61
Anova-P Valor	0,999	0,999	0,028	0,088
CV (%)	3,04	1,62	3,78	3,27

*Relación Met+Cis / Lis Dig para las fases de 1-10; 10-21 y 21 – 42 días

Interacción entre Valina, Isoleucina y Leucina

Con estructura química semejante los AAs de cadena ramificada (AACR) comparten enzimas comunes para los procesos de catabolismo en el hígado y musculo (transaminación y descarboxilación oxidativa). El exceso de Leu en las dietas de pollos es catabolizado aumentando la actividad de la aminotransferasa y la desidrogenasa, que también aumentan el catabolismo de la Val e Ile, lo que puede causar deficiencia y reducción en el desempeño. Por tanto, el exceso de Leu en las dietas puede resultar en deficiencia de AAs (Ile y Val), principalmente cuando son utilizados niveles dietéticos en el requerimiento (sin exceso). Se puede concluir que niveles altos de Leu, contribuyen significativamente para el aumento del catabolismo de los demás AACR por la activación del sistema de catabolismo (figura 3).

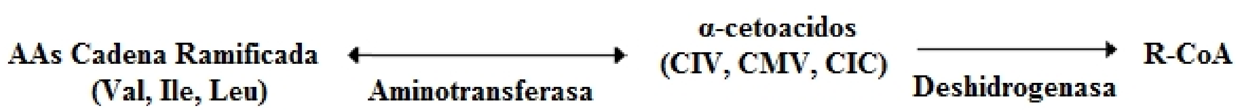

Figura 3. Metabolismo de los AAs de cadena ramificada (Adaptado de Brosnan et al., 2006)

Es posible que el aumento (mayor que el requerimiento) en los niveles de Val o de Ile en dietas con niveles excesivos de Leu resulten en mejorías en los índices de desempeño. En este contexto, Maia (2013) evaluó el efecto de la utilización de diferentes relaciones dieteticas de Ile:Lis, Val:Lis y Leu:Lis sobre el desempeño en pollos de engorde en el periodo de 14 a 23 días (Tabla 4).

El aumento de la relación Leu:Lis dig de 107 (requerimiento) para 150 perjudicó la ganancia de peso (628,7 vs 612,9 g) y la conversión alimenticia (1,436 vs 1,467) de los pollos de engorde.

Debe resaltarse que el nivel 150 de Leu:Lis dig es fácilmente alcanzado en dietas a base de maíz y harina de soja. El aumento en las relaciones Val:Lis y Ile:Lis no afecto el desempeño de las aves. A pesar de no presentar efecto significativo (P>0,05) para ganancia de peso y conversión, fue verificada que la relación Val:Lis dig de 90% en dietas con exceso de Leu aumentó la ganancia de peso en 3% (608,9 vs 626.5 g). Se puede concluir que una mayor relación Val:Lis en dietas con exceso de Leu mantiene desempeño adecuado en pollos de engorde (Tabla 4).

Tabla 4. Efecto de la relación Val:Lis, Ile:Lis y Leu:Lis sobre la ganancia de peso (g) de pollos de engorde machos de 14 a 23 días de edad (Maia, 2013)

	Val 77		**Val 90**		**Promedio Ile**
	Leu 107	**Leu 150**	**Leu 107**	**Leu 150**	
Ile 67	630,4	608,9	627,6	626,5	623.3
Ile 80	625,4	613,8	631,8	602,3	618,3
Promedio Leu	628,7 a	612,9 b	629,7	614,4	CV (%)
Promedio Val	619,6		622,1		3,60

(a,b) – Valores promedios en la misma línea seguidos por letras diferentes, difieren entre sí por el test F (P < 0,0065)

Aminoácidos Limitantes en las Dietas para Pollos de Engorde

En la nutrición proteica es usado el concepto de “AA Limitante” el cual puede ser definido como el AA proporcionalmente más deficiente en relación al requerimiento nutricional del animal para mantenimiento, crecimiento y salud. El aminoácido limitante es normalmente un AA esencial que está presente en la dieta en poca cantidad en comparación al requerimiento diario del animal (Wu, 2013).

Varios experimentos fueron realizados por Waguespack et al. (2009) con el objetivo de determinar que AAs eran limitantes en dietas a base de maíz y harina de soja. Los autores concluirán que para pollitos de 1 a 18 días de edad el orden fue: 1. Met, 2. Lis, 3. Thr, 4. Gli, 5. Val y/o Arg. Utilizando los datos de composición y requerimientos nutricionales para pollos de engorde (7 a 21 días) de las Tablas Brasileñas (Rostagno et al, 2011) fueron realizadas simulaciones y estimados los AAs limitantes de una dieta a base de maíz y harina de soja: 1. Met, 2. Lis, 3. Thr, 4. Val, 5. Gli +Ser, 6. Arg y/o Ile. Aplicando el mismo procedimiento para las fases de 21 a 33 y de 33 a 42 días de edad para pollos de engorde el orden de los AAs limitantes fue: 1, Met, 2. Lis, 3. Thr, 4. Val, 5. Ile y/o Arg.

Según Wu (2013) en las dietas a base de maiz y harina de soja para pollos de engorde en la fase de crecimiento, la Arg puede ser el quinto AA limitante, despues de la Met+Cis, Lis, Thr y Val.

Cuando son formuladas dietas a base de maiz, harina de soja, harina de carne y huesos 44% PC (cantidad fija 4%) y harina de plumas 75% PC (cantidad fija de 2%) el orden de los aminoacidos limitantes para pollos en el periodo de 21 a 33 días de edad es: 1. Met, 2. Lis, 3. Thr, 4. Trp y/o Ile, 5. Val.

En una revisión hecha por Prymot et al. (2010) con el objetivo de dilucidar cual AA es limitante después de Met, Lis y Thr en dietas para pollos de engorde, los autores concluyeran que la Val era el cuarto AA limitante, en dietas formuladas con ingredientes de origen vegetal, sin embargo cuando son utilizados alimentos de origen animal la Ile pasa a ser el cuarto AA limitante.

Relación Nitrógeno Esencial: Nitrógeno Total

La proteína cruda fue utilizada por muchos años en la formulación de dietas para pollos de engorde. Su aplicación promueve el exceso de AAs dietéticos que deberán ser catalizados y excretados al ambiente resultando en grandes pérdidas, tanto energéticas como económicas, principalmente cuando hay desequilibrio de AAs. Actualmente es recomendado formular dietas con la proporción ideal de AAs a modo de que no existan ni exceso ni deficiencia de AAs.

Las aves no tienen requerimiento nutricional de proteína cruda, más si de cada uno de los AAs esenciales (AAe) que componen la proteína y una cantidad suficiente de N para biosíntesis de AA no esencial (AAne) (Costa e Goulart, 2010).

Diferentes trabajos apuntan a la reducción proteica con suplementación de AAs industriales en la dieta, como herramienta para mantener el óptimo desempeño y reducir la excreción de N al ambiente. La reducción proteica también se muestra ventajosa porque disminuye el incremento calórico de la dieta, lo que es recomendado en condiciones de estrés térmico.

La gran disparidad para obtener una estimativa de la óptima relación AAe:AAne es atribuida a las diferentes formas de expresar esas relaciones entre los dos grupos de AAs y la clasificación diferente entre la esencialidad o no de algunos AAs (Heger, 2003). Existen varias formas de expresar la relación entre AAe:AAne, pudiendo el AAe ser relacionado con la cantidad total de AAs (AAt), AA no esencial (AAne), N total (Nt) o proteína total.

La principal función del AAne es proporcionar N no especifico, la proporción de los grupos de los AAs debe ser expresada con base en el N, de la misma forma como Ne:Nne y Ne:Nt. Esta última relación parece ser la más aceptable una vez que la relación Ne:Nne tiende al infinito cuando la concentración de AAne es próxima a cero. El N total presente en una dieta es dada como la suma entre Ne + Nne.

La optima relación Ne:Nt para crecimiento o deposición de proteína estimada para un nivel constante de Nt, usando la misma clasificación de los AAs, no difiere substancialmente entre especies y varía entre 43 y 55% (Heger, 2003).

Estudios han demostrado que la suplementación de AAne en las dietas también es usada como una manera de mejorar el desempeño, y prevenir el uso de AA esencial para la síntesis de AAne (Costa e Goulart, 2010).

Existen investigaciones mostrando la reducción en el desempeño de las aves alimentadas con dietas de bajo nivel de proteína lo que según Afta et al. (2006) puede estar asociado a varios factores, entre ellos: a) Variaciones dietéticas en los niveles de potasio y el balance electrolítico, b) Insuficiente N para síntesis de AAne, c) Reducción en el consumo de ración de las aves, d) Alteración de la relación AAe:AAne, e) Deficiencia de algunos AAs esenciales, f) Diferencias en la eficiencia de utilización de los AAs de la proteína intacta vs AAs cristalinos, g) Aumento en la relación Energía Neta: Energía Metabolizable.

En dietas con niveles altos o adecuados de proteína, los AAne pueden ser sintetizados a partir de los AAs en exceso presentes en la dieta. Sin embargo con la reducción de

tres o cuatro puntos porcentuales del nivel proteico, la síntesis y disponibilidad de AAne y N pasa a ser limitante lo que puede resultar en bajo desempeño (Dean et al.,2006; Payne, 2007). Por tanto, el uso de niveles adecuados de AAne con el propósito de encontrar el equilibrio correcto entre Ne:Nt en las dietas de pollos de engorde resulta en una mejora en la eficiencia de utilización de la proteína dietética.
Heger et al (1998), realizaron un ensayo con objetivo de evaluar el efecto de la relación Ne:Nt (25 a 86%) sobre la retención de N con cerdos en la fase de crecimiento. Los autores verificaron que la retención de N se mantuvo constante hasta alcanzar la relación de 48% disminuyendo linealmente con valores superiores. Estos resultados sugieren que con valores de Ne:Nt arriba de 50 los AAes son parcialmente degradados y usados para la síntesis de AAnes.

Uso de Aminoácidos Industriales en las Dietas para Pollos de Engorde

En los últimos años niveles reducidos de proteína cruda han sido investigadas en pollos de engorde, sin embargo los resultados obtenidos son controversiales. En una revisión hecha por Aftab et al. (2006) fue concluido que es posible reducir la proteína de una dieta para aves en 10% sin afectar estadísticamente el desempeño. Los autores recopilaron datos de 16 experimentos con pollos de engorde de diferentes edades, publicados entre los años 1991 y 2006, donde la proteína cruda media de la dieta testigo fue de 21,4% versus 18,8% de la dieta con proteína reducida + AAs, la ganancia de peso y la eficiencia alimenticia relativa de las aves (Control = 1) fue de 0,99 y 0,98, respectivamente.
El uso de AAs industriales, aplicando el concepto de proteína ideal, permitió la utilización de un perfil adecuado de AAs en dietas para pollos de engorde (Baker et al. 2002). Diversos ensayos fueran realizados por Baker (2003) para determinar las relaciones AAs:Lis. Para esto, los autores utilizaron una dieta testigo a base de maíz y harina de soja y una dieta semi-purificada con 14,6% de AAs cristalinos y 18,34% de harina de gluten de maíz, como única fuente de proteína intacta. El desempeño de pollos de engorde alimentados con la dieta semi-purificada en la fase de 8 a 20 días fue estadísticamente similar a las aves que recibieran la dieta testigo.
Waguespack et al. (2009) estudiaran el efecto de dietas con reducción proteica y suplementación con AAs cristalinos sobre el desempeño de pollos de engorde de 0 a 18 días. Los autores verificaron que aves alimentadas con dietas a base de maíz y harina de soja conteniendo 19% de PC y suplementadas con Met, Lis, Thr, Gli, Ile, Arg y Val (total de 25,84 kg/ton.) presentaron una ganancia de peso y eficiencia alimenticia semejante a las aves alimentadas con dietas conteniendo 22% de proteína (tabla 5). Estos resultados sugieren que la suplementación de varios AAs en dietas con reducción proteica es una práctica indicada en la formulación de raciones.
El orden de limitación de AAs en dietas para pollos de engorde de 8 a 22 días de edad fue también estudiada por Han et al. (1992), donde fue verificado que aparte de la suplementación con AAes en dietas con 19% PC, existe la necesidad de suplementar con una fuente de Nne a través de la utilización de Glu. La suplementación con Glu aumento la eficiencia alimenticia y redujo la grasa corporal (tabla 6). Se debe resaltar que el aumento en la relación carne:grasa fue mayor para las aves que recibieran la suplementación con Glu.

Tabla 5. Ganancia de peso diaria (GPD) y eficiencia alimenticia (EA) de pollos de engorde de 0 a 18 días alimentados con diferentes relaciones Ne:Nt. (Experimento 4 y 5, Waguespack 2009)

Tratamientos	Ne:Nt (PB) (%)	AAs cristalinos (kg/ton.)	GPD (g)	EA
		Experimento 4		
Proteína Normal	47,43 (22,00)	5,03	29,96ª	0,788ª
Baja Prot + AAs	54,29 (19,00)	25,84	28,72ª	0,790ª
		Experimento 5		
Proteína Normal	47,44 (22,5)	5,00	28,75ª	0,770ª
Baja Prot + AAs	55,05 (19,5)	25,84	26,89a	0,775ª

a, b (P<0,05)

De forma general, los resultados indican que la cantidad de Nne y de algunos AAes, son factores limitantes en dietas con reducción proteica. Similarmente, Berres et al, (2010) observaron un adecuado desempeño y rendimiento de pechuga en pollos de engorde alimentados con dietas bajas en proteína y suplementadas con Met, Lis, Thr, Val, Ile, Gli y Glu. Los autores sugieren que en este tipo de dieta, la suplementación de N, principalmente de Glu, es necesaria para sintetizar AAne.

Tabla 6. Ganancia de peso, eficiencia alimenticia y grasa corporal de pollos de engorde de 8 a 22 días suplementados o no con AAs cristalinos y/o ácido glutámico (Han et al.,1992).

Tratamiento	AAs Cristalinos (kg/ton.)	Ganancia de Peso (g)	Eficiencia Alimenticia	Grasa Corporal (%)
23% PC	2,00	290 a	675 ab	8,9 a
19% PC	2,00	271b	606 c	11,5 c
19% PC+AAs	11,80	291 a	660 b	10,3 b
19% PC+AAs+ Glu	16,42	298 a	686 a	9,0 a

a, b, c (P<0,05).

Similarmente, Barboza et al. (2010 a,b), realizaron 4 experimentos por con el objetivo de evaluar diferentes niveles de proteína y relaciones de Ne:Nt para pollos de engorde de 8 a 21 y de 22 a 40 días. Las dietas experimentales, a base de maíz y harina de soja, fueron formuladas isolisina siendo solamente adicionados AAs cristalinos esenciales para atender los requerimientos (proteína ideal) recomendadas por Rostagno et al (2011). Los resultados de desempeño son presentados en la Tabla 7, y permiten concluir que: para pollos de engorde en las fases inicial y crecimiento la proteína puede ser reducida para 21% y 18% respectivamente. Cuando es llevada en consideración la relación Ne:Nt, los mejores resultados de desempeño en las dos fases evaluadas fueron obtenidas con valores entre 45 y 50% de Ne:Nt. Se debe resaltar, que en los cuatro experimentos realizados por los autores, no fueron encontradas diferencias significativas en el peso y el rendimiento de la pechuga de las aves que recibieron las dietas con los diferentes niveles proteicos.

De la misma forma, Maia (2014) evaluó usando pollos de engorde de 8 a 21 días dos relaciones de Ne:Nt (47,66 vs 51,48%). Las dietas fueron formuladas a base de maíz, harina de soja y gluten de maíz + AAs, utilizando el Glu como fuente de Nne para alcanzar la relación de 47,66% de Ne:Nt. El desempeño de las aves alimentadas con la dieta de 20,5% de proteína (51,48% Ne:Nt) fue inferior a los de las aves que recibieron la dieta control con 22% de proteína (47,66% Ne:Nt), sin embargo, la ganancia de peso y la conversión alimenticia del tratamiento con 20,5% de proteína + Glu y con una relación Ne:Nt de 47,66% fue similar al tratamiento testigo y superior al tratamiento con una relación de 51,48% sin la adición de Glu (tabla 7).

Tabla 7. Ganancia de peso (GP) y conversión alimenticia (CA) de pollos de engorde de 8 a 21 días y de 28 a 40 días alimentados con dietas conteniendo diferentes niveles de proteína y relaciones Ne:Nt (Barboza et al., 2010 a,b)[1]

8-21 días (Media de 2 Experimentos)				**28-40 días (Media de 2 Experimentos)**			
PC, % (Ne:Nt)	**AAs cristalinos (kg/ton.)**	**GP (g)**	**CA**	**PC, % (Ne:Nt)**	**AAs cristalinos (kg/ton.)**	**GP (g)**	**CA**
23 (45,77)	2,06	614,7 a	1,41 a	20 (44,8)	3,54	971,7	1,98
22 (47,61)	3,97	611,4 a	1,40 a	19 (46,8)	6,15	973,9	1,97
21 (49,53)	6,32	601,5 a	1,43 a	18 (48,7)	9,64	954,6	1,99
20 (52,78)	9,10	587,2 b	1,46 b	17 (50,5)	13,79	940,5	2,01
19 (53,42)	12,57	589,5 b	1,47 b	16 (52,4)	17,95	937,8	2,05
CV(%)		**3,39**	**2,63**			**4,38**	**3,01**
Lineal		**0,01**	**0,01**			**0,05**	**0,01**

[1] Contrastes, medias con letras diferentes en la misma columna son significativamente diferentes del tratamiento con 23% PC por el test de Dunnett (P<0,05)

Tabla 8. Ganancia de peso (GP), Consumo de ración (CR) y conversión alimenticia (CA) de pollos de engorde de 8 a 21 días alimentados con diferentes relaciones Ne:Nt (%) (Maia, 2013)

Ne:Nt (PC) %	**AAs cristalinos (kg/ton)**	**GP (g)**	**CR (g)**	**CA**
47,66 (22%)	4,10	824a	1124	1,365ª
51,48 (20,5%)	10,39	775b	1115	1,444b
47,66 (20,5+Ac. Glu)	13,19	812a	1097	1,351ª
CV(%)		4,80	4,16	5,05
P-Valor		0,022	0,425	0,013

Medias seguidas por letras diferentes, difieren por el test SNK (P<0,05).

Conclusiones

La actualización de la Proteína Ideal es necesaria para acompañar los cambios del mercado consumidor y el desafío sanitario de las aves.
El orden de los AAs limitantes en las dietas de pollos de engorde varía con la edad y el tipo de dieta.
La relación Ne:Nt recomendada es 50% o menor. Nuevas investigaciones deberían ser realizadas con pollos de engorde para mejorar el conocimiento de la relación entre los AAs esenciales y no esenciales en dietas con reducción proteica.
El nivel de los AAs industriales en las dietas de pollos de engorde puede ser aumentado significativamente dependiendo de la viabilidad económica.

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9

Infertility in Production Animals: Causes and Potential Mitigation Strategies

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Summary

Infertility and subfertility in production animals represent important economic, health and welfare issues. Non-successful breeding and embryonic mortality are major limitations to reproductive efficiency. Additionally, with increased requirements for efficient production of animal protein to feed a growing world economy, compromised fertility represents a global food security concern. This review will focus chiefly on swine and ruminant reproduction for which the majority of knowledge has been acquired. The primary objective of this paper is to review an environmental stress (hyperthermia) and a physiological condition (bacterial infection) that both impair fecundity and fertility in domestic animal species. The secondary objective is to provide a brief summary of potential mitigation strategies to improve animal reproductive efficiency in the face of such stressors.

Resumen

Infertilidad en animales: Causas y potenciales estrategias de mitigación

La infertilidad y subfertilidad en los animales de producción representan problemas importantes en la economía y la salud y bienestar de los animales. Una reproducción sin éxito y la mortalidad embrionaria son limitaciones importantes para la eficiencia reproductiva. Adicionalmente, con el aumento de los requerimientos para la producción eficiente de proteína animal para alimentar a una economía mundial en crecimiento, mejorar la fertilidad representa una preocupación para la seguridad alimentaria mundial. Esta revisión se centrará principalmente en la reproducción porcina y de rumiantes para la que se ha adquirido la mayoría de conocimientos. El objetivo principal de este trabajo es evaluar un estrés ambiental (hipertermia) y una condición fisiológica (infección bacteriana) que perjudican la fecundidad y la fertilidad en las especies de animales domésticos. El objetivo secundario es proporcionar un

breve resumen de las posibles estrategias de mitigación para mejorar la eficiencia reproductiva de los animales afrontando estos factores de estrés.

Overview of Mammalian Reproductive Physiology:

The ovary is the site of female gamete production and maturation as well as steroid hormone synthesis. At birth, the ovary is endowed with a finite number of oocytes housed in follicular structure. The pre-antral follicle is comprised of the oocyte and granulosa cells. Following antrum formation, another layer of cells, theca, are recruited to surround the granulosa cells. Within the antrum, follicular fluid provides nourishment to the developing oocyte (Hirshfield, 1991). The oocyte is arrested in the prophase I stage of meiosis, and will not resume and complete meiosis I and II until ovulation and fertilization, respectively. The vast majority of oocytes are lost to a process known as atresia, and only approximately 1% of oocytes which initially endow the ovary will ovulate. The female sex steroid hormones, 17β-estradiol (E_2) and progesterone (P_4) are produced by pre-ovulatory, dominant follicles and the corpus luteum (CL), respectively. Briefly, an upper E_2 threshold is required to induce a surge of luteinizing hormone (LH) from the anterior pituitary in order to induce ovulation. In addition, LH regulates ovarian steroidogenesis and the process of luteinization by which CL formation occurs. The CL produces P_4, a hormone needed for implantation and pregnancy maintenance. In addition to inducing the LH surge, E_2 is required for appropriate display of secondary female sex characteristics and is the dominant hormone involved in the demonstration of behavioral estrus. Should pregnancy occur, E_2 synthesis and release from the developing porcine conceptus prevents the luteolytic (CL degradation) action of PGF2α, and the CL's, and thus the pregnancy, are maintained. Both E_2 and P_4 have specific ovarian receptors – the estrogen receptors α (ER α) and β (ERβ) and the progesterone receptor isoforms A and B (PRA and PRB). The process of folliculogenesis which comprises oocyte development and maturation within the follicular structure, and steroidogenesis for hormone production are essential for efficient reproduction. Any stressor that negatively affects either process will compromise fertility and fecundity.

Reproductive Impacts of Heat Stress

<u>Hyperthermia in swine</u>:

The swine industry suffers considerably due to impaired reproductive performance during periods of seasonal infertility, particularly during late summer and early autumn months (Pollmann, 2010). The impact is particularly visible in the U.S. with day 28 pregnancy rates reaching a nadir in August into October and subsequently reduced farrowing in November and December. This phenomenon is not limited to specific regions and occurs internationally (Auvigne *et al.*, 2010; Pollmann, 2010). Several components can contribute to seasonal infertility, such as photoperiod and environmental conditions (i.e. temperature) and deciphering the precise contribution of each on swine reproductive performance is difficult. Despite that, heat stress has been repeatedly demonstrated to negatively impact reproductive efficiency in pigs by affecting gamete development, pregnancy establishment, maintenance of gestation, and lactation performance.

Folliculogenesis:

The impact of heat stress during oocyte maturation and early embryonic development is evidenced in that sows exposed to hyperthermia for 5 days following breeding have significantly reduced number of viable embryos after day 27 of gestation, with control pigs possessing an average of 11.0 (68.8% survival) viable embryos and heat stressed sows containing only 6.8 (39.1% survival) viable embryos (Tompkins *et al.*, 1967). In this study, heat stress was administered following breeding, which generally occurs prior to ovulation and complete oocyte maturation, as pigs typically ovulate in the mid to latter half of estrus (Soede *et al.*, 1992).

Due to the difficulty for such studies *in vivo*, characterization of heat stress effects during oocyte growth and maturation and early embryonic development in pigs has been demonstrated using *in vitro* oocyte maturation and embryo culture systems. Some evidence of *in vitro* heat stress models during the transition between germinal vesicle breakdown and the 4-cell stage of development demonstrates the susceptibility of this stage to heat stress. A nine hour culture of pig embryos at 42°C following porcine *in vitro* fertilization reduced blastocyst formation rate from 20.6% to 8.8% (Isom *et al.*, 2007) and heat shock of 41.5°C for 4 hours following *in vitro* maturation also reduced oocyte development (Tseng *et al.*, 2006).

We have also demonstrated the impact of *in vitro* heat stress during oocyte maturation and its impact on subsequent developmental competency. Oocytes exposed to heat stress (41°C) for the first half (21h) or the duration of (42-44 h) of *in vitro* maturation demonstrated impaired ability to reach metaphase II arrest while heat stress during only the second half (21h) of *in vitro* maturation did not impact maturation rate (Wright and Ross, unpublished data). Metaphase II arrested oocytes following heat stress during *in vitro* maturation demonstrated impaired developmental competency compared to oocytes matured at 39°C, as measured by their ability to develop to the blastocyst stage following *in vitro* fertilization and culture in thermal neutral conditions. We have subsequently used this model to demonstrate differences in gene expression in developing 4- to 8-cell embryos as a result of heat stress conditions during *in vitro* maturation (Wright and Ross, unpublished data).

Gestational impacts:

The effect of heat stress during pregnancy in pigs is variable as different stages of gestation can be variably affected. This is demonstrated by a study conducted by

Omtvedt *et al*. (1971) in which exposed pregnant gilts to heat stress for 8 days during different stages of gestation. Heat stress (37.8°C for 17 hours and 32.2°C for 7 hours) beginning either on day 0 or day 8 of gestation compared to thermal neutral conditions (constant 23.3°C) reduced the number of viable embryos by day 30 of gestation. Interestingly, the same heat stress conditions administered on days 53-61 did not affect farrowing performance while heat stress during late gestation (days 102-110) resulted in a significantly increased number of dead piglets born and a 4 piglet reduction in the number of piglets born alive (Omtvedt *et al.*, 1971). However, a more moderate, cyclic heat stress, on bred gilts beginning on day 3 and extended to either day 24 or 30 of gestation did not impact embryo survival (Liao and Veum, 1994).

Lactation:

Heat stress during lactation can also have a profound impact on production. Temperatures exceeding the evaporative critical temperature during lactation resulted in reduced feed intake and lowered milk production (Black *et al.*, 1993). Elevated core body temperature results in the redirection of blood flow from the mammary gland towards the skin in an effort to facilitate heat dissipation. In response, lactation and piglet growth (during lactation) are reduced (McGlone *et al.*, 1988; Black *et al.*, 1993; Johnston *et al.*, 1999). In addition to reduced performance, heat stress during lactation can also reduce the number of sows returning to estrus within 15 days post weaning (Johnston *et al.*, 1999).

Semen Quality:

While the effects of heat stress on pig reproduction is notable, it is difficult to distinguish the consequences resulting from heat stress between the male or female. While it is clear that reproductive parameters in gilts and sows are affected by heat stress, exposure of boars to heat stress can also be detrimental to swine reproduction through impacts on semen quality. Boars subjected for heat stress for 90 days (34.5 °C and 31.0°C for 8 and 16 h per day, respectively) demonstrated reduced motility and increased percentage of abnormal sperm within 2 weeks from the initiation of heat stress compared to thermal neutral boars (constant 23°C) (Wettemann *et al.*, 1976). Utilization of semen from heat stress boars resulted in reduced number of embryos on day 30 post-insemination compared to thermal neutral boars (Wettemann *et al.*, 1976). Similar results were demonstrated by Cameron and Blackshaw in boars exposed to heat stress demonstrated a significant increase in abnormal sperm in 2-3 weeks following initiation of heat stress (Cameron and Blackshaw, 1980).

Thermal Stress Effects on Ruminant Reproduction:

The physiological effects of heat stress on productivity can be financially devastating for the animal production systems. During periods of heat stress, dry matter intake (DMI) decreases and maintenance requirements increase as livestock attempt to dissipate excess heat load (West, 1999). In addition, changes in blood flow and the production of various hormones ultimately result in decreased reproductive performance. During summer months, conception rates can decline by 20-30% (Rensis and Scaramuzzi, 2003). This observed reduction in fertility, is attributed to several factors, including a reduction in estrus detection ability, early embryonic death, inhibition of follicular dominance, and reduced ovarian steroidogenic output (Putney *et al.*, 1988; Wolfenson *et al.*, 2000; Rensis and Scaramuzzi, 2003). Thus, heat stress has a wide range of reproductive effects beginning with the developing follicle and continuing through early embryonic development. The biological mechanisms that mediate these effects, however, are not completely understood.

Inevitably, the decrease in DMI that occurs during periods of heat stress is accompanied by changes in circulating concentrations of several metabolic hormones. In turn, these metabolic adaptations alter the production and secretion of the hormones controlling the reproductive cycle (Wolfenson *et al.*, 2000). Such consequences are far-reaching and may involve detrimental effects on ovarian follicular development, oocyte competence, early embryonic development and the maternal recognition of pregnancy.

During heat stress, the development of the dominant ovarian follicle is attenuated and circulating concentrations of E_2 are lower. In addition, the luteal phase of heat-stressed cattle is extended and follicular wave dynamics are altered (Wilson *et al.*, 1998). These changes in ovarian function appear to be the result of decreased LH pulse amplitude (Gilad et al., 1993). As a reminder, LH is directly involved in the processes of follicular growth, E_2 production, ovulation and P_4 production. These changes in LH pulsatility may simply be a consequence of lower feed consumption during heat stress (nearly a 35% decrease compared to thermal-neutral controls; (Rhoads *et al.*, 2007). Decreased feed intake is associated with changes in circulating insulin, leptin and ghrelin, which have all been shown to affect LH pulsatility in several species (Szymanski *et al.*, 2007).

Effects on the Oocyte and Embryo

Preovulatory oocytes can be damaged directly by heat stress, and indirectly by prolonged estrous cycles. These longer estrous cycles presumably result in the ovulation of an aged oocyte that has reduced potential for developmental competence. Oocytes contained within antral follicles appear to be the most susceptible to the damaging effects of heat stress. As a result, conception rates remain depressed extending into the fall as the oocytes that were damaged during the summer heat stress are cleared from the ovary via ovulation or degradation.

Reproductive Impacts of Infection

Lipopolysaccharide (LPS) is a marker of bacterial infection and is elevated in animals suffering from mastitis, as well as from leaky gut in the transition period. Additionally and interestingly, LPS is increased in hyperthermic animals. From a reproductive perspective, the LPS-induced poor fecundity phenomena is reported throughout the literature. Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte contains LPS levels reflective of the systemic circulation. Thus, LPS is reaching the ovary via the systemic circulation and directly interacts with the oocyte proportionately as extra-ovarian tissues (Herath *et al.*, 2007).

Folliculogenesis:

Bovine ovarian cortical explants exposed to LPS had reduced numbers of primordial follicles, concomitant with increased atresia of the ovarian reserve (Bromfield and Sheldon, 2013). Similarly, mice exposed to LPS *in vivo* had reduced primordial follicle number which was described as a TLR4-mediated effect, since *Tlr4*$^{-/-}$ mice were refractory to LPS-mediated primordial follicle depletion (Bromfield and Sheldon, 2013).

Steroidogenesis:

LPS alters the level of anterior pituitary hormones, through direct or indirect mechanisms. Using anestrous ewes as a model, LPS infusion decreased LH but stimulated systemic prolactin (PRL) and cortisol levels. Additionally, mRNA abundance of genes encoding LH (LHβ) and the LH receptor (LHR) were reduced by approximately 60% in both cases (Herman *et al.*, 2010). Interestingly, the FSH and FSH receptor as well as PRL and PRL receptor genes were increased by LPS infusion (Herman *et al.*, 2010).

LPS exposure did not impact cell number or androstenedione production from cultured theca cells from either small, medium or large ovarian follicles, but did reduce the amount of E_2 produced from cultured granulosa cells isolated from all three follicular sizes (Williams *et al.*, 2008). In an *in vitro* system where ovarian cortical explants were cultured with LPS and provided with FSH or androstenedione, E_2 and P_4 conversion was reduced; potentially due to the observed decreased expression of *Cyp19a* mRNA and protein, an enzyme critical for production of E_2 (Price *et al.*, 2013). Cultured granulosa cells had increased expression of TLR4, likely in response to mediating LPS signaling, and negative impacts of LPS on E_2 production were demonstrated (Herath *et al.*, 2007). While no overall impact of LPS on E_2 was observed *in vivo*, a temporal decrease in bovine P_4 concentrations and lower ovulation rates resulted from LPS treatment (Williams *et al.*, 2008). In agreement with reduced E_2 level, when LPS was infused into the uterine lumen, the pre-ovulatory LH surge was attenuated and may be the result of an insufficient stimulation from E2 driving the LH surge (Peter *et al.*, 1989). Furthermore, LPS-treated females had delays in the time to the LH surge (Fergani *et al.*, 2012).

In a regularly cycling animal, in the absence of fertilization and pregnancy, endometrial synthesis and release of PGF2α causes CL regression. LPS itself also causes CL regression by inducing the production of PGF2α (Moore *et al.*, 1991; Hockett *et al.*, 2000). LPS administration causes delayed ovulation, and lengthens the time to CL formation and sufficient P_4 production (Suzuki *et al.*, 2001; Lavon *et al.*, 2011). Additionally, the size of CL are reduced by LPS (Herzog *et al.*, 2012), perhaps due to activation of pro-apoptotic pathways (Herzog *et al.*, 2012). Interestingly, a temporal pattern of LPS on circulating P_4 has been demonstrated, whereby P_4 is initially increased and then declines in LPS-treated, relative to their control females (Herzog *et al.*, 2012).

Estrus behavior:

Not surprisingly both heat stress (Doney *et al.*, 1973; Sejian *et al.*, 2010) and LPS (Battaglia *et al.*, 2000) impact female estrus behavior and frequency. As in the case of the LH surge, a threshold of E_2 is needed to induce behavioral display of estrus, however the amount required for the latter is thought to be at a lower level (Saifullizam *et al.*, 2010). LPS-induced reductions in E_2 production may explain the observed impacts on behavior estrus display since E_2 is required for this female phenotypic response.

Pre-term labor:

P_4 is essential for pregnancy maintenance, and LPS reduces the PR in uteri of pregnant mice, thus impacting pregnancy maintenance (Agrawal *et al.*, 2013). The effect of LPS on the ability of P4 to sustain gestation could cause spontaneous abortion, a phenotypic event frequently also associated with hyperthermia. Infection from gram negative bacteria or their outer wall components (LPS) triggers pre-term labor in many species (Koga and Mor, 2010), and, as a testament to the efficacy of LPS at inducing preterm labor, intraperitoneal LPS injection is an established experimental model for inducing pre-term labor (Deb *et al.*, 2004; Agrawal *et al.*, 2013). In addition, infertility can be the result of gynecological infections in both humans and production animals (Williams *et al.*, 2008; Price *et al.*, 2013).

Potential Mitigation Strategies

As might be expected, a major effort has gone into designing housing facilities for production animals that provides shade, misting and fans to cool animals, and these efforts have greatly ameliorated the occurrence of hyperthermia induced reproductive calamities in production animals. In addition, it is recognized that heat stressed animals do not consume the same amount of feed as their thermal neutral counterparts, however, "off feed" only accounts for approximately 50% of the heat stress-induced alterations to lactation (Baumgard, 2013), thus likely is also not the sole contributor to negative consequences of heat stress of reproduction. Thus, greater research remains to be done to bridge our knowledge gaps in terms of how to overcome the negative impacts of hyperthermia on reproduction.

Heat-stressed induced changes in ovarian dynamics ultimately result in unique challenges for reproductive management and potentially translating to the production of a substandard oocyte. Many producers now rely on timed artificial insemination programs during periods of heat stress because estrus detection ability is reduced as a result of reduced behavioral demonstrations of estrus. Indeed mounting activity declines by nearly half and is likely the result of lower circulating E_2 concentrations. Extended luteal phases during periods of heat stress also make it more difficult to predict when individual animals will come into estrus. Timed artificial insemination alleviates these challenges by allowing the producer to control the time of ovulation.

One management technique has shown promise for overcoming the oocyte-specific problems associated with heat stress. Conception rates by transferring fresh *in vitro*-produced embryos into heat stressed cattle (Al-Katanani et al, 2002; Stewart et al., 2010). Used as a management practice, this allows the producer to completely bypass the challenges associated with substandard oocyte quality. Currently, however, the advantage is only evident with the use of fresh embryos. Using frozen embryos yields conception rates that are similar to those resulting from timed artificial insemination during heat stress. This presents a logistical challenge since few producers have access to an economical source of fresh *in vitro*-produced embryos. The source of the oocytes is also a concern if the offspring are needed as replacement animals: collecting oocytes from genetically superior females (housed in a cool environment) is more costly, while the least expensive alternative is indiscriminately collecting oocytes from ovaries at the slaughterhouse. Depending on the geographical region, animals sent to the slaughterhouse vary widely in breed and genetics, and therefore would not be desirable as replacement animals. Future advances in *in vitro* embryo production and freezing will make this technique a more viable alternative for use during periods of heat stress.

There are a number of other avenues for exploration, however, including improving intestinal integrity to prevent "toxic" compounds from reaching the reproductive tract. Heat stressed animals suffer from hyperinsulinemia (Baumgard, 2013), a biological paradox since they are consuming less feed. Systemic hyperinsulinemia could be reduced via pharmaceutical insulin-sensitizing agents, which could lessen blood insulin levels. In addition, compromised PR level and function could perhaps be overcome through supplementing with P_4, a strategy routinely used in humans at risk for preterm spontaneous abortion. These potential avenues for mitigation of infertility that results from exogenous exposures remain reliant on generation of science-based understanding of the problem.

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Genómica y Producción Animal

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Summary

Developing countries have the challenge of achieving food security in a world context that is affected by climate change and global population growth. Molecular genetics and genomics are proposed as technologies which will help achieve sustainable food security. Technologies that have been developed in the last decade such as development of genetic markers, genetic maps, genomic selection, next generation sequencing and DNA editing systems are discussed. Examples of some discoveries and achievements are provided.

Resumen

Genómica y producción animal

Los países en desarrollo tienen el reto de alcanzar seguridad alimenticia en un contexto mundial que se ve afectado por el cambio climático y el crecimiento global de la población. La genética molecular y la genómica se proponen como tecnologías que ayudaran a alcanzar seguridad alimenticia sostenible. Se discuten las tecnologías de desarrollo de marcadores genéticos, mapas genéticos, selección genómica, tecnología de secuenciamiento de ADN de nueva generación y sistemas de edición precisa de ADN que se han desarrollado en la última década y se dan ejemplos de descubrimientos y logros.

Introducción

Para los países en desarrollo el reto de alcanzar seguridad alimenticia es probablemente mucho más complejo y desalentador que en el siglo pasado. Tenemos ahora un mundo de aproximadamente 7,000 millones de habitantes en el que se estima que 1,000 millones se encuentran subalimentados y un número parecido sobrealimentados. Esta falta de alimentos para unos y exceso para otros crea serios problemas de salud humana que dominan la agenda agropecuaria y de salud mundial y se constituye en uno de los grandes retos mundiales. Esta situación se agrava debido al hecho de que la población mundial en el 2050 se estima que alcanzara 9,000 millones de habitantes y, de acuerdo con estimados de las Naciones Unidas, se necesitara 35% más alimentos, 40% más agua y 50% más energía para sostener este aumento poblacional. Estos incrementos tendrán que ser alcanzados en un periodo corto de tiempo con casi la misma área cultivable que ahora existe en un mundo que se enfrenta a cambios climáticos que van a afectar la disponibilidad de agua para agricultura y consumo humano entre otros retos. Claramente el futuro demanda mejor coordinación y colaboración entre naciones, mejor distribución de recursos alimenticios dentro y entre naciones, e intensificación sostenible a nivel global de la producción agrícola (Rothschild and Plastow, 2014). Creemos que los avances alcanzados en los últimos 10 años con la genética molecular y la genómica pueden ser usados para desarrollar una producción avícola y pecuaria más productiva y eficiente basada en planes de mejoramiento genético y el uso de tecnologías reproductivas y de edición de ADN (Neeteson-van Nieuwenhoven et al, 2013). El propósito de este manuscrito es proporcionar información sobre algunos de estos avances y sugerir su uso para desarrollar una pecuaria intensiva sostenible.

Marcadores Genéticos

Uno de los primeros enfoques de la genómica fue el desarrollo de mapas genéticos basados en marcadores moleculares. Los marcadores más usados para el desarrollo de estos mapas han sido los microsatélites (MS) y los polimorfismos de nucleótidos simples (PNS, el acrónimo en Inglés es SNP). Los primeros son secuencias únicas de ADN que flanquean un fragmento que puede variar de 2, 3 o 4 nucleótidos que se repiten consecutivamente. La variación en el número de repeticiones crea fragmentos de diferentes tamaños y por ende alelos. Las secuencias únicas que flanquean el MS son usadas para diseñar cebadores que permiten amplificar el MS por medio de la reacción en cadena de la polimerasa (PCR). Los fragmentos podrán visualizarse por medio de su separación por electroforesis. Los MS pueden tener desde dos alelos a más de 15 alelos informativos. Alelos informativos son aquellos que se encuentran con una frecuencia de por lo menos 0.05 en la población.

Los PNS en cambio son sustituciones de un nucleótido por otro en una posición específica en un fragmento dado de ADN. A pesar que los PNSs pueden tener un máximo de cuatro alelos en su mayoría solo se observan dos. Por ejemplo, en un fragmento dado de ADN algunos individuos de la población tienen un nucleótido, digamos, Citosina, y otros en ese mismo lugar del fragmento de ADN tienen Adenina. Sin embargo un marcador con dos alelos es un marcador que tiene muy baja resolución porque solo divide la población en dos grupos. Sin embargo debido a la abundancia de estos marcadores se pueden hacer agrupaciones de marcadores a lo largo de una hebra de ADN con lo cual se establecen haplotipos (un conjunto especifico de marcadores ordenados consecutivamente en un fragmento de ADN) que en conjunto son más informativos (mejor resolución) y son usados para estudios de asociación con rasgos productivos cuantitativos. El método para analizar los PNSs ha sido automatizado de tal manera que se puede hacer análisis simultáneo de miles de estos marcadores en una sola reacción para cada animal con lo que se obtiene el genotipo PNS del animal pudiendo cubrir más del 95% del genoma.

Mapas Genéticos

Los marcadores genéticos moleculares fueron generados usando diferentes estrategias. Los MS se aíslan de bibliotecas de fragmentos cortos de ADN usando sondas con 2, 3 o 4 nucleótidos en tándem seguida por secuenciamiento de los fragmentos detectados para identificar secuencias únicas que flanqueen el MS para diseñar cebadores de PCR. Los PNS se identifican por secuenciamiento y comparación de las secuencias de fragmentos similares de ADN en grupos de animales de la misma especie. Sin embargo la localización de los marcadores a lo largo de ADN de un genoma no se puede saber a menos que se construyan mapas de marcadores genéticos. La tecnología para desarrollar los mapas está basada en el análisis de ligamiento entre genes y/o marcadores genéticos que fue descrito por Sturtevant (1913) cuando construyo el primer mapa cromosómico a principios del siglo pasado. Dos genes están ligados (cercanos) en una misma hebra de ADN cuando la frecuencia de recombinación entre ellos es baja. A menor distancia entre dos genes o marcadores, menor será la probabilidad de recombinación o de quiasmas que se puedan generar entre ellos durante la división celular meiótica. Para desarrollar estos mapas se tuvieron que generar cruzamientos apropiados entre animales dentro de especie que resultaran en la mayor proporción de meiosis informativas. También se usaron sistemas "seudogenéticos" (híbridos de radiación) para facilitar el ordenamiento secuencial de los varios miles de marcadores moleculares que existen para cada especie. Contamos en este momento con mapas genéticos de marcadores PNS para rumiantes (vacuno, búfalo, ovino y cabra) no rumiantes (conejo, puerco, caballo), aves (pollo, pavo, pato) entre otras especies de granja. Algunos mapas son más informativos que otros porque contienen más marcadores con distancias promedio entre ellos que van desde 7,500 pares de bases (pb) hasta 100,000 pb. De una manera u otra estos mapas han facilitado el ordenamiento de fragmentos de ADN (mapas físicos) provenientes de bibliotecas de ADN genómico facilitando de esta manera el ordenamiento de secuencias de ADN obtenidas de los proyectos de secuenciamiento de ADN de animales de granja. Para mayor información sobre las especies secuenciadas ver las páginas del National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Los marcadores moleculares se usan para análisis de historia evolutiva, flujo génico, variabilidad genética, detección de secuencias específicas de ADN, mejoramiento genético, selección asistida, sistema de apareamiento controlado, identificación de individuos y paternidad entre otros usos.

PNS “chips”

Dependiendo del número de PNSs disponibles para cada especie se han escogido PNSs que se encuentran distanciados entre ellos por 3,500 (vacunos) y 54,000 bps (pollo, ovinos, puerco) para generar lo que se conoce como “PNS chips” que son láminas portaobjetos de microscopio que contienen todos los PNSs escogidos distribuidos, ordenados y fijados a la lámina como puntos microscópicos. Cada uno de estos puntos microscópicos contiene una secuencia específica de un PNS el mismo que será detectado por hibridación de secuencias de ADN del animal siendo genotipado (Affimetrix, Santa Clara, CA 95051, USA) or por hibridación y extensión de la secuencia del PNS basado en la guía del ADN del animal siendo genotipado (Illumina, San Diego, CA 92122, USA). El número de PNSs en el chip puede ser diseñado a voluntad dependiendo del grado de resolución que se quiera tener. A mayor numero de PNS, menor será la distancia entre ellos y mayor la resolución y precisión de identificación de los genes o del segmento de ADN asociado con características fenotípicas. La mayoría de las investigaciones sobre asociaciones fenotípicas con marcadores se hacen con “PNS chips” que contienen entre 50,000 y 60,000 marcadores.

Mapeo de *loci* de caracteres cuantitativos (QTL)

El QTL se entiende como una región cromosómica específica que se ha identificado, por métodos estadísticos, estar asociada con un fenotipo cuantitativo. La región en si contiene uno o varios genes responsables por el control y segregación genética del fenotipo. Fundamentalmente el principio en el que se basa la identificación de un QTL es en detectar el desequilibrio de ligamiento que existe entre un gene o genes que controlan una variable cuantitativa y un marcador o marcadores moleculares en el genoma. La disponibilidad de los “PNS chips” y bases de datos con información cuantitativa de fenotipos ha permitido identificar QTL para la mayoría de los caracteres fenotípicos bajo mejoramiento genético en algunas especies. En las páginas de “AnimalQTLdb” (http://www.animalgenome.org/cgi-bin/QTLdb/index) (Zhi-Liang et al., 2013; Zhang et al., 2012) se podrá encontrar más detalle sobre los QTL específicos para cada especie estudiada. Solo basta aquí con resaltar que 11,543 QTLs representando 481 rasgos productivos se han identificado en bovinos; 4,337 QTL representando 305 rasgos en pollos; 11,610 QTL representando 649 rasgos en suinos; 789 QTL representando 217 rasgos en ovinos; 345 QTL representado 9 rasgos en equinos y 127 QTL representando 14 rasgos en truchas. Esta información está siendo usada para estimar el valor genético de un animal basado en la asociación de marcadores con rasgos productivos (Meuwissen, et al., 2001).

Selección genómica en vacunos de leche

Los aproximadamente 38,000 PNSs informativos que se encuentran asociados con rasgos de producción lechera están distribuidos a lo largo del genoma de cada animal y teoréticamente el espacio entre cada uno de ellos es de aproximadamente 80,000 nucleótidos. Esto significa que los marcadores PNSs son consecutivos y se encuentran relativamente cerca uno del otro. Los progenitores transfieren a sus descendientes este ordenamiento. El descendiente, debido a la recombinación del ADN materno y paterno que ocurre en sus células reproductivas, transfiere a los nuevos descendientes (o nietos de los progenitores evaluados) fragmentos del ADN del abuelo mezclados con fragmentos de ADN de la abuela. La información genética que se busca en la evaluación genómica de cada animal es la identificación de los fragmentos de ADN que han sido heredados y que están asociados positivamente o negativamente con los rasgos de producción a mejorar. Esto se logra haciendo un análisis de los PNSs que cada animal hereda. Así se obtiene un mapa de marcadores PNS para cada animal o el genotipo de cada animal y entonces se puede determinar que fragmentos de ADN el animal ha heredado de sus ancestros los que a su vez han sido evaluados por pruebas de progenie o por evaluación genómica. Basados en estudios de asociación de marcadores y rasgos productivos hechos en una población de referencia se adjudican valores específicos a cada fragmento heredado y se obtiene el PTA genómico (GPTA) para cada rasgo en evaluación. Creo apropiado recordar aquí que el ordenamiento consecutivo de marcadores PNS que se encuentran en un fragmento de ADN se le conoce por el nombre de "haplotipo". Por lo tanto a cada haplotipo se le da un valor que representa su contribución (positiva o negativa) al rasgo productivo al que está asociado (Ponce de León, 2014). La confiabilidad de este estimado ha sido evaluada y se ha determinado que es superior a la confiabilidad del PTA. Van Raden et al., (2009) concluyo que el promedio de confiabilidad obtenido para 28 rasgos de producción evaluados en la raza Holstein de EE.UU. fue 23% superior al promedio de confiabilidad solamente basado en el promedio de los padres (PTA). Esta ventaja es equivalente a evaluar la producción de once hijas. La confiabilidad aumenta más doblando el número de sementales evaluados que incrementando el número de marcadores PNSs. Estas conclusiones se obtuvieron después de usar las evaluaciones obtenidas en agosto del 2003 provenientes de 3,576 toros nacidos antes de 1999 y que sirvieron para predecir las desviaciones de producción de las hijas de 1,759 toros nacidos entre 1999 y 2002. Así por ejemplo, la confiabilidad para producción de leche basada en el promedio de los padres fue de 28% y la confiabilidad basada en la predicción genómica no linear fue de 49%, lo que indica una ventaja del 21%. El rasgo que presentó mayor ventaja fue el rasgo de porcentaje de grasa en el que el promedio de los padres alcanzó el 25% y la predicción genómica un 63%. La razón principal para este resultado es la existencia de un gene con efecto mayor. Similares evaluaciones y resultados se han obtenido en Australia, Francia y Nueva Zelandia lo que estimulo el uso del GPTA en toros con 2 años o menos lo que reduce significativamente el intervalo generacional y permite duplicar la ganancia genética anual (Hayes et al. 2009). De la misma manera en otros estudios VanRaden et al. (2011) identificaron cinco haplotipos que no se encuentran en forma homocigota en la población de animales genotipados de la raza Holstein y que tienen un efecto negativo en el porcentaje de concepciones cuando los haplotipos son segregados por el padre y el abuelo materno. El porcentaje de animales portadores de estos haplotipos varía entre el 2.7 al 6.4%. Los genes que producen este efecto negativo aún no se conocen, lo único que podemos inferir es que en los segmentos de ADN identificados por estos haplotipos negativos existen algunos genes que cuando están en condición homocigota causan perdidas por baja fertilidad o por muerte embrionaria temprana.

El que existan estos haplotipos negativos no quiere decir que los animales portadores deban ser eliminados como futuros progenitores. Más bien, que la información con que se cuenta sea usada para programar cruzas controladas que no permitan la posibilidad de generar individuos homocigotos. Otros ejemplos de esta naturaleza existen en la literatura científica y el conglomerado de instituciones y compañías relacionadas con la industria están proveyendo la información a productores tan pronto como identifican haplotipos detrimentales o negativos.

Selección genómica en otras especies

Van Eenennaam et al., (2014) ha publicado una revisión sobre el uso pragmático de selección genómica en vacunos (leche y carne), suinos y pollos. Estos autores concluyen que la selección genómica en vacunos de leche es exitosa debido a la existencia de bancos de datos fenotípicos acumulados a través de varias décadas de pruebas de progenie que permiten alcanzar niveles significativos de exactitud y de confiabilidad. En suma las poblaciones de referencia ya existían en las bases de datos. Sin embargo el grado de adopción y uso de la selección genómica por otras industrias pecuarias y avícolas estará influenciado por: 1) limitaciones biológicas de la especie, 2) la estructura de organización de la industria, 3) determinación del tamaño ideal de las poblaciones de referencias lo que representa una inversión significativa, 4) el desarrollo de estrategias de genotipado económico y eficiente, 5) la practicidad de su implementación en el campo, y 6) el costo de la selección genómica en comparación con los beneficios obtenidos por la ganancia real genética anual. Por ejemplo el genotipado de PNSs con alta densidad de PNSs en machos seleccionados y baja densidad en hembras está siendo usado con éxito para imputar genotipos en los descendientes haciendo que la selección genómica sea económicamente más eficiente para la industria de suinos y de pollos (Dekkers et al., 2010; McKay, 2009; van der Steen et al., 2005).

Tecnologías de secuenciación de nueva generación

En los últimos cinco años el desarrollo de tecnologías de secuenciación de nueva generación (NGS del Ingles, New Generation Sequencing), también llamadas de secuenciación masiva en paralelo permiten obtener entre 1 millón y 43,000 millones de lecturas (cada lectura es un fragmento pequeño de ~ 50 bp a 400 bp) por instrumento secuenciador en una corrida dependiendo de la plataforma de secuenciación que se use. El costo de secuenciación también ha bajado significativamente desde $9.00 por mega-base en el año 2001 a aproximadamente $ 0.08 por mega-base en el año 2014 (National Human Genome Research Institute http://www.genome.gov/sequencingcosts/). Este hecho permite, a un costo razonable, hacer estudios de comparación de genomas entre animales dentro de una raza, entre razas y entre especies para detectar variación genética a nivel de genes y secuencias reguladoras de genes. Estas tecnologías se aplican en el secuenciamiento *de novo* de genomas, re-secuenciación de áreas específicas del genoma, descubrimiento de marcadores genéticos, descubrimiento de variación estructural del ADN, secuenciación de transcriptomas, secuenciamiento del metiloma, descubrimiento de variantes de ARN, identificación de smARNs y ncARNs, etc.

Estas tecnologías están revolucionando nuestro entendimiento de lo que es variación genómica y nos permite descubrir los mecanismos genéticos que gobiernan la expresión de fenotipos que antes solo podíamos medir cuantitativamente o cualitativamente. Por ejemplo, recientemente Pausch et. al. (2014) basados en investigación genómica y secuenciamiento profundo de NGS han encontrado en la raza Fleckvieh una mutación sin sentido en el exón 6 del gen que codifica la proteína 95 transmembrana que se localiza en la membrana de espermatozoides normales. La mutación consiste en que el cambio de un solo nucleótido (PNS) crea un codón de terminación prematuro con lo cual la proteína queda truncada y no funcional. Esta proteína no se encuentra presente en espermatozoides de animales homocigotos recesivos resultando en una reducción significativa de fertilidad. De la misma manera en la raza vacuna Piedmontesa la mutación de un nucleótido (PNS) en el gen de la miostatina causa hipertrofia muscular que es conocida como el fenotipo de "doble musculatura" (McPherron y Lee, 1997). Los animales que presentan este fenotipo tienen, en promedio, 20% más masa muscular. En el primer ejemplo el gen de la proteína 95 transmembrana sería un candidato ideal para corregir en animales elite de la raza que presenten ventajas en otros rasgos. En el segundo ejemplo el gen mutado de miostatina podría ser un gen candidato para introgresión en otras razas.

Tecnologías de Edición de ADN

Una de las maneras de determinar la función de un gene es alterando la secuencia del gene o silenciando de alguna manera la actividad del gene y observando cambios en el fenotipo. Con este fin se han desarrollado sistemas que usan enzimas nucleasas para efectuar cambios en la secuencia de ADN que permiten reparar y/o mutar un gene o secuencias reguladoras de genes. Las nucleasas son enzimas que son conocidas como "tijeras moleculares" porque permiten cortar la cadena doble del ADN en lugares precisos basados en el reconocimiento de una secuencia específica de ADN que puede ser leída por la enzima con la ayuda de una guía y después usar los mecanismos endógenos naturales de recombinación homologa de la célula para reparar el ADN y copiar la secuencia estipulada en la guía como parte del fragmento reparado. A estas tecnologías se les considera como las más importantes de los últimos años porque permitirán hacer lo que se conoce ahora como "cirugía genética" y que consiste en la edición dirigida de secuencias de ADN en células vivas. En la actualidad se usan con más frecuencia tres nucleasas que deben ser diseñadas para reconocer las secuencias específicas del ADN a ser editado.

La primera nucleasa en ser usada para editar ADN es la conocida como "Dedos de Zinc" (ZFN, del Inglés, Zinc Finger Nuclease). Estas nucleasas consisten en una cadena de aproximadamente 30 aminoácidos que reconocen un triplete de nucleótidos. En la actualidad se han diseñado ZFNs que reconocen las 64 combinaciones de tripletes que se pueden encontrar en el ADN. Con esto se construyen dímeros que reconocen 6 bases o dos tripletes y de esta manera se pueden preparar dímeros que reconozcan una secuencia especifica arriba (5') y otra abajo (3') de la secuencia que se quiere modificar (Carroll, 2011).

La identificación de las nucleasas conocidas como TALENs (del Inglés, Transcription Activator-Like Effector Nucleases) que son semejantes a enzimas activadoras de transcripción actúan en forma similar a las ZNFs. Estas usan bloques de aminoácidos para reconocer un nucleótido específico de tal manera que ensamblando varios bloques en un orden específico se puede reconocer una secuencia específica en el ADN. Se necesita entonces diseñar un TALEN para reconocer una secuencia especifica arriba (5') y otro TALEN para reconocer una secuencia abajo (3') de la secuencia que se quiere modificar (Joung and Sander, 2013).

La más reciente nucleasa usada para editar ADN es la que reconoce repeticiones de secuencias cortas palindrómicas inter-espaciadas y regularmente agrupadas CRISPR (del Inglés, Clustered Regularly Interspaced Short Palindromic Repeats) en el ADN y que se asocian con otra enzima conocida como Cas para generar el sistema CRISPR/Cas. Este sistema está basado en el uso de ARN que sirve como guía para reconocer la secuencia especifica de ADN que se quiere editar. La molécula guía de ARN (gARN) que consta de una parte que reconoce a la enzima y otra de aproximadamente 20 nucleótidos que reconoce la secuencia de ADN a editar forma un complejo enzima/ARN que localiza la secuencia de ADN a editar (Sanger and Joung, 2014).

De los tres sistemas descritos el que está siendo usado más es el sistema CRISPR/Cas con el que se han logrado modificar genes en células vivas y producir animales y plantas portadores de los cambios producidos. También se han corregido errores genéticos por infusión directa en órganos de animales adultos. Por ejemplo Yin et al., (2014) han logrado mediante inyección hidrodinámica de los componentes del sistema CRISPR/Cas corregir la expresión del gene *Fah* en ~1/250 células del hígado de ratones adultos. La expansión de hepatocitos positivos *Fah* rescato a los animales de la pérdida de peso observada cuando el gen no funciona. Este sistema también permite editar varios *loci* a la vez en un solo embrión. La simplicidad, alta eficiencia y su amplitud de aplicabilidad van a permitir diseñar experimentos más complejos que permitirán elucidar interacciones entre genes, cosa bastante difícil de hacer hasta el momento. Desde luego que estos sistemas están aún siendo investigados y mejorados para aumentar su precisión y determinar cuál es su efecto en el genoma. En el futuro las técnicas de clonación (Wilmut et al., 1997; Cibelli et al., 1998), edición precisa del ADN y la información genómica que se obtiene mediante la secuenciación del ADN se combinaran para incrementar el mejoramiento genético mediante la introducción a alelos benéficos de una raza en el genoma de animales elite de otra raza evitando así la introducción de material genético no deseado que acompaña la introgresión de alelos por cruzamiento tradicional (Tan et al., 2012).

Conclusión

Progresos en genética molecular y genómica han demostrado su utilidad en el desarrollo de la selección genómica. Esto ha sido posible debido al desarrollo de marcadores moleculares que han permitido identificar segmentos del genoma que contienen genes que controlan rasgos cuantitativos. La aplicación de la selección genómica en vacunos de leche es, por el momento, la más exitosa. El uso de selección genómica en otras especies está evolucionando y dependerá de la estructura de crianza y mejoramiento genético que se aplica a esas especies. Progreso para su uso dependerá también del costo de la aplicación de la tecnología. La mayoría de las especies de animales de granja cuentan ya con genomas secuenciados a diferentes niveles de profundidad, pero en general el nivel de secuenciamiento logrado es informativo y debido al abaratamiento constante de la tecnología de secuenciación se lograra incrementar la resolución de la que ahora se dispone. Las nuevas tecnologías de clonación, edición de ADN, desde que tengan aceptación social, unidas al conocimiento de secuencias de genes y sus variantes alélicas permitirán la "introgresión molecular" de alelos benéficos entre razas y la corrección de alelos detrimentales mutantes. Estas técnicas también podrían ayudar a disminuir la huella ambiental de la industria pecuaria. La reducción de generación de metano, uno de los gases que contribuye al efecto de invernadero, en vacunos se obtiene produciendo más producto (leche o carne) por animal o se obtiene modificando la flora del rumen. La genómica en general y la selección genómica en particular podrían contribuir muy rápidamente a incrementar de producción lechera por animal así como también ayudar a entender la composición de la flora del rumen. Por otro lado la modificación del gen de miostatina en razas productoras de carne a través de los sistemas de edición genética podría aumentar la producción de carne en 20% por animal en estas razas. En ambos casos el volumen de metano producido por unidad de producto (litros de leche o kilos de carne) sería menor que el actual con lo que se adquiriría una mayor eficiencia productiva y una reducción significativa de la huella ambiental de la industria pecuaria (Hayes et al., 2013).

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Approaches to improve production by small holders

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Summary

While the "Green Revolution" has greatly changed production of crops worldwide and helped feed over a billion people, improved production of livestock has been more limited across the globe. Modern advances in livestock production generally have only benefitted two groups: large scale livestock producers and consumers in the developed world. In some parts of the world many of the animal production practices have not changed for the last 1000 years and in other regions small holders have benefited only marginally by the scientific advances that now are an integral part of large scale commercial production. However, increased food insecurity and a worldwide food production crisis loom in the future as the most significant scientific challenge facing us in the next 30 years. Expectations are that human population growth will soon go from 7.3 billion to 9.6 billion by 2050, and food production must increase rapidly to meet the demand. These increases must come despite evidence of climate change and limited land and water resources. Furthermore, the so called "livestock revolution" has fueled a significant increased demand for animal source foods especially in the poorer countries of the developing world where most livestock are produced by small holders but also in countries like China and Brazil which are transforming quickly. Many researchers have touted that modern feeding systems, advanced reproductive technologies and advanced genetics and genomics offers solutions to increasing food in the developing world. These opportunities certainly exist, but direction and focus of research, funding issues, human capacity training and training of small holders will all be required for increasing livestock production. These activities will need to be embedded within sustainable programs that address implementation from the outset, and benefiting small holder production will be crucial to meeting this challenge.

Resumen

Estrategias para mejorar la producción animal en pequeñas crianzas

Mientras que la "Revolución Verde" ha cambiado en gran medida la producción de los cultivos en todo el mundo y ha ayudado a alimentar a más de mil millones de

personas, la mejora de la producción de ganado ha sido más limitada en todo el mundo. Los avances en la producción de ganado en general, sólo han beneficiado a dos grupos: productores a gran escala y consumidores del mundo desarrollado. En algunas partes del mundo, muchas de las prácticas de producción animal no han cambiado durante los últimos 1000 años y en otras regiones los pequeños productores se han beneficiado sólo marginalmente por los avances científicos que ahora son una parte integral de la producción comercial a gran escala. Sin embargo, el aumento de la inseguridad alimentaria y la crisis mundial de producción de alimentos se vislumbra en el futuro como el reto científico más importante al que nos enfrentaremos en los próximos 30 años. Las expectativas son que el crecimiento de la población humana crecerá de 7.3 billones a 9.6 billones en el 2050, y la producción de alimentos debe aumentar rápidamente para satisfacer esta demanda. Estos incrementos deben ocurrir a pesar de la evidencia del cambio climático y de los recursos limitados de tierra y agua. Por otra parte, la llamada "revolución ganadera" ha generado un aumento importante en la demanda de alimentos de origen animal, especialmente en los países en desarrollo más pobres del mundo, donde la mayoría del ganado es producido por pequeños ganaderos, pero también en países como China y Brasil, que están cambiando rápidamente. Muchos investigadores han pregonado que los sistemas de alimentación modernos, las tecnologías reproductivas avanzadas, la genética avanzada y la genómica ofrecen soluciones para aumentar los alimentos en el mundo en desarrollo. Ciertamente estas oportunidades existen, pero la dirección y el enfoque de la investigación, la financiación, la formación de capacidades humanas y la formación de los pequeños productores serán necesarios para aumentar la producción ganadera. Estas actividades tendrán que ser incluidas dentro de programas sostenibles que abordan desde el principio de su implementación, y que el beneficio en la producción de los pequeños productores será crucial para enfrentar este desafío.

Introduction

For many applied crop and livestock researchers, the greatest scientific and moral challenge of the 21st century likely will be the challenge of feeding the growing human population. Today, it has been estimated that nearly 1 billion people suffer from daily hunger and that as many as an additional 1.5 billion people have food insecurity issues (Smith et al., 2013). Estimates further suggest the population will grow from an estimated 7.3 billion people (at time of publication, http://www.worldometers.info/world-population) to approximately 9.6 billion people in 2050 and food production will have to increase by an additional 50 to 70% of today’s production (Ingram et al., 2010). This, of course, will come with limited land and water availability. Growth in population numbers and increases in Gross Domestic Product (GDP) have fueled a livestock revolution (Delgado et al., 1999) since the 1970s. Total meat consumption has tripled worldwide from 1980 to 2002 (World Bank, 2009). In the developing world total meat consumption is expected to double between now and 2050 as will milk consumption, while in the developed world both meat and milk consumption are likely to increase by less than 15% (Thorton, 2009).

The importance of animal protein in relation to balanced nutrition and its role in the future is well described in the review by Ludu and Plastow (2013).

Given the present day limited production efficiency of livestock in the developing world drastic increases in production levels and efficiency will be required to meet these demands. These increases can be accomplished by increasing livestock numbers very significantly—but with enormous environmental as well as production issues/impacts—or by increasing production efficiency per animal. The latter will be essential as these pressures will also apply to the production of crops especially those that are used for feeding both animals and man. A challenge of similar magnitude was met in the developed world (and some advanced developing countries like China and Brazil) over the last 75 years, although without the extra constraints of reducing resources (land, water, energy) and the impact of climate change. These increases in production efficiency have been accomplished in the developed world, in large part, by improvements in the technologies that are integral to animal production and in the adoption of ad libitum feeding in most developed countries. The impact of these efforts, especially genetics, is beautifully demonstrated in chickens (Havensteen et al., 2003) and pigs (Fix et al., 2010) and has been reviewed by others (Hume, Whitelaw and Archibald, 2011).

This paper is no way meant to be an exhaustive review of all opportunities and difficulties that exist in this area. Rather, the objectives are to define some of the opportunities to use existing and new technologies and to address some of the limitations facing use of improved technologies in the developing world by small holders. Given the authors' background much of the discussion will be centered on genetics and genomics approaches.

Use of existing technologies

In the 20th century increases in production traits in all major livestock species have taken place in developed countries largely due to 1) improved nutrition, 2) *ad libitum* availability of water and feed, 3) increases in genetic merit resulting from genetic improvement programs using advanced quantitative genetic methodology, 4) improved reproductive technologies including wide spread use of AI and embryo transfer and 5) improvements in animal health and disease control (including biosecurity as well as the development of vaccines and other treatments). Some of these technologies are listed in Table 1.

Feed technologies to improve production

In modern commercial systems, especially for non-ruminants, feed costs often exceed over 40% of the production cost and the two largest feeding factors affecting these costs are 1) quality of the feed and 2) availability of sufficient amounts of feed. For small holders these represent considerable challenges except for ruminant production where and when small holders have access to good forage. For other small holders, especially those in peri-urban settings, the cost and availability of sufficient quality feed is extremely constraining. For ruminants many small holders are left to chop grasses and forages from the roadside or from nearby open lots. Other small holders owning chickens or pigs often let the animals scavenge and this causes real biosecurity concerns. Many small holders do not fully understand the concept of growth and hence to make purchased feeds last longer they feed at levels that maintain the animal's weight and do not provide the needed feed for increased growth and weight gain. Also improved/formulated feeds, premixes, mineral and vitamin supplements are not affordable or easy to use for most small producers in developing countries. Additionally, considerable nutrition advice is required for small holders and clearly extension workers with nutritional training would be advantageous to help in ration formulation, and other assistance. If these existing technologies could be transferred at a sustainable and affordable manner then major increases in efficiency could be achieved and food security and economic growth among small holders would be improved. This suggests that some effort should be applied to making information more accessible and supporting its uptake. For example, using information and communication technologies that are tailored to the target audience. Radio may be one of the ways that such knowledge could penetrate in some parts of the world. Educating farmers was one of the aims of the BBC in creating The Archers in the early 1950s (http://www.bbc.co.uk/historyofthebbc/collections/archers_merl.shtml) and this along with web-based video may provide much needed improvements in basic husbandry.

Genetics and genomics for livestock production

Artificial selection began with domestication some 10-15,000 years ago and development of breeds, especially those suited for small holders and livestock keepers followed. Such developments depended heavily on selection and other genetic tools practiced by master breeders and indigenous people. For the developed world, adoption of other animal genetics practices such as animal identification, record keeping, selection of fast growing animals to be future parents and the adoption of mating systems such as crossbreeding for heterosis have been highly effective. Unfortunately many of these standard practices are not followed or poorly adhered to.

For example in many locations where improved livestock are donated by aid agencies local breeders may use them repeatedly, ignoring inbreeding. Also, given market pressures, small holders often sell the fast growing animals first instead of selecting and retaining them as future improved parents. The principles of genetic improvement are relatively simple, however, they do require a disciplined systematic approach and this is can be difficult to organize across groups and different ecosystems. Many of these issues could be resolved with a major education effort to improve the information and its application to specific breeding plans. One attempt at this is the Community Based Breeding Programs (CBBP) which have sprung up thanks to some governmental groups. These CBBP practice record keeping, selection, avoidance and inbreeding and group based marketing. In all these combinations of standard animal genetic practices combined with marketing and health programs have worked well but their sustainability in some regions is tenuous given their cost.

Again human capacity building (see below) would aid in training small holders and adopting approaches like "train the trainer" to further the spread of knowledge would be useful.

Table 1. New and improved technologies for improved commercial animal production.

- Improved Feeds
  - Ad libitum feeding
  - Premixes
  - Improved levels of amino acids
  - Higher levels of protein
  - Alternative feed sources in balanced rations
  - Processing to improve digestibility
  - Adding trace minerals and vitamins
- Reproductive technologies and interventions
  - Artificial insemination
  - Synchronized estrus and ovulation
  - Timed insemination
  - Embryo transfer
  - Semen sexing
  - Cloning
- Genetics and genomic technologies
  - Animal Identification
  - Record keeping
  - Estimation of breeding values from performance recording
  - Crossbreeding systems to harness hybrid vigor
  - Use of genetic markers
  - Genomic selection
- Disease reduction and health improvement
  - Access to affordable veterinary care
  - Providing better climate controlled shelter
  - Removing animals from their wastes
  - Proper biosecurity
  - Improved antibiotic use
  - Improved pathogen detection
  - Improved vaccines

Improvement with genetic markers and genomic approaches

The last part of the 20st century saw the initial use of genetic markers to improve selection or animal identification. For example, to confirm parentage in systems using natural mating and multiple sires. Advances in genomics are happening at an increasing pace including sequencing, development of large panels of single nucleotide polymorphisms (SNPs), genome wide association studies (GWAS) and employing genomic selection (see Garrick paper in this symposium). As Hayes et al.

(2013) pointed out, such genomic selection methods are being applied to traits such as milk production in cattle and feed efficiency in chickens, cattle and pigs and could eventually be applied for traits like reduced methane production in cattle (see Pickering et al., 2013) or even water requirements. This fine tuning of selection approaches using genomics has come after many decades of the use of conventional genetic methods and has been developed in systems that support animal identification, recording of phenotypes, and good-paying reward systems for increased production.

Unlike milk production in many developed counties, where cows may produce over 100 liters a day, cows in some developing countries can only produce 1–2 liters per day. Of course interventions that increase the amount and quality of feed and availability of water will be major inputs. With better environmental conditions, improved genetic quality of livestock certainly will be advantageous. This has been demonstrated in countries as diverse as India and Ethiopia (Duncan et al., 2013) and could potentially help address, at least in part, the problems encountered by the introduction of improved breeds from the developed world. Improvement through use of genetically superior breeds generally has been perceived as a failure for small holders, with genetic potential being lost in these poorer or more challenging environments. A better understanding of the genetic architecture and strengths of local breeds through genomics also may allow a more precise use of exotic germplasm to support these improvement efforts. . Some groups are looking to see if these tools can be used for the characterization of breeding stock to help overcome the failure to make systematic improvement by traditional means. Indeed, this may help unlock new opportunities where trait and pedigree recording is impossible to organize. The provision of improved sires or semen within CBBP may overcome this hurdle if proper account is taken of factors impacting producer decision making.

Use of large SNP panels to identify signatures of artificial and natural selection, of benefit in different production settings and environments, are underway in many studies (i.e. Ai et al., 2013). SNP panels also have been used to examine genetic differences between cattle in large production herds compared to those of small holders (Gorbach et al., 2010). All these genomic approaches are likely to lead to discovery of genes or genomic regions associated with increased production for breeds in harsh environments in developing countries. An initial example is preliminary work on Gir cattle from Brazil, where some signatures of selection were in regions of the genome known to contain genes that might be associated with adaptation (Liao et al., 2013). Use of genomics in other Latin American cattle production has also been well explored (Montaldo et al., 2012). Combined with information from similar discoveries using improved breeds, genomic solutions should be very useful for improving production efficiency and outputs, provided breeding systems can be developed to ensure application of the improved genetics (see later section in this paper and previous paragraph).

Improvement in traits affecting climate resilience

Improving standard production traits, such as milk production, growth rate and production of total animal protein, are important. However, climate change is expected to affect animal production significantly, especially for small holders. These effects are likely to include limited feed, increased drought, changes in disease prevalence and increased incidence of heat stress. Heat stress reduces production efficiency, decreases product outputs, increases animal welfare issues and is expected to result in significant death losses in some cases (Baumgard and Rhoades, 2013). For heat stress, short-term solutions include building shelters and providing cooling mechanisms. However, in many developing countries facilities and management are often limited, so solutions to combat climate change can be difficult. Even so, efforts are likely to be needed on all fronts, and this aspect cannot be ignored, especially in the short term.

Long-term genetic solutions may require the use of genomics to identify signatures of selection related to heat stress (for example in *Bos indicus*, Liao et al., 2013) and individual genes associated with mechanisms to combat climate issues. Research examining climatic stress in sheep and goats (Huson et al., 2013; Elbeltagy et al., 2014) has revealed possible signatures of selection. Of particular importance to Peru and this region is that South American camelids need further study to determine the genomic regions associated with their resilience.

Severity of disease and disease prevalence also are likely to be affected by climate change through the impact of stresses as well as through changes in the geographical range of diseases (Purse et al., 2005). Long-term solutions also may benefit from genomic approaches. To date most disease resistance research efforts have been devoted to diseases existing in modern production settings, but examples do exist in the developing world, such as resistance to lentiviruses in small ruminants (White and Knowles, 2013) that demonstrate the possible power of modern genomic approaches. Other examples include the long-term research effort devoted to examining differences among some native breeds of cattle in Africa for resistance to *Trypanosoma congolense* infection, which causes sleeping sickness (Noyes et al., 2011). A novel variation on this approach is to examine diseases that exist in the wild without serious consequences but that affect similar domesticated species. In all such cases, sequencing of genomes and comparisons among resistant and susceptible breeds or resistant and susceptible species offers hope in understanding the underlying genes responsible for resistance.

Characterization and management of genetic resources

Modernization and genetic improvement of many livestock species has led to a limited number of breeds being used in most production settings and increased losses of local native breeds. The FAO estimates that there are now 1491 (20%) breeds at risk worldwide (FAO, 2007). Genomic tools have been used to measure genetic diversity and population structure in many studies (see for example Blott et al., 2003, Amaral et al., 2008 for pigs). This work has largely been done in cooperation with scientists from institutions in the developed world and hence may reflect their own approaches and biases. It has been proposed that such genomic knowledge would be useful for designing effective strategies for management and conservation of farm animal genetic resources (FAO, 2007). Measures of allelic differences between populations (i.e., Fst) often have been employed using a limited number of highly polymorphic microsatellites which have been less expensive for developing country research budgets. Good reviews on this subject have been presented (e.g. Lenstra et al., 2012).

However, with the advent of genome sequencing and the production of thousands of SNPs, and the subsequent development of SNP chips, researchers routinely employ them for genetic diversity and GWAS. Researchers are now more effectively comparing different breeds/populations from different geographical regions (sometimes called landscape genomics). In turn this should provide them with more accurate measures of genetic diversity, architecture and perhaps natural selection for local environments. Although signatures of selection relating to local adaptations may be identified (see below), in general these studies need to be allied to industry efforts to characterize the different aspects of performance in these environments. Clearly if breed conservation is to be maintained then cost of genomic evaluation efforts must be funded and performance of conserved breeds must be of economic value to all livestock producers.

Reproductive technologies and interventions

Reproductive technologies have greatly advanced animal improvement by generally making it possible to use fewer select animals to produce the next generation. In doing so the genetically superior animals can be used more widely. For small holders, especially dairy producers, use of artificial insemination (AI) and superior bulls for milk production could greatly increase production ability. Of course this must be coupled with improvements in the production environment. Other advances, such as embryo transfer (ET) can allow for the ability to multiply significant numbers of superior embryos for production. In many countries in South and Central America increased use of AI and ET have been very successful. Development of CBBP to help in semen distribution can certainly be useful for small producers. Many production systems for cattle are both meat and milk and hence extra male calves have value. However, sexed semen would be particularly beneficial for small producers trying to produce only heifers to increase milk production.

Other technologies such as timed insemination and synchronized estrus have benefit for many larger operations but may in the near future be useful for small producers who wish to share males for breeding. Again a CBBP may be most helpful.

Disease reduction and health improvement

Most animal production units in the developing world are operated by small holder famers with extremely limited resources and limited access to proper affordable veterinary care. Many shelters are poor and have little or no biosecurity enforcement and poor environmental control. In many cases animals are not separated from their waste and live on earthen floors which allows continual parasite problems. Removal of these problems and increased biosecurity will go a long way towards increasing animal production efficiency for small holders. Many of these diseases also are zoonotic, and hence as many as a 1 billion livestock keepers are at risk worldwide (Grace et al., 2012). One strand of thought is that many of these problems can be addressed by increasing the pace of intensification. This would help address the issue of disease and rapidly increase the availability of animal protein through improved efficiency. However, it is not clear if this can be achieved on sufficient scale or in a short enough time to overcome problems of disease spread between the sectors, or the social issues that would be faced as the structure of communities is changed. Although some of the production gap will be addressed in this way through large scale intensification and increased biosecurity (e.g. as seen in Brazil and China), more sustainable change is likely to be delivered by small holders. Therefore, protection from pathogens is extremely important and requires accurate assessment of the pathogen itself as well as the possible development of an inexpensive and efficacious vaccine that can be delivered in these environments and situations. The use of genomics, in particular, new methods of sequencing, to more effectively identify the strain of a pathogen and to help in the isolation of specific antigens for development of new and more effective vaccines has been proposed. Tracing the source of the pathogen and monitoring its spread over regions also can be effective in future disease prevention strategies.

Genetic engineering of livestock

The introduction of genetically modified crops (GMOs) has revolutionized plant agriculture, at least in some parts of the world. Even so, there continues to be resistance to the technology, especially in Europe and even for a product such as Golden Rice, which was designed to help improve the lives of the poor in the developing world. Resistance to the engineering of food animals has been even greater for a number of reasons, including animal welfare. However, genetic manipulation provides the potential to make genetic changes that may be impossible through other approaches, at least in relatively short time frames. An area of great opportunity and potential benefit for this technology is therefore in animal health and disease resistance. For example, the development of chickens resistant to avian flu or those that reduce the spread of the disease, could have a huge impact on the economics and supply of chicken as well as potentially playing a significant role in reducing the threat of a flu pandemic (Lyall et al., 2011). Other opportunities include the production of Trypanomiasis-resistant cattle or animals resistant to African Swine Fever which have the potential to revolutionize the lives of small holders in parts of Africa where these diseases are endemic. The development of new tools and technologies mean that these changes can be introduced more precisely and more efficiently than ever before (Tan et al., 2012). Results from genomic studies, for example those investigating different breeds and species, will provide new targets for such manipulation to help improve the suitability of genetics for many of the environmental challenges faced in the developing world and can remove some of the problems in organizing improved animal production. However, it will not be enough that we can create these potential solutions. In addition, proactive efforts to win approval will be required to ensure the acceptability of such solutions where they are needed.

Implementation of technology solutions

Criteria have been suggested to evaluate the performance of livestock-related projects. These include "1) relevance of projects to the poor, and to national and local development objectives; 2) extent of satisfaction of project objectives through successful completion of activities; 3) sustainability in delivery of project benefits; 4) market access and utilization enabled by the project; and 5) value addition enabled by the project" (Wanyoike and Baker, 2013). Such criteria should be applied to applications of technology to livestock improvement and production.

How will this be achieved? At present a large dairy cattle evaluation project, funded by the Bill and Melinda Gates Foundation, is underway using SNP genotyping panels to determine genomically what is the "best" breed/genotype combinations for production in sub-Saharan Africa, and recently has been extended to include Ethiopia and Tanzania. Similar efforts are underway for goats through partnerships with European and US scientists (Huson et al., 2013). Assuming that the best genotypes can be identified, how then will they be delivered? In the developed world, recording systems, animal identification, breeding companies and artificial insemination companies exist to deliver improved sires, provide embryos for transfer or make planned matings. Such industrial infrastructure as has been discussed is lacking in the poorest countries of the developing world, even though governments attempt to put this infrastructure in place. Even if breeding systems can be developed and semen from genomically improved sires become available, improvements may fail to reach small holders due to lack of physical infrastructure including insufficient stores of liquid nitrogen, AI tools, quality roads and transportation services. The lack of clear paths to market can also mean that technological solutions will fail. Clearly industry and government need to work together to support such efforts.

Use of technology to develop better vaccines offers real promise, but impediments do exist in many poor developing countries. Because many vaccines require refrigeration and cold chain storage is unlikely in many developing countries, therefore vaccines not requiring refrigeration ensure higher use and efficacy. The manufacturing of high-quality biologics and vaccines also may be an impediment since these require considerable technological advances. Furthermore counterfeit vaccines and drugs in some regions of the world are a real problem. These aspects also are true for human health, and attention should be paid to understanding how these issues are being addressed in this arena—to transfer learning to agricultural issues. Finally many of the very poor developing countries may view technology solutions as genetic engineering and may have policies that are not supportive. Similar problems may be encountered for technologies such as genome editing, which some researchers consider to be outside of the definition of genetically modified organisms (GMOs). These are the sort of considerations that need to be dealt with proactively by cross-disciplinary teams and by utilizing new opportunities for communicating with consumers. In summary, if technology improvements are to be realized, infrastructure and policy considerations, as well as communication, need to be included in long-range planning and implementation. Planning for adoption should be part of all research proposals in this area of development.

Other issues for consideration

A number of other important considerations will affect the application of technology to improvement of livestock production in developing countries. Long-term strategies to circumvent these possible roadblocks are required.

Research funding

The agricultural research enterprise in most developed countries has limited funding but far outpaces that of developing countries. It has been estimated that high- and middle-income countries account for nearly 90% of all investment in agricultural research and development (Beitema and Stads, 2010). Such funding often is a mix of basic and applied research efforts. This is further complicated by the realization that larger investments are often directed toward the plant sciences than the animal sciences. This fact is even more pronounced in research devoted to agricultural improvement in the poorer developing countries (e.g. excluding Brazil and China). While not officially published or known it has been estimated that less than 20% of the several agencies and foundations (e.g. US Agency for International Development and the Bill and Melinda Gates Foundation) agricultural research efforts are devoted to animal-related research. The Bill and Melinda Gates Foundation recently created an enlarged team dedicated to livestock and revised its priorities.

Data from other countries and donors are limited, but until there are increases in animal agricultural research funding, improvements, especially as they relate to animal production research for developing countries, are likely to lag. Even so, countries such as the US, Canada and the UK are beginning to recognize the importance of food security and that this extends beyond their own borders. After all, more than ever, having sufficient food is one of the elements that contributes to stability, and ultimately security, across today's connected world.

Need for human and institutional capacity building

Technological advances for example in the field of genomics have been quickly adopted in the developed world as witnessed by the use of animal breeding technologies, SNP chips, GWAS, genomic selection and sequencing for most species and in many of the livestock industries (Hayes et al., 2013). Such developments require well-trained scientists and laboratories to support their activities, and these advances traditionally have been supported by and developed in strong universities and government agricultural research units.

In the developing world, large investments in higher education were made from the 1960s to the 1990s by donors such as USAID, the Rockefeller Foundation and the World Bank, but in recent years investments have declined and the negative effects are obvious. In many cases, with the numbers of students in these countries increasingly coupled with lack of support, quality has declined (Mouton, 2008b). The National Agricultural Research Systems (NARSs) or other government research organizations, called National Agricultural Research Institutes (NARIs), in many developing countries provide the majority of the agricultural research (Pardey and Alson, 2010). The NARS and NARIs both suffer in many countries from aging staff, few female staff, limited funding and old facilities (Pardey and Alson, 2010). Although

international research organizations, such as the Consultative Group on International Agricultural Research (CGIAR) cover many important crops and animal issues they cannot make up for limited institutional and human capacity deficiencies. Again, these issues have been recognized and are at least being built into plans, especially in funding from agencies like USAID in the US and in efforts with CGIAR.
The advancement and adoption of modern technologies will require research institutions and an increasing number of significantly better trained individuals with advanced degrees, and such training likely will need to occur in the US and other developed countries or be addressed by new ways of developing these resources *in situ*. This also will necessitate that these trained scientists return to and/or remain in their home countries to help train small holders, food producers and the next generation of researchers and farmers. There are opportunities to use new communication tools to support these efforts as well as to identify existing approaches that have worked in the past (see above). Other activities that could advance the ability to employ modern technologies include technical assistance, mentoring, workshops, conferences (like this Ensminger conference), study tours (especially those aimed at providing training of future trainers), institutional linkages and increased access to the internet for webinars, publications and technical information. Many donors are increasing support for such capacity-building activities, although support of those activities related to livestock research lags behind that of crops. These and other issues are beginning to be recognized along with the need for developments and initiatives that are "relatively simple, cheap and low-risk" as set out by Rege et al. (2011).

Market Access

Even with advanced technology, improved nutrition and access to better health care, small holders still suffer from lack of market access. Government intervention to insure proper roads, facilities to slaughter animals and provide safe post-harvest handling are all required. This will even the "playing field" for all farms, small and large, alike.

Conclusions

By 2050 the need to feed 2 more billion people will require 50–70% more food production, and there will be a significant increase in demand for animal sourced foods. Limitations in land and water and climate change issues will challenge livestock producers, especially small holders worldwide. The biological sciences in the 21st century already have been transformed by new technologies and their applications to agriculture. However, these changes have affected largely only those who live in the developed world. Employing technology generated solutions to increase livestock production efficiency in the developing world to meet these demands will be required. Opportunities for such solutions are many and include all aspects of livestock production. Delivery of many of these solutions in these production settings, especially in the case of breeding programs involving improved livestock, have yet to be developed and will require novel solutions. Although, there has been a general belief in the power of technology to rescue humanity, time is short and there is evidence that attitudes are changing. Technology itself will not provide all of the answers. Instead, we need to look at all aspects, from animal production to food distribution and international trade. This includes improving our understanding of small holder and consumer attitudes and their impact on adoption and purchasing decisions. Additionally, improved development of human and institutional capacities also will be required. The challenges are many, but the need to feed a hungry world will require that all animal producers, scientists, social scientists, funding agencies and policy makers work together to find solutions.

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