2020

RESEARCH SHOWCASE

ADVANCING KNOWLEDGE

POULTRY INNOVATION PARTNERSHIP

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ADVANCING THE CANADIAN POULTRY ENTERPRISE

The Poultry Innovation Partnership (PIP) is a collaboration of the Poultry Industry, Government of Alberta and University of Alberta created to foster a healthy Canadian poultry enterprise. Excellence in research and innovation, knowledge management, technology transfer and mentoring tomorrow's poultry professionals are the Centre's hallmark.

Together, PIP partners collaborate to create an environment where research, extension and tech transfer can flourish and grow far beyond the reach of a single entity. This research showcase highlights the cutting-edge research published in 2022 by Poultry Innovation Partnership scientists.

The University of Alberta and the poultry industry have a long history of collaborating on leading edge research. Each partner brings diverse knowledge, skills, resources and perspectives in exploring solutions to systems-level issues such as achieving high standards for animal care, respect for the environment and genetic resources, economic efficiency and poultry by-product utilization. The Showcase covers the entire spectrum of research from discovery to farm-ready application.

'This Research Showcase highlights what is possible when academia, industry and farmers work together to explore and develop solutions within the unique Western Canadian context.'

Dr. Valerie Carney, Poultry Innovation Partnership Lead

Contents

IMPACT OF BROILER BREEDER GROWTH TRAJECTORY ON PLASMA CORTICOSTERONE CONCENTRATION: A COMPARISON OF ANALYTICAL METHODS

M. Afrouziyeh, M.J. Zuidhof

KEYWORDS: broiler breeder, corticosterone, ELISA, metabolomics, feed restriction

ABOUT

Blood concentration of corticosterone (CORT) is a measure of welfare in feed-restricted broiler breeders. The RIA and ELISA have been routinely used for measuring CORT in blood, excreta, and feather. Due to the presence of some confounding factors in the methods mentioned, this study had the objective to 1) determine correlation between plasma concentrations of CORT measured by ELISA and LC-MS/MS methods and 2) investigate the effects of the high and low photostimulation BW and breeder age on plasma CORT levels.

APPROACH

A total of 36 broiler breeder pullets were used, of which 30 were randomly assigned to one of 10 unique growth trajectories, and 6 were assigned to an unrestricted group. We designed the growth trajectories using a 3-phase Gompertz growth model with 10 levels of BW gain in the prepubertal and pubertal growth phases, ranging from the breeder-recommended target BW (CON) to 22.5% higher (CON+22.5%), in 2.5% increments. The BW trajectories were applied to each individual bird using a precision feeding (PF) system, which collected BW and feed intake data. The birds were classified based on age at first egg (AFE), and 12 birds each having the highest and lowest AFE were selected for the CORT study. Then median photostimulation BW of the candidate birds was used to define the upper (heavy BW) and lower (standard BW) extremes and plasma CORT levels were evaluated by ELISA and LC-MS/MS methods from their blood collected at 18, 20, 22, 24, and 26 wk of age.

ANALYSIS OF RESULTS

Concentrations of plasma CORT measured using ELISA method were highly correlated ($r = 0.95$; $P < 0.001$) with values measured using LC-MS/MS method, validating interchangeably usage of both methods to measure plasma CORT in broiler breeders. Plasma CORT levels were not affected by photostimulation BW or breeders' age, indicating same welfare status between the precision-fed high and low BW groups. Probably, because the PF system provided frequent meals per day, the length of fasting between meals was not long enough to affect the plasma CORT level.

APPLICATION

The results of this experiment indicate that there is a highly positive correlation between CORT measures using ELISA and LC-MS/MS methods. This suggests that both methods are valid for measuring plasma CORT.

