

Low potassium diets with different levels of calcium in comparison with different anionic diets fed to prepartum dairy cows: Effects on sorting behaviour, total tract digestibility, energy metabolism, oxidative status and hormonal response Abbas Rajaeerad, Gholam R. Ghorbani, Mohammad Khorvash, Ali Sadeghi-Sefidmazgi, Amir H. Mahdavi, Mirja R. Wilkens

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The effect of different dietary ratios of lysine, arginine and methionine on biochemical parameters and hormone secretion in turkeys

Katarzyna Ognik, Zuzanna Całyniuk, Dariusz Mikulski, Anna Stępniowska, Paweł Konieczka, Ian Jankowski

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Yellow mealworm, *Tenebrio molitor* (Col: Tenebrionidae), larvae powder as dietary protein sources for broiler chickens: Effects on growth performance, carcass traits, selected intestinal microbiota and blood parameters

Shadi Sedgh-Gooya, Mehran Torki, Maryam Darbemamieh, Hassan Khamisabadi, Mohammad Amir Karimi Torshizi, Alireza Abdolmohamadi

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Biochemical and molecular investigation of oxidative stress associated with urolithiasis induced by increased dietary calcium or protein in chickens

Mahmoud G. El Sebaei, Nagah Arafat, Reham A. El-Shafei, Mohamed A. El-Adl, Amany Farag, Abeer E. Aziza, Abdelfattah H. Eladl

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Effects of dietary supplementation with herbal extract mixture on growth performance, organ weight and intestinal morphology in weaning piglets

Meiwei Wang, Huijun Huang, Yangping Hu, Yiting Liu, Xiao Zeng, Yu Zhuang, Huansheng Yang, Lei Wang, Shuai Chen, Lanmei Yin, Shengwen He, Shuo Zhang, Xiaozhen Li, Shanping He

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SHORT COMMUNICATION

Impact of an anti-Salmonella. Typhimurium Bacteriophage on intestinal microbiota and immunity status of laying hens

Mijin Lee, Abdolreza Hosseindoust, SeungMin Oh, HanSeo Ko, EunSeok Cho, SooJin Sa, YoungIn Kim, JungWoo Choi, JinSoo Kim

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Effects of reducing dietary crude protein concentration and supplementation with laminarin or zinc oxide on the faecal scores and colonic microbiota in newly weaned pigs

Ruth Rattigan, Torres Sweeney, Stafford Vigors, Gaurav Rajauria, John V. O'Doherty

SHORT COMMUNICATION

Blood mineral profile of the critically endangered Western Derby eland (*Taurotragus derbianus*) in two conservation breeding reserves in Senegal

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Effect of essential oils or saponins alone or in combination on productive performance, intestinal morphology and digestive enzymes' activity of broiler chickens

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Effects of transport stress on pathological injury and main heat shock protein expression in the respiratory system of goats

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Effects of silymarin supplementation during transition and lactation on reproductive performance, milk composition and haematological parameters in sows

XiaoJun Jiang, Sen Lin, Yan Lin, ZhengFeng Fang, ShengYu Xu, Bin Feng, Yong Zhuo, Jian Li, LianQiang Che, XueMei Jiang, De Wu

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Effect of dietary pomegranate peel (*Punica granatum* L.) and Aloe vera gel (*Aloe barbadensis miller*) supplementation on testicular antioxidant biomarkers and spermatogenesis enzymes in mature V-Line rabbit bucks

Manal R. Bakeer Ph.D., Mahmoud M. El-Attrouny Ph.D., Alzahraa M. Abdelatty Ph.D.

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Effects of dietary fibres on gut microbial metabolites and liver lipid metabolism in growing pigs

Yaolian Hu, Daiwen Chen, Bing Yu, Hui Yan, Ping Zheng, Xiangbing Mao, Jie Yu, Jun He, Zhiqing Huang, Yuheng Luo, Junqiu Luo, Xianghui Zhang, Luhong Luo

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Beneficial effects of curcumin as a native or nanoparticles form on productive efficiency, liver and kidney functions, antioxidative status and immunity of heat-stressed growing rabbits

Ibrahim T. El-Ratel, Tag El-Din Hassan Tag El-Din, Merna Mohamed Bedier

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Consumption of identically formulated foods extruded under low and high shear force reveals that microbiome redox ratios accompany canine immunoglobulin A production

Matthew I. Jackson, Christopher Waldy, Chun-Yen Cochrane, Dennis E. Jewell

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Effects of *Opuntia ficus-indica* in the diet of primiparous sows on the metabolic profile during late gestation and lactation and feed intake during lactation

Gerardo Ordaz, Aureliano Juárez, Rosa E. Pérez, Héctor E. Martínez, Ruy Ortiz

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Melatonin supplement induced the hair follicle development in offspring rex rabbits

Yang Feng, Shuangbao Gun

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SHORT COMMUNICATION

Supplementation of diets with tannins from Chestnut wood or an extract from *Stevia rebaudiana* Bertoni and effects on in vitro rumen fermentation, protozoa count and methane production

Chiara Sarnataro, Mauro Spanghero, Andrej Lavrenčič

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A low protein maternal diet during gestation has negative effects on male fertility markers in rats – A Systematic Review and Meta-analysis

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Expression of selected genes encoding mechanistic pathways, nutrient and amino acid transporters in jejunum and ileum of broiler chickens fed a reduced protein diet supplemented with arginine, glutamine and glycine under stress stimulated by dexamethasone

Reza Barekatain, Peter V. Chrystal, Saad Gilani, Clive J. McLaughlan

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Oral administration of royal jelly may improve the preservation of rooster spermatozoa

Golsomeh Rahnama, Hamid Deldar, Zarbakht Ansari Pirsaraei, Mohammad Kazemifard

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Tonicity of oral rehydration solutions affects water, mineral and acid-base balance in calves with naturally occurring diarrhoea

Iuliette N. Wilms. Juanita Echeverry-Munera. Lauren Engelking. Leonel N. Leal.

lavier Martín-Tereso

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Dietary guanidinoacetic acid supplementation improved carcass characteristics, meat quality and muscle fibre traits in growing-finishing gilts

Zhengpeng Zhu, Changsong Gu, Shengdi Hu, Bin Li, Xiangfang Zeng, Jingdong Yin

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Alterations in fermentation parameters during and after induction of a subacute rumen acidosis in the rumen simulation technique

Theresa Orton, Karl Rohn, Gerhard Breves, Melanie Brede

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Developmental changes in physiological amino acids and hepatic methionine remethylation enzyme activities in E10-21 chick embryos and D1-49 broilers

Jianwei Lu, Jordan Weil, Sandro Cerrate, Craig Coon

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Milk urea nitrogen concentration is higher in Brown Swiss than in Holstein dairy cows despite identical feeding

Evelyne C. Kessler, Rupert M. Bruckmaier, Josef J. Gross

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Regulating appetite in broilers for improving body and muscle development – A review

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Influence of the effective microorganisms (EM) on performance, intestinal morphology and gene expression in the jejunal mucosa of pigs fed different diets

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Dietary regimen of the woodpigeon (Columba palumbus)

Coralie Negrier, Marco Fantinati, Jean-Yves Jouglar, Faouzi Lyazrhi, Valérie Cohou, Nathalie Priymenko

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Biorefined grass-clover protein composition and effect on organic broiler performance and meat fatty acid profile

Lene Stødkilde, Morten Ambye-Jensen, Søren Krogh Jensen

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Effects of crude protein and sodium intake on water turnover in cats fed extruded diets

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L-Carnitine metabolism, protein turnover and energy expenditure in supplemented and exercised Labrador Retrievers

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The immune adjuvant effect of *Astragalus* polysaccharide on *in ovo* injection of Newcastle disease vaccine

Ligang Xue, Dan Wang, Dongxing Zhang, Anqi Ju, Aoyi Duan, Junhong Xing, Yanjun Qin, Shubao Yang, Weimin Luan

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In vitro metabolism of red clover isoflavones in rumen fluid

Kateřina Dadáková, Andrea Trnková, Jitka Kašparovská, Ludmila Křížová, Jan Lochman, Tomáš Kašparovský

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Transcriptome changes associated with fat deposition in the *longissimus thoracis* of Korean cattle following castration

Sang Weon Na, Seung Ju Park, Soo Jong Hong, Myunggi Baik

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Blastodermal development, hatchability and chick quality of Marshall[®] broiler breeders of different flock ages during egg storage

Victoria A. Uyanga, Okanlawon M. Onagbesan, John A. Abiona, Lawrence T. Egbeyale, Oyegunle E. Oke, Obafemi F. Akinjute

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In vitro degradability of corn silage and *Leymus chinensis* silage and evaluation of their mixed ratios on performance. digestion and serum parameters in beef cattle

Xianglun Zhang, Hongliang Wang, Wei You, Hongbo Zhao, Chen Wei, Qing Jin, Xiaomu Liu, Guifen Liu, Xiuwen Tan, Xingling Wang, Fachun Wan, Xiaoyu Sun

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The possibility of using citric phosphate dextrose in chilling ram semen instead of egg yolk and soybean lecithin to improve fertility

Mahmoud Yassin Mohamed, Tarek M. M. Mahdy, Ezz I. Khalifa

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Water and Food restriction decreases immunoreactivity of oestrogen receptor alpha and antioxidant activity in testes of sexually mature *Coturnix coturnix japonica*

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Dietary cold-pressed red and black pepper oil mixture enhances growth, carcass, blood chemistry, antioxidant, immunity and caecal pathogens of quails

Mahmoud Alagawany, Ayman S. Salah, Mohamed A. Mahmoud, Fayiz M. Reda

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Changes in long-chain fatty acid composition of milk fat globule membrane and expression of mammary lipogenic genes in dairy cows fed sunflower seeds and rumen-protected choline

Saman Lashkari, Jeppe W. Moller, Søren K. Jensen, Lars I. Hellgren, Martin T. Sørensen, Peter K. Theil, Kristen Sejrsen

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Tolerance evaluation of Moringa oleifera extract to Hailan brown laying hens

Zhi-min Chen, Wen-huan Chang, Ai-juan Zheng, Hui-yi Cai, Guo-hua Liu

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The effect of Rosemary (*Rosmarinus officinalis* L.) distillation residues and linseed supply on fatty acid profile, meat colour, lipid oxidation and sensorial and hygienic quality of cull Barbarine ewes' meat

Yomna Ben Abdelmalek, Samir Smeti, Ines Essid, Yathreb Yagoubi, Souha Tibaoui, Naziha Atti

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Alterations of serum vitamin E and vitamin A concentrations of ponies and horses during experimentally induced obesity

Carola Schedlbauer, Dominique Blaue, Jens Raila, Ingrid Vervuert

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The effect of crude protein content of the diet on renal energy losses in horses

Marleen Kuchler, Annette Zeyner, Andreas Susenbeth, Ellen Kienzle

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Chlorella vulgaris microalgae and/or copper supplementation enhanced feed intake, nutrient digestibility, ruminal fermentation, blood metabolites and lactational performance of Boer goat

Ahmed E. Kholif, Hatem A. Hamdon, Ayman Y. Kassab, Eman S. A. Farahat, Hossam H. Azzaz, Osama H. Matloup, Ashraf G. Mohamed, Uchenna Y. Anele

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Ruminal degradation and estimated energy and protein values for ruminants of Durum wheat varieties grown in three locations

Jochen Krieg, Natascha Titze, Herbert Steingass, C. Friedrich H. Longin, Markus Rodehutscord

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Comparative study of adaptation in three chicken genotypes under humid tropical conditions of Nigeria

Monsuru O. Abioja, Olufemi P. Omotara, Oluwaseun S. Iyasere, John A. Abiona, Lawrence T. Egbeyale, Obafemi F. Akinjute

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Effects of reducing crude protein concentration in starter feed containing constant rumen undegradable protein on dairy calves performance

Mohammad Boorboor, Ali A. Alamouti, Nasser Karimi, Mohsen Sahraei Belverdy

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Effects of feeding extruded flaxseed on layer performance, total tract nutrient digestibility, and fatty acid concentrations of egg yolk, plasma and liver

Siyuan Huang, Bushansingh Baurhoo, Arif Mustafa

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Supplement Article

The effect of dietary supplementation with globin and spray-dried porcine plasma on performance, digestibility and histomorphological traits in broiler chickens

Sihem Dabbou, Angela Trocino, Gerolamo Xiccato, Joana Nery, Josefa Madrid, Silvia Martinez, Fuensanta Hernández, Isabelle D. Kalmar, Maria Teresa Capucchio, Elena Colombino, Ilaria Biasato, Lucia Bailoni, Laura Gasco, Cecilia Mugnai, Achille Schiavone

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Effects of dietary amino acid levels and ambient temperature on mixed muscle protein turnover in *Pectoralis major* during finisher feeding period in two broiler lines

Pramir Maharjan, Garret Mullenix, Katie Hilton, Antonio Beitia, Jordan Weil, Nawin Suesuttajit, Diego Martinez, Cole Umberson, Judith England, Justina Caldas, Victor Daniel Naranjo Haro, Craig Coon

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Fibrolytic enzymes improving in vitro rumen degradability of tropical forages

Gabriel Zanuto Sakita, Thiago Francisco Ventoso Bompadre, Dhanasekaran Dineshkumar, Paulo de Mello Tavares Lima, Adibe Luiz Abdalla Filho, Tania Sila Campioni, Pedro de Oliva Neto, Herman Bremer Neto, Helder Louvandini, Adibe Luiz Abdalla

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Effects of maternal dietary vitamin E on the egg characteristics, hatchability and offspring quality of prolonged storage eggs of broiler breeder hens

Jun Yang, Xuemei Ding, Shiping Bai, Jianping Wang, Qiufeng Zeng, Huanwei Peng, Zhuowei Su, Yue Xuan, Gregory Scott Fraley, Keying Zhang

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In ovo injection of nano-selenium spheres mitigates the hatchability, histopathology image and immune response of hatched chicks

Mahmoud H. El-Deep, Khairy A. Amber, Salwa Elgendy, Mahmoud A. O. Dawood, Abdulrahman Zidan

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Weaning affects the glycosidase activity towards phenolic glycosides in the gut of piglets

Noémie Van Noten, Elout Van Liefferinge, Jeroen Degroote, Stefaan De Smet, Tom Desmet, Joris Michiels

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Health benefits of carotenoids and potential application in poultry industry: A

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Fazul Nabi, Muhammad A. Arain, Nasir Rajput, Mahmoud Alagawany, Jamila Soomro, Muhammad Umer, Feroza Soomro, Zhongqing Wang, Ruiling Ye, Juan Liu

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The effects of dietary medium-chain fatty acids on ruminal methanogenesis and fermentation in vitro and in vivo: A meta-analysis

Yulianri Rizki Yanza, Małgorzata Szumacher-Strabel, Anuraga Jayanegara, Andre Meiditama Kasenta, Min Gao, Haihao Huang, Amlan Kumar Patra, Ewelina Warzych, Adam Cieślak

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Dietary nitrate and presence of protozoa increase nitrate and nitrite reduction in the rumen of sheep

María Laura Villar, Roger Stephen Hegarty, Jonathon William Clay, Katherine Anne Smith, Ian Robert Godwin, John Vivian Nolan

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SHORT COMMUNICATION

Faecal pH throughout the reproductive cycle of sows in commercial pig herds

Dominiek Maes, Ilias Chantziaras, Eline Vallaey, Kristel Demeyere, Evelyne Meyer, Geert P. J. Janssens

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The Solanum glaucophyllum Desf. extract reduces mineralized matrix synthesis in osteogenically differentiated rat mesenchymal stem cells in vitro

Fabrício Gomes Melo, Natália Melo Ocarino, Amanda Maria Sena Reis, Eduardo Juan Gimeno, Adriana Raquel Massone, Marília Martins Melo, Ana Flávia Machado Botelho, João Renato Stehmann, Rogéria Serakides

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Campomanesia sp. flour attenuates non-alcoholic fatty liver disease on rats fed with a hypercaloric diet

Paulo Sérgio Loubet Filho, Thayná Gil Santos, Vitória Helena de Oliveira Teixeira Reis, Cynthia Monteiro Santee, Matheus Pereira da Costa, Camila Jordão Cândido, Wander Fernando de Oliveira Filiú, Luciane Candeloro Portugal, Elisvânia Freitas dos Santos

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Trace elements homeostasis in brain exposed to 900 MHz RFW emitted from a BTSantenna model and the protective role of vitamin E

Mansour Azimzadeh, Gholamali Jelodar

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Efficacy of some feed additives to attenuate the hepato-renal damage induced by aflatoxin B1 in rabbits

Ismail E. Ismail, Mayada R. Farag, Mahmoud Alagawany, Hemat K. Mahmoud, Fayiz M. Reda

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Urinary excretion of advanced glycation end products in dogs and cats

Pornsucha Palaseweenun, Esther A. Hagen-Plantinga, J. Thomas Schonewille, Gerrit Koop, Claire Butre, Melliana Jonathan, Peter A. Wierenga, Wouter H. Hendriks

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Effects of increasing alfalfa (*Medicago sativa*) leaf levels on the fattening and slaughtering performance of organic broilers

Lydia Pleger, Petra Nicole Weindl, Peter Andreas Weindl, Luz Salomé Carrasco, Céline Leitao, Minjie Zhao, Benjamin Schade, Karen Aulrich, Gerhard Bellof

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SHORT COMMUNICATION

Exogenous melatonin and male foetuses improve the quality of sheep colostrum

José-Alfonso Abecia, Claudia Garrido, Marianne Gave, Ana-Isabel García, David López, Silvia Luis, José-Antonio Valares, Luis Mata

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Efficacy of ajwain (*Trachyspermum ammi* L.) seed at graded levels of dietary threonine on growth performance, serum metabolites, intestinal morphology and microbial population in broiler chickens

Alireza Kolbadinejad, Vahid Rezaeipour

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Dynamic changes in circulating levels of metabolites in the portal-drained viscera of finishing pigs receiving acute administration of Larginine

Jianying Yang, Bi'e Tan, Qinghua He, Yulong Yin, Guoyao Wu, Xiangfeng Kong

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Dietary guanidinoacetic acid improves the growth performance and skeletal muscle development of finishing pigs through changing myogenic gene expression and myofibre characteristics

Yafei Lu, Tiande Zou, Zirui Wang, Jin Yang, Lanhai Li, Xiaobo Guo, Qin He, Liling Chen, Jinming You

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Productive performance, lipid profile and caecum microbial counts of growing rabbits treated with humic acid

Shimaa Abdl Razek Mohmed Mohmed, Azza Elsebai, Osama A. Elghalid, Ahmed M. Abd El-Hady

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ORIGINAL ARTICLE

Adaptation of aerobic training essentially involved autophagy, mitochondrial marker and muscle fibre genetic modulation in rat cardiac muscles

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Abstract

Information about the role of moderate acute treadmill training in modulating autophagy and mitochondrial markers that might be correlated with alteration of muscle fibre gene expression in rat cardiac muscles is very limited. In this present study, the researchers divided twenty male Wistar rats into four groups: sedentary control, 3, 6 and 15 days and subjected them to treadmill training with moderate intensity (20 m/ min), 30 min each day. RNA was extracted from cardiac muscles and stored in temperature of -80°C. Specific primers were utilized for semi-quantitative PCR. Treadmill training decreased autophagy-related gene expression (LC3, p62) and upper stream signalling of autophagy (PIK3CA, Akt and mTOR) in 3 and 6 d, but stimulated gene expression of mitochondrial markers (PGC1 α , Cox1, Cox2 and Cox4) in 15 days. α MHC gene expression increased while β MHC gene expression decreased in 15 days. In line with this, autophagy-related genes increased in 3 and 6 days and returned to baseline in 15 days. The increment in mitochondrial gene expression might be correlated with shifting gene expression of α MHC and β MHC in 15 days. Taken together, acute adaptation in cardiac muscles is stimulated by genetic modulation of autophagy, mitochondrial marker and muscle fibre that may explain physiological cardiac adaptation after 2 WILEY Animal Physiology and Animal Nutrition

training. This study can be used as a reference for optimizing performance in period of cardiac muscle adaptation stimulated by treadmill training.

KEYWORDS

adaptation, autophagy, cardiac, mitochondria, myosin heavy chain, training

1 | INTRODUCTION

Regular exercise improves physical health especially the cardiovascular system that protects the heart from injury (Platt, Houstis, & Rosenzweig, 2015; Wei, Liu, & Rosenzweig, 2015). During aerobic exercise, such as treadmill training, there are several phases of cardiac adaptation. It starts with immediate improvement of the cardiac function, increasing the heart rate and stroke volume that augments the cardiac output (Fulghum & Hill, 2018; Weiner & Baggish, 2012), and then followed by an increase in the vascular function together with cardiac output to increase blood flow to skeletal muscles (Fulghum & Hill, 2018). However, the molecular mechanism that explains mitochondria and muscle fibre genetic modulation correlated with autophagy in cardiac muscles during early treadmill training adaptation remains unclear. In order to cope with cellular stress induced by training, cardiac adaptation might involve cell death that occurs in several ways, such as apoptosis, necrosis and potentially autophagy (Bernardo, Ooi, Weeks, Patterson, & Mcmullen, 2018). Autophagy may play an important role to enhance optimal adaptation to damage due to cellular stress since cardiomyocyte does not have a regenerative capacity (Lee et al., 2017). Autophagy is a generic term for all pathways by which cytoplasmic materials are delivered to the lysosome in animal cells (Mizushima & Komatsu, 2011). Autophagy is divided into macroautophagy, micro-autophagy and chaperone-mediated autophagy. In macroautophagy, initiation of autophagy is carried out by a phagophore, a non-selective sequestering cytosolic substrate within a double membrane, and then conjugated with ubiquitination-linked proteins, together with light chain 3 (LC3) and p62/sequestosome 1 in a structure called autophagosome. Then, autophagosome will be fused to lysosome, where the

TABLE 1 Primers used for semi-quantitative PCR analysis

Gene symbol	Primer Sequence (5′–3′) Upper strand: sense Lower strand: antisense	Product size (bp)	Annealing (°C)	Cycle	Accession number	References
PIK3CA	ACCTCAGGCTTGAAGAGTGTCG	137	59	35	NM_133399.2	Krusen et al. (2016)
	CCGTAAGTCGTCGCCATTTTTA					
Akt	CTAACTTGAGCCGCAGGAAC	165	57	35	XM_008764918.2	Primer3
	GCTTGCTCAGTTTGCTACCC					
mTOR	CTGATGTCATTTATTGGCACAAA	170	57	35	NM_019906.1	Yin et al. (2013)
	CAGGGACTCAGAACACAAATGC					
LC3	GGTCCAGTTGTGCCTTTATTGA	153	59.5	35	NM_022867.2	Yin et al. (2013)
	GTGTGTGGGTTGTGTACGTCG					
p62	CTAGGCATCGAGGTTGACATT	116	56	35	NM_175843.4	Kowalik et al. (2016)
	CTTGGCTGAGTACCACTCTTATC					
PGC1a	CGCACAACTCAGCAAGTCCTC	263	62	37	NM_031347.1	Sylviana et al. (2018)
	CCTTGCTGGCCTCCAAAGTCTC					
Cox1	GGAGCAGTATTCGCCATCAT	244	55.3	35	X14848	Javadov, Purdham, Zeidan,
	CGGCCGTAAGTGAGATGAAT					and Karmazyn (2006)
Cox2	ACTTGGCTTACAAGACGCTACA	162	56.7	35	S67722.1	Primer3
	TCTTGGGCGTCTATTGTGCTT					
Cox4	CTCCCATCTTATGTTGATCG	144	57	35	NM_017202.1	Lesmana, Sinha, et al.
	GTACAATTGGACTTTCTCATCC					(2016)
αMHC	GCCCTTTGACATCCGCACAGAGT	152	60	35	NM_017239.2	Radik, Doka, Malikova,
	TCTGCTGCATCACCTGGTCCTCC					Krenek, and Klimas (2016)
βΜΗC	GCGGACATTGCCGAGTCCCAG	133	59.5	35	NM_017240.2	Radik et al. (2016)
	GCTCCAGGTCTCAGGGCTTCACA					
GAPDH	GTTACCAGGGCTGCCTTCTC	177	61	35	NM_017008.4	Wang et al. (2017)
	GATGGTGATGGGTTTCCCGT					



FIGURE 1 Rat body weight and heart weight during treadmill training after several periods of training. Baseline characteristics of the rats in each group. (a) Body weight of each group of the rats. (b) Heart weight of each group of the rats. Data are presented as average mean ± standard error of the mean (*SEM*)

substrate is then degraded by enzymes (Mizushima & Komatsu, 2011; Pankiv et al., 2007).

Stimulation of autophagy is important and essential for mitochondrial biogenesis and activity in skeletal muscles. The role of autophagy in muscle adaptation is connected with autophagy-induced mitochondrial biogenesis in skeletal muscles (Lesmana, Sinha, et al., 2016). Autophagy in mitochondria (mitophagy) determines mitochondrial function and content in skeletal muscles (Chen, Erlich, & Hood, 2018). Mitophagy is a selective autophagy of mitochondria that eliminates damaged mitochondria, as part of quality control mechanism. There is an interplay between mitochondrial dynamics—their movements, fissions and fusions—and the progression of mitophagy; arrest and fragmentation of mitochondria after damage are likely to facilitate the selective clearance of damaged organelles (Ashrafi & Schwarz, 2013; Bloemberg & Quadrilatero, 2019). Mitochondrial biogenesis is regulated by peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC1 α) (Fernandez-Marcos & Auwerx, 2011; Puigserver Journal of d Animal Nutrition

et al., 1998), while mitochondrial energy metabolism is assessed using Cytochrome c oxidase (Cox) enzyme activity (Kadenbach, Hüttemann, Arnold, Lee, & Bender, 2000). Another important process that plays a role in the adaptation to aerobic training in skeletal muscles is fibre type transformation (Farenia et al., 2019; Yan, Okutsu, Akhtar, & Lira, 2011). This study hypothesizes that adaptation of skeletal muscle by autophagy may also have a role in cardiac muscles adaptation.

Cardiac muscles fibres have myosin heavy chain (MHC) as a chemical and mechanical transducer to drive the sliding of myofilaments (Nadal-Ginard & Mahdavi, 1989). MHC provides structural integrity, and its isoform is a major determinant of contractile and functional properties. Cardiac MHC isoforms exist as homodimer or heterodimer, and a unique gene encodes each subunit. At the protein level, cardiac α or β MHC proteins have a high degree (94%) of sequence identity, and divergence only occurs in clusters, some of which are functionally important sites such as the adenosine triphosphatase (ATPase) catalytic site (Mcnally, Kraft, Bravo-Zehnder, Taylor, & Leinwand, 1989; Rafalski, Abdourahman, & Edwards, 2007). α MHC has twofold to threefold higher actinactivated ATPase activity and actin filament sliding velocity than β MHC (Herron & Mcdonald, 2002; Krenz & Robbins, 2004).

Recent studies have shown that aerobic training induces autophagy gene expression in skeletal muscles (Tarawan et al., 2019) and in cardiac muscles (He et al., 2012; Lee et al., 2017). Aerobic training stimulated mitochondrial biogenesis markers and increased PGC1α levels in cardiac muscles (Kim, Ahn, & Jung, 2018). Exercise increased α MHC while repressing β MHC genes in rats compared with sedentary control in infracted heart (Hashimoto, Kambara, Nohara, Yazawa, & Taguchi, 2004; Wan, Xu, Zhao, Garza, & Zhang, 2014). Cardiac muscle fibres may shift MHC isoforms (from α MHC to βMHC) as a compensatory response to training, due to increased glycolytic demand and lack of functional capacity in the muscle fibre. This shift occurs in early period and might be responsible in increasing myocardial contractility and preserving cardiac functions (Rafalski et al., 2007). Taken together, modulation of autophagy, mitochondrial marker and muscle fibres may take part in cardiac muscles adaptation stimulated by training.

2 | MATERIALS AND METHODS

2.1 | Animals

Ten-weeks-old male Wistar rats were obtained from the Animal Breeding Centre of PT Biofarma in Bandung, Indonesia. The baseline characteristics for the rats were 10 ± 2 weeks in term of age and 287.8 \pm 4.74 g in term of weight. In terms of environment, the rats were subjected to a dark light cycle (12 hr of light cycle and 12 hr of dark cycle) and a stable temperature (± 22 °C). The rats were fed a pellet rodent diet ad libitum and had free access to water. After one week of acclimatization, 20 male rats were divided into four groups, sedentary control group and three training groups (3, 6 and 15 days). All experimental procedures followed the guidelines for the care and use of laboratory animals (National Research Council, 2011)



FIGURE 2 Aerobic training modulates autophagy-related gene expression in physiologic adaptation of rat cardiac muscles. (A) Representative electrophoresis result of autophagy-related gene expression in 3, 6 and 15 days after treadmill training. (B) Relative mRNA expression of LC3 was significantly decreased in 3 and 6 days (a) compared with control, and between 3 and 15 days (b), 6 and 15 days (c). (C) Relative mRNA expression of p62 was significantly decreased in 3 days (a) and 6 days (b) compared with control, and between 3 and 15 days (c), and 6 and 15 days (d). Data are presented as average mean ± standard error of the mean (*SEM*); with *p* < .05 considered as significant (**)

and approved by Research Ethics Committee of Faculty of Medicine Universitas Kristen Maranatha-Rumah Sakit Immanuel Bandung No 013/KEP/V/2019.

2.2 | Treadmill training protocol

A moderate treadmill training protocol (20 m/min), 30 min a day was designed, for three different groups of male Wistar rats, divided into three periods: 3, 6 and 15 days. The study chose moderate tread-mill training based on a previous study in rats (Gunadi et al., 2019; Lesmana, Iwasaki, et al., 2016). For the control group, the rats were put on the treadmill without training. After given one week to acclimatize, the rats were put on the treadmill for 3 days with increasing speed and time. The rats were subjected to the treadmill for 30 min a day and a speed of 20 m/min. They were then killed in 3, 6 and 15 days immediately after the last session of the training. All their body weights were monitored and recorded weekly. Control and training

groups were euthanized using isoflurane gas, and then, their cardiac muscles were removed, snap-frozen in liquid nitrogen and stored in temperature of -80°C until used. The researchers who analysed the tissues did not know to which group the rats belonged.

2.3 | RNA extraction and semi-quantitative PCR

RNA from cardiac muscles was extracted using TRIsure reagent (Bioline). Total RNA was measured using Multimode Microplate Reader at 268/280 nm absorbance spectrophotometry (M200 Pro; Tecan). Semi-quantitative PCR was performed using the One-Step RT-PCR Kit (Bioline, United Kingdom). Gene expression levels were normalized using GAPDH. BluePad Detection system was used to visualize electrophoresis gels. Visualization and quantification of PCR band were done using ImageJ 1.52 version (Schneider, Rasband, & Eliceiri, 2012). Primer sequences used in this study are presented in Table 1.

5



FIGURE 3 Aerobic training modulates PIK3CA, Akt and mTOR signalling gene expression that is involved in modulating autophagy. (A) Representative electrophoresis result of PIK3CA, Akt and mTOR signalling gene expression in 3, 6 and 15 days after treadmill training. (B) Relative mRNA expression of PIK3CA was significantly decreased in 6 days compared with control (a), between 3 and 15 days (b), and between 6 and 15 days (c). (C) Relative mRNA expression of Akt was significantly decreased in 3 days (a), 6 days compared with control (b), and between 3 and 15 days (c). (D) Relative mRNA expression of mTOR was significantly decreased in 3, 6 days compared with control (a), and between 3 and 15 days (b), and 3 and 15 days (c). Data are presented as average mean ± standard error of the mean (SEM), with p < .05 considered as significant (*), p < .01 considered as very significant (**)

2.4 | Statistics

SPSS 19.0 software was used to analyse the data obtained in this study. Results were presented as mean \pm standard error of mean (mean \pm SEM). Mean differences between groups were examined with one-way ANOVA and LSD post hoc test with 95% confidence interval with p < .05.

3 | RESULTS

3.1 | There were no significant differences in body weight and heart weight

The rats from the four experimental groups had similar initial body weight (287.8 \pm 4.74 g). There were no significant differences found

in body weight (Figure 1a) and heart weight (Figure 1b) between 3, 6 and 15 days training groups and control after the rats were subjected to treadmill training for 30 min/day with speed of 20 m/ minute.

3.2 | Training stimulated dynamic modulation of autophagy-related gene expressions in cardiac muscles of Wistar rats

There was modulation of autophagy-related gene expression in cardiac muscles of Wistar rats (Figure 2a). LC3 and p62 bands were normalized using GAPDH. Treadmill training decreased autophagy-related gene expression: LC3 by 0.897-fold in 3 days and 0.9 in 6 days (Figure 2b) and p62 by 0.874-fold in 3 days and 0.913 in 6 days (Figure 2c).



FIGURE 4 Modulation of mitochondrial marker gene expression during aerobic training adaptation in rat cardiac muscles. (A) Representative electrophoresis result of mitochondrial marker gene expression in 3, 6 and 15 days after treadmill training. (B) Relative mRNA expression of PGC1 α was significantly increased in 15 days compared with control (a), and between 3 and 15 days (b). (C) Relative mRNA expression of Cox1 was significantly increased in 6 days (a) and 15 days (b) compared with control. (D) Relative mRNA expression of Cox2 was significantly increased in 15 days compared with control (a). (E) Relative mRNA expression of Cox4 was significantly increased in 15 days compared with control (a). Data are presented as average mean ± standard error of the mean (*SEM*); with *p* < .05 considered as significant (**)

(B)

3.3 | Upper stream signalling of autophagy was also modulated after training in cardiac muscles of Wistar rats

This study explored autophagy upper stream signalling and observed the modulation of PIK3CA-Akt-mTOR in cardiac muscles of Wistar rats (Figure 3a). PIK3CA, Akt and mTOR bands were normalized using GAPDH. Treadmill training decreased autophagy upper stream signalling gene expression in 3 and 6 days: PIK3CA by 0.894-fold in 6 days (Figure 3b), Akt by 0.807-fold in 3 days and 0.813-fold in 6 days (Figure 3c), and mTOR by 0.844-fold in 3 days and 0.870-fold in 6 days (Figure 3d).

3.4 | Training induced mitochondrial markers gene expressions in cardiac muscles of Wistar rats

Mitochondrial markers gene expressions were upregulated in rat cardiac muscles through training (Figure 4a). PGC1a, Cox1, Cox2 and Cox4 bands were normalized using GAPDH. This study found increased gene expressions for mitochondrial markers in 15 days: PGC1 α increased by 1.111-fold in 15 days (Figure 4b), Cox1 increased by 1.218-fold in 15 days (Figure 4c), Cox2 increased by 1.148-fold in 15 days (Figure 4d) and Cox4 increased by 1.173-fold in 15 days (Figure 4e).

3.5 | Training increased α MHC and decreased βMHC gene expressions in cardiac muscles of Wistar rats

This study also examined muscle fibre gene expression in cardiac muscles of Wistar rats. α MHC and β MHC bands were normalized using GAPDH (Figure 5a). This study found increase in α MHC gene expression by 1.145-fold in 6 days and 1.314-fold in 15 days (Figure 5b) and decrease β MHC gene expression by 0.907-fold in 3 days and 0.887-fold in 15 days (Figure 5c).

3.6 Summary of gene expression modulation after aerobic training in rat cardiac muscle

Adaptation to aerobic training essentially involved autophagy, mitochondrial marker and fibre shifting in rat cardiac muscles. Autophagy







FIGURE 6 Summary of gene expression modulation after aerobic training in rat cardiac muscles. (a) Dynamic modulation description of autophagy, mitochondrial marker and muscle fibre gene expression in cardiac muscles during adaptation to aerobic training. (b) Proposed mechanism of autophagy, mitochondrial marker, and muscle fibre gene expression in cardiac muscles during adaptation to aerobic training

started to increase in 3 and 6 days, then back to baseline in 15 days, while mitochondrial marker and fibre shifting started to occur in 15 days (Figure 6a). The proposed mechanism for this modulation is described in Figure 6b.

4 | DISCUSSION

Cardiac myocytes in adult rat heart represent 70%–85% of the heart's volume, but possess extremely limited regenerative capacity. Thus, it is important to preserve the function of cardiac myocytes to maintain healthy living (Lee et al., 2017; Pinto et al., 2016). There are some key processes in adaptation of training in cardiac myocytes including cellular stress response (proteasome-ubiquitin system, autophagy and apoptosis), mitochondrial adaptation (energy metabolism and redox status) and fibre shifting (Bernardo et al., 2018; Yan et al., 2011). However, the understanding about coregulation of these systems during aerobic training adaptation remains unclear.

This study suggests a time-dependent modulation of autophagy, mitochondrial biogenesis and fibre shifting gene expression during treadmill as an adaptation processes in cardiac muscles of Wistar rats. We try to understand how cardiac muscle autophagy related to and signalling gene expression via PI3KCA-Akt-mTOR are altered by aerobic training at certain periods (3, 6 and 15 days). The results indicate that autophagy increased significantly in 3 and 6 days and then returned to baseline in 15 days supported by decreased gene expression of LC3, p62, PI3KCA, Akt and mTOR in 3 and 6 days (Figures 2 and 3). Other studies have also shown that endurance exercise training induces autophagy in cardiac muscles (He et al., 2012; Lee et al., 2017).

Autophagy plays a role in regulating mitochondrial markers such as PGC1 α and Cox enzyme activity (Lesmana, Sinha, et al., 2016). These markers increased due to aerobic training in 15 days (Figure 4). The previous study conducted by Kim et al have also shown that during chronic aerobic training, mitochondrial biogenesis markers were upregulated in the cardiac muscle (Kim et al., 2018).

An interesting physiological transition of cardiac muscle gene expression stimulated by aerobic training was observed, proved by an increase of α MHC and decrease of β MHC in 15 days (Figure 5). Other studies showed that exercise significantly increased α MHC while repressing β MHC gene expression in exercise rats compared with sedentary control, and this shift occurred in early period (Hashimoto et al., 2004; Rafalski et al., 2007; Wan et al., 2014).

This study showed that autophagy, mitochondrial biogenesis and fibre shifting might be correlated and jointly regulated by aerobic training. Autophagy genes increased in 3 and 6 days (Figures 2 and 3), while genes responsible for mitochondrial function (Figure 4) and fibre shifting (Figure 5) were stimulated in 15 days. A summary of this modulation and its proposed mechanism is presented in Figure 6. However, other factors may also regulate these processes and need to be investigated, such as temperature (Puigserver et al., 1998) and nitric oxide (Tengan, Rodrigues, & Godinho, 2011). We had sacrificed all the rats immediately after the last session of the training and reduce all confounding factors by using rats with the same age, standardized food, controlled environment and same intensity of training induction. However, gene expression might be altered by other factors due to posttranscriptional modifications. Thus, it may be beneficial to measure the protein levels in future studies.

5 | CONCLUSION

This study suggests a time-dependent modulation of autophagy, mitochondrial biogenesis and fibre shifting during treadmill adaptation in cardiac muscles of Wistar rats. Autophagy increased in 3 and 6 days and returned to baseline after 15 days of treadmill training. Mitochondrial biogenesis increased after 15 days of treadmill training, along with shift of β MHC to α MHC in cardiac muscles of Wistar rats. The study believes that the adaptation period needs a specific genetic modulation, protein and tissue rearrangements, to improve functional capability improvement. An athlete's performance can be optimized through a time structured training programme by understanding the physiological adaptation period in cardiac muscles.

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Journal of and Animal Nutrition

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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10

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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