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PLASMA METABOLOMIC PROFILING REVEALS POTENTIAL ONSET OF LAY BIOMARKERS IN BROILER BREEDERS

M. Afrouziyeh, N.M. Zukiwsky, D.R. Korver, M.J. Zuidhof

KEYWORDS: parent stock, sexual development, metabolomics, metabolic status, maturation

ABOUT

Broiler breeder pullets need to reach a certain metabolic status to achieve sexual maturation at photostimulation. Variations in nutrient intake and subsequent energy status are communicated to the liver and hypothalamic-pituitary axis by alterations in the plasma levels of hormones. Reproductive system maturation and changes in some plasma metabolites and reproductive hormones occur during the pullet-to-hen transition period. Profiling the plasma metabolome may provide a new perspective for studying the metabolic response of sexual maturity in breeders, a better understanding of its biological mechanisms, and potential biomarkers for predicting the onset of lay. The objectives of the current study were to evaluate the effect of lay status (pullet vs. hen), photostimulation BW, and onset of lay timing (early vs. late) on plasma metabolomic dynamics to identify potential biomarkers of sexual development in broiler breeders.

APPROACH

A total of 36 pullets were used, in which 30 pullets were randomly assigned to one of 10 unique growth trajectories and 6 birds were assigned to an unrestricted group. The growth trajectories were designed using a 3-phase Gompertz growth model with 10 levels of BW gain in the prepubertal and pubertal growth phases ranging from the breeder-recommended target BW to 22.5% higher, in 2.5% increments. The BW trajectories were applied to each individual bird using a precision feeding (PF) system, which collected BW and feed intake data for each individual bird. The birds were classified based on age at first egg (AFE), and 12 pullets were chosen from the lower and upper AFE extremes (early and late onset of lay) at 18, 20, 22, 24, and 26 wk of age to run repeated blood plasma metabolomic assays.

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ANALYSIS OF RESULTS

Univariate analysis identified 87 differential metabolites between the early- and late-onset of lay groups at 24 wk of age and 104 differential metabolites between the pullet and hen groups. Further investigation of differential metabolites showed 15 potential biomarkers for pullet to hen transition by analyzing the receiver operating characteristic (ROC) curve, mainly consisting of carnitine and choline metabolites. Differential metabolites during the pullet-to-hen transition were mainly associated with lipid, energy, and amino acid metabolism pathways, which gave clues to the physiological and metabolic shifts resulting from sexual maturation. At 24 wk of age, the main pathways involved in differentiation of the early- and late-onset of lay groups were related to lipid and amino acid metabolism. These metabolites could be involved in biosynthesis of egg yolk precursors in the liver.

APPLICATION

In conclusion, this study indicated that metabolic transition during the onset of lay in broiler breeders is accompanied by certain metabolic signatures that can be used to predict the metabolic status linked to the bird's maturity. More research is needed to reach the goal of designing a point-of-care device (similar to a portable blood glucometer) to measure broiler breeder plasma metabolome in real-time at the flock level and in a matter of seconds. Thereby, the poultry industry can use the extracted data to evaluate sexual development status in a flock.

ARCHITECTURE OF BROILER BREEDER ENERGY PARTITIONING MODELS

M. Afrouziyeh, N.M. Zukiwsky, J. Youi, D.R. Korver, M.J. Zuidhof

KEYWORDS: broiler breeder, feed restriction, energy partitioning model, random term, prediction optimization

ABOUT

A robust model that estimates the ME intake over broiler breeder lifetime is essential for formulating diets with optimum nutrient levels. The objectives of the current study were to 1) evaluate inclusion of random terms associated with individual MEm, ADG, and age in a ME partitioning model on residual dependency, model fitting and predictive performance; 2) evaluate how including random terms associated with individual maintenance ME, ADG, and age could bias the ME partitioning model; 3) evaluate the effect of chunking BW, ADG, and egg production data into different chunk sizes (daily, 4-d, weekly, 2-wk, or 3-wk) on fitting and predictive performance of ME partitioning model; and 4) determine the effect of an increased (10%) prepubertal BW gain and earlier pubertal phase growth on energy efficiency of broiler breeders.

APPROACH

The experiment was conducted as a randomized controlled trial with 40 Ross 708 broiler breeder pullets reared on 1 of 10 target growth trajectories, which were designed with 2 levels of cumulative BW gain in prepubertal growth phase and 5 levels of timing of growth around puberty. The BW trajectories were implemented for each individual bird using a PF system.This study investigated the effect of growth pattern on energy efficiency of birds and tested the effects of dividing data into daily, 4-d, weekly, 2-wk, and 3-wk periods and the inclusion of random terms associated with individual maintenance ME and ADG requirements, and age on ME partitioning model fit and predictive performance.

ANALYSIS OF RESULTS

Model [I] was: $MEI_d = a \times BW^b + c \times ADG_p + d \times ADG_n + e \times EM + E$, where MEI_d was daily ME intake (kcal/d); BW in kg; ADG_p was positive ADG; ADG_n was negative ADG (g/d); EM was egg mass (g/d); E was the model residual. Models [II to IV] were nonlinear mixed models based on the model [I] with inclusion of a random term for individual maintenance requirement, age, and ADG, respectively. Model [II] – 3 wk was chosen as the most parsimonious based on lower autocorrelation bias, closer fit of the estimates to the actual data (lower model MSE and closer R2 to 1), and greater predictive performance among the models. Estimated ME partitioned to maintenance in model [II] – 3 wk was 100.47 ± 7.43 kcal/$kg^{0.56}$, and the ME requirement for ADG_p, ADG_n, and EM were 3.49 ± 0.37; 3.16 ± 3.91; and 2.96 ± 0.13 kcal/g, respectively. Standard treatment had lower residual heat production (RHP; -0.68 kcal/kg $BW^{0.56}$) than high early growth treatment (0.79 kcal/kg $BW^{0.56}$), indicating greater efficiency in utilizing the ME consumed. Including random term associated with individual maintenance ME in a 3-wk chunk size provided a robust, biologically sound life-time energy partitioning model for breeders.

APPLICATION

To increase robustness of broiler breeder energy partitioning models, a novel chunking procedure was applied on precision feeding system data. Increasing chunk size of data provided closer fit of the models estimated coefficients to the actual data by accounting for more variation in the residuals. A mixed effect ME partitioning model containing a random term associated with individual maintenance requirement in a 3-wk chunked data (model [II] – 3wk) increased inferential efficiency. The model can be used as a tool to estimate ME requirements and to facilitate choosing a precise energy level in feed formulation practices. Furthermore, applying Ross 708 guideline data in the model suggested a revision on the breeder-recommended target BW. The current study results indicated that an earlier pubertal growth strategy could reduce energy efficiency in broiler breeders.

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EVOLUTION OF MATERNAL FEED RESTRICTION PRACTICES OVER 60 YEARS OF SELECTION FOR BROILER PRODUCTIVITY

V.L. Carney, F.E. Robinson, B.L. Reimer, D.R. Korver, M. J. Zuidhof, M. Afrouziyeh

KEYWORDS: broiler breeder, sexual maturity, quantitative feed restriction, genetic progression, feed efficiency

ABOUT

After decades of quantitative genetic selection, modern broiler chickens have substantially greater growth rates, carcass yield and feed. The objectives of the current study were to re-evaluate the growth, efficiency, and yield characteristics of 4 strains of random-bred broiler breeders representing genetics from 1957, 1978, 1995, and 2015 and also to investigate the timing of sexual maturation of female stocks under conditions of ad libitum feeding or nutrient restriction to a modern broiler breeder BW standard. Furthermore, the study evaluated how the degree of feed restriction has changed over the last seven decades to maintain birds on a modern-day broiler breeder target BW.

APPROACH

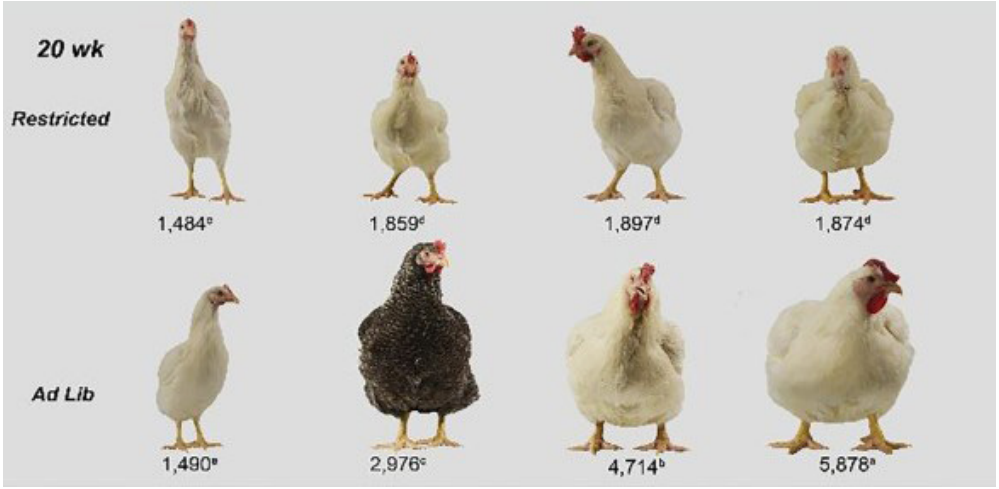
The effect of commercial selection by poultry breeders on the growth, efficiency, and sexual maturity of broiler breeders was studied using 2 University of Alberta Meat Control strains unselected since 1957 and 1978 (AMC-1957 and AMC-1978, respectively) and 2 strains originating from the University of Arkansas; 1995 Random-bred (1995RB) and 2015 Random-bred (2015RB). A study with a 4 × 2 factorial arrangement was conducted with the 4 strains fed at either ad libitum, or restricted levels to achieve a current commercial breeder target BW profile. Growth rate, feed intake, feed efficiency, age at sexual maturity, carcass components, and body conformation were measured. To assess reproductive development, birds were assigned to 2 fates: dissected at photostimulation or dissected after the second oviposition.

ANALYSIS OF RESULTS

At 22.4 wk of age, the restricted-fed AMC-1957, AMC-1978, 1995RB, and 2015RB reached 100, 61, 46, and 38% of their ad libitum-fed counterparts' BW, respectively. During the rearing phase, the amount of feed needed to maintain restricted-fed birds on the target BW was 99.4, 57, 29.5, and 24.9% of their ad libitum-fed counterparts for AMC-1957, AMC-1978, 1995RB, and 2015RB, respectively. Feed restricted birds in the 2015RB had lower heat production relative to the AMC-1957 and AMC-1978, which shows that modern strains utilized feed more efficiently compared to the antique strains. This might be related to the increasing severity of feed restriction of broiler breeders over the past 60 years. Relative to AMC-1957 and AMC-1978 strains, the 1995RB and 2015RB strains had heavier breast muscle and lower fat pad weight.

APPLICATION

Although the pubertal threshold for age and BW have increased over the last 6 decades, changes in selection programs for feed efficiency have resulted in broiler breeders that prioritize nutrient allocation to growth and breast development rather than adipose storage. As a result, feed restricted modern broiler breeders may have marginally sufficient fat resources to support reproduction. Thus, potential strategies to optimize broiler breeder growth trajectory should be considered to alleviate the negative effects of severe feed restriction on broiler breeders. More research is needed to optimize broiler breeder feeding programs.



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Restricted and ad lib weight in 4 different strains
V.L. Carney et al, Poultry Science 101 (2022)

SYSTEMATIC PROFILING OF THE CHICKEN GUT MICROBIOME REVEALS DIETARY SUPPLEMENTATION WITH ANTIBIOTICS ALTERS EXPRESSION OF MULTIPLE MICROBIAL PATHWAYS WITH MINIMAL IMPACT ON COMMUNITY STRUCTURE

K. Nadeau, D.R. Korver

KEYWORDS: chicken, gut mibrobiome, antibiotics, growth promoters, dietary supplementation

ABOUT

The emergence of antimicrobial resistance is a major threat to global health and has placed pressure on the livestock industry to eliminate the use of antibiotic growth promotants (AGPs) as feed additives. To mitigate their removal, efficacious alternatives are required. AGPs are thought to operate through modulating the gut microbiome to limit opportunities for colonization by pathogens, increase nutrient utilization, and reduce inflammation. However, little is known concerning the underlying mechanisms. Previous studies investigating the effects of AGPs on the poultry gut microbiome have largely focused on 16S rDNA surveys based on a single gastrointestinal (GI) site, diet, and/or timepoint, resulting in an inconsistent view of their impact on community composition. Our aim was to perform a comprehensive investigation of the role of diet, age, and AGP treatment on community structure across the chicken GI tract.

APPROACH

In this study, we perform a systematic investigation of both the composition and function of the chicken gut microbiome, in response to AGPs. Birds were raised under two different diets and AGP treatments, and 16S rDNA surveys applied to six GI sites sampled at three key timepoints of the poultry life cycle. Functional investigations were performed through metatranscriptomics analyses and metabolomics.

ANALYSIS OF RESULTS

Our study reveals a more nuanced view of the impact of AGPs, dependent on age of bird, diet, and intestinal site sampled. Although AGPs have a limited impact on taxonomic abundances, they do appear to redefine influential taxa that may promote the exclusion of other taxa. Microbiome expression profiles further reveal a complex landscape in both the expression and taxonomic representation of multiple pathways including cell wall biogenesis, antimicrobial resistance, and several involved in energy, amino acid, and nucleotide metabolism. Many AGP-induced changes in metabolic enzyme expression likely serve to redirect metabolic flux with the potential to regulate bacterial growth or produce metabolites that impact the host.

APPLICATION

As alternative feed additives are developed to mimic the action of AGPs, our study highlights the need to ensure such alternatives result in functional changes that are consistent with site-, age-, and diet-associated taxa. The genes and pathways identified in this study are therefore expected to drive future studies, applying tools such as community-based metabolic modeling, focusing on the mechanistic impact of different dietary regimes on the microbiome. Consequently, the data generated in this study will be crucial for the development of next-generation feed additives targeting gut health and poultry production.

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PROTECTED BIOFACTORS AND ANTIOXIDANTS REDUCE THE NEGATIVE CONSEQUENCES OF VIRUS AND COLD CHALLENGE WHILE ENHANCING PERFORMANCE BY MODULATING IMMUNOMETABOLISM THROUGH CYTOSKELETAL AND IMMUNE SIGNALING IN THE JEJUNUM

D.R. Korver

KEYWORDS: immunometabolism, kinome peptide array, protected biofactors and antioxidants, cold stress, gut health

ABOUT

Quality and formulation of feed is important to meeting producers' goals of increasing average daily gains (ADG) and reduced feed conversion ratio (FCR) of broilers; especially following government restrictions on antibiotics in many countries. Feed additives can act to improve poultry responses to environmental and immune challenges, and we can improve these responses by understanding their mechanisms of action. The objective of this study was to evaluate and compare the immunometabolic effects and mechanisms of action of 2 feed additives, protected biofactors and antioxidants (P(BF+AOx)), and protected biofactors and antioxidants with protected organic acids and essential oils (P(BF+AOx)+P(OA+EO)) in broilers exposed to an early life cold stress and viral immune stimulation; and to identify their mechanism of action in the broiler gut.

APPROACH

We determined the effects of protected biofactors and antioxidants (P(BF+AOx)), and protected biofactors and antioxidants with protected organic acids and essential oils (P(BF+AOx)+P(OA+EO)) on the immune and metabolic health of Ross 308 broiler chickens. These biofactors and antioxidants were derived from vitamins, and Aspergillus niger, Aspergillus oryzae and Bacillus subtilis fermentation extracts. All Ross 308 chickens were exposed to a double-dose of live bronchitis vaccine at d 0 and environmentally challenged by reducing the temperature from 32°C to 20°C at d 3 for 48 h. Control birds were fed without feed additives in the diet. Each treatment consisted of 12 replicate pens with 30 birds each. Performance data and jejunum samples were collected to evaluate the effects of these treatments on growth, cytokine expression, and protein phosphorylation via kinome peptide array.

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ANALYSIS OF RESULTS

The P(BF+AOx) and P(BF+AOx)+P(OA+EO) treatments significantly increased bird weight gain and decreased feed conversion. The kinome peptide array data analysis showed increased activity of cytoskeletal, cell growth and proliferation proteins, and metabolic signaling in the jejunum of P(BF+AOx)+P(OA+EO) treated chickens. There was a significant decrease in IL-6 gene expression in the jejunum of P(BF+AOx)+P(OA+EO) samples compared to control at d 15. P(BF+AOx)+P(OA+EO) treatments in the jejunum showed strong immunomodulatory effects, perhaps to control inflammation. P(BF+AOx)+P(OA+EO) improves gut health via growth and metabolic signaling in the jejunum while inducing stronger immunomodulation.

APPLICATION

Treatment with both P(BF+AOx) and P(BF+AOx)+P(OA+EO) decreased FCR significantly. Performance data should be considered in the context that comparisons were made to a challenge (cold stress and IBV) control. Thus, an increase in performance suggests a decrease in the stress effects of the physiological and environmental challenge. Increased growth performance due to each treatment compared to control may be linked to changes in cell growth and metabolic signaling, P(BF+AOx)+P(OA+EO) appears to induce a unique effect in the growth and metabolic signaling in the gut compared to the P(BF+AOx) treatment.

A POULTRY SUBCLINICAL NECROTIC ENTERITIS DISEASE MODEL BASED ON NATURAL CLOSTRIDIUM PERFRINGENS UPTAKE

E. Goes, D.R. Korver

KEYWORDS: broiler chicken, necrotic enteritis, clostridium perfringens, disease model

ABOUT

Necrotic enteritis (NE) in poultry is an opportunistic infection caused by Clostridium perfringens. Well-known as a multifactorial disease, NE development is under the influence of a wide range of environmental risk factors that promote the proliferation of pathogenic C. perfringens at the expense of nonpathogenic strains. Current in vivo NE challenge models typically incorporate pre-exposure to disease risk factors, in combination with exogenous C. perfringens inoculation. Our objective was to validate a natural, subclinical NE challenge model. Aiming to optimize the current natural NE model, we incorporated a novel stressor, a 24-h feed withdrawal at day 18 post-hatch, apart from other commonly used risk factors for inducing experimental NE.

APPROACH

Ross 708 broilers (n=752) were divided into three experimental flocks. Animals in flocks 1 and 3 were randomly assigned to two dietary treatments to evaluate the impact of antibiotic removal on NE development (antibiotic treatment and drug-free treatment). Animals in flock 2 were used for evaluating the immunomodulation effect of ß-glucan and were randomly assigned to three injection treatments (each with 5 cages of 8 birds). All three flocks were treated with a natural NE challenge procedure but with different levels of coccidiosis challenge intensity. Our goal was to enhance current models using a natural uptake of C. perfringens from the barn environment to produce a subclinical infection. We incorporated access to litter, coccidial exposure (either 10× or 15× of the manufacturer-recommended Coccivac B52 Eimeria vaccine challenge; provided unspecified doses of E. acervulina, E. mivati, E. tenella, and two strains of E. maxima), feed composition, and feed withdrawal stress, and achieved the commonly observed NE infection peak at 3 weeks post-hatch. NE severity was evaluated based on gut lesion pathology, clinical signs, and mortality rate.

ANALYSIS OF RESULTS

Under cage-reared conditions, 15× coccidial vaccine-challenged birds showed overall NE lesion prevalence that was 8-fold higher than 10× coccidial vaccine-challenged birds. NE-associated mortality was observed only in a floor-reared flock after a 15× coccidial vaccine challenge. Our results suggested timely application of stress factors resulted in a consistent NE infection similar to the field situation, characterized by a high incidence of gut lesions in the flock with a low mortality rate.

APPLICATION

The NE infection model presented in this study is based on the natural uptake of C. perfringens presented in the housing environment by the chicken. We incorporated multiple NE-associated risk factors to promote the natural development of pathogens, and successfully reproduce subclinical NE. This will contribute to future research aiming at understanding and preventing this disease, by mimicking the natural development of NE in commercial poultry production.

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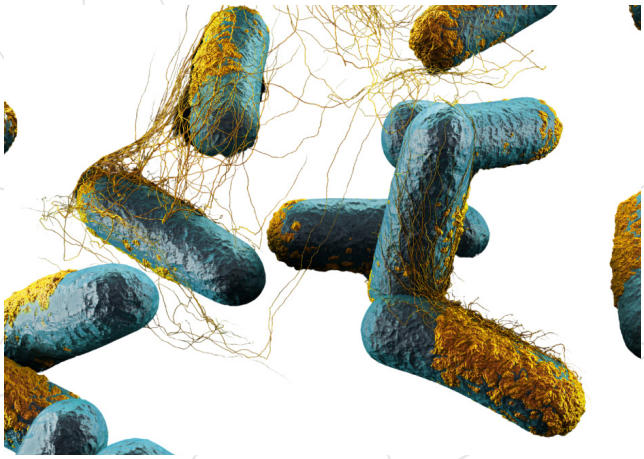

BIO-COMPOSITES FROM SPENT HEN DERIVED LIPIDS GRAFTED ON CNC AND REINFORCED WITH NANOCLAY

A. Ullah

KEYWORDS: Spent hen lipids, Cellulose nanocrystals, Composites, Thermal properties, Mechanical properties

ABOUT

A novel cellulose nanocrystal (CNC)-grafted macromonomer containing both saturated and unsaturated fatty acids was synthesized from spent hen lipids, which was further homopolymerized and copolymerized with styrene and in-situ nano-reinforced into nanocomposites. The purpose of the current study was to harvest the massive and cheap bioresource of the poultry industry, the spent hen derived lipids with the ultimate goal of developing nanocomposites for different potential applications.

APPROACH

Spent hens were collected with the help of Alberta Hatching Egg Producers, Edmonton, AB, Canada. Lipids (mainly composed of triacylglycerols (TAGs)) were extracted from spent hens using microwave-assisted technology. Spent hen lipid-based nanocomposites were fabricated by copolymerization of CNC-grafted macromonomer (Ac-CNC-ELSH) with 30 wt% styrene using 1 wt% AIBN as a catalyst. The chemical grafting on CNC, its polymerization and effect of nanofiller were characterized and investigated by various analytical techniques.

ANALYSIS OF RESULTS

The results revealed improved thermal stability for all nanocomposites with higher onset of degradation temperature and more char production for 5% and 10% nanoclay composites, while increased tensile strength was obtained for all nanoclay composites. However, reduced storage modulus and higher moisture uptake was observed for nanocomposites compared to the control polymer composite, which can be attributed to poor interfacial adhesion between nanofiller and matrices, and hydrophilic nature of nanofillers, respectively.

APPLICATION

In this study, we have successfully prepared a novel spent hen derived lipid-based CNC-grafted acrylated macromonomer and its corresponding homopolymer and styrene copolymer composites reinforced with different percentages of nanoclay (1%, 5%, and 10% by weight), which were characterized by various analytical techniques and investigated for their thermal, mechanical and water absorption behavior. The synthesized cross-linked nanoreinforced biomaterial from spent hen derived lipids with enhanced thermal and mechanical properties offers an environment friendly material that can be used potentially as composites or coatings in different industrial sectors.

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CHICKEN MUSCLE HYDROLYSATE REDUCES BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS, UPREGULATES ACE2, AND AMELIORATES VASCULAR INFLAMMATION, FIBROSIS, AND OXIDATIVE STRESS

H. Fan, J. Wu

KEYWORDS: spent hen, chicken muscle, vascular inflammation, blood pressure

ABOUT

Spent hens are egg-laying chickens reaching the end of their egg-laying cycle and are seen as a by-product to the egg industry. A spent hen muscle protein hydrolysate prepared by food-grade thermoase PC10F (SPH-T) has previously shown antihypertensive potential. In the present work, we further investigated its antihypertensive effect and underlying mechanisms in spontaneously hypertensive rats.

APPROACH

Spent hen carcasses were purchased from a local supermarket. Spent hen muscle proteins were extracted using a pH-shift method. Spontaneously hypertensive rats were divided into three groups: untreated, low dose (250 mg SPH-T/kg/day body weight), and high dose (1,000 mg SPH-T/kg/day body weight); each group consisted of six animals. SPH-T was dissolved in 10 ml of Ensure and orally administered to rats once per day from day 1; untreated group was given Ensure only. Systolic/diastolic blood pressure (SBP / DBP), mean arterial pressure (MAP), and heart rate (HR) were recorded.

ANALYSIS OF RESULTS

Oral administration of SPH-T over a period of 20 days reduced SBP by 25.7 mm Hg ($p < 0.001$) and 11.9 mm Hg, respectively, for the high- and low-dose groups. The high-dose treatment decreased the circulating level of angiotensin II (from 25.0 to 5.7 pg/ml) while increased angiotensin-converting enzyme 2 (ACE2) (from 1.3 to 3.3 IU/ml) and angiotensin (1–7) (from 37.0 to 70.1 pg/ml) significantly. Furthermore, the high-dose group doubled the aortic expression of ACE2 while reduced the expression of angiotensin (Ang) II type 1 receptor (by 35%). Circulating inflammatory cytokines including tumor necrosis factor alpha and monocyte chemoattractant protein-1 as well as vascular inflammatory proteins including inducible nitric oxide synthase and vascular cell adhesion molecule-1 were attenuated by ~15%–50% by the treatment; nitrosative stress (35%) and type I collagen synthesis (50%) in the aorta were also attenuated significantly. Moreover, SPH-T possessed an umami taste (no obvious bitter taste) as analyzed by electronic tongue.

APPLICATION

Hypertension is a global health concern, afflicting more than 20% of adults worldwide. Uncovering the antihypertensive effect of spent hen protein hydrolysate underpinned its functional food nutraceutical applications for the prevention and treatment of hypertension. Future research is needed to ascertain whether or not the antioxidant, anti-inflammatory, and anti-fibrotic effects of SPH-T are due to the increased ACE2 and Ang (1–7) levels. Besides, SPH-T was featured with an umami taste and without an obvious bitter taste, supporting its potential functional food and nutraceutical applications.

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CHICKEN MUSCLE PROTEIN-DERIVED PEPTIDE VVHPKESF REDUCES TNFα-INDUCED INFLAMMATION AND OXIDATIVE STRESS BY SUPPRESSING TNFR1 SIGNALING IN HUMAN VASCULAR ENDOTHELIAL CELLS

H. Fan, J. Wu

KEYWORDS: spent hen, muscle proteins, vascular endothelial cells, bioactive peptides

ABOUT

Excessive and sustained inflammatory responses in the endothelial cells trigger vascular dysfunction, the key contributor to hypertension and atherosclerosis. Tumor necrosis factor alpha (TNFα), a pro-inflammatory cytokine, plays an important role in endothelial inflammatory responses cyclooxygenase 2 (COX2) and adhesion molecules including vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Over the years, food-derived bioactive peptides have been an emerging option for the prevention and treatment of hypertension and cardiovascular diseases. This study aims to investigate the protective effects of four chicken muscle-derived peptides Val-Arg-Pro (VRP), Leu-Lys-Tyr (LKY), Val-Arg-Tyr (VRY), and Val-Val-His-Pro-Lys-Glu-Ser-Phe [VVHPKESF (V-F)] on tumor necrosis factor alpha (TNFα)-induced endothelial inflammation and oxidative stress in human vascular endothelial EA.hy926 cells.

APPROACH

EA.hy926 cells (passages 3–10) were grown in the growth medium in an incubator (37 °C) at 5% CO2 and 100% humidity. Chicken muscle-derived peptides were added into the cultured media to treat the cells at the concentration of 25–100 μM for different periods of time (within 24 h). Cells were grown on 24-well plates until reaching confluence, followed by peptide treatment for 18 h before another 6 h of TNFα stimulation (10 ng mL^{-1}). Cells without peptide or TNFα treatment were used as the control group.

ANALYSIS OF RESULTS

Inflammation and oxidative stress are induced in EA.hy926 cells by TNFα (10 ng mL^{-1}) treatment for different periods of time. Inflammatory proteins and signaling molecules including inducible nitric oxide synthase, intracellular cell adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), cyclooxygenase 2 (COX2), nuclear factor kappa B (NF-kB), mitogen-activated protein kinases (MAPKs), and TNFα receptor 1 (TNFR1) are measured by qRT-PCR or western blotting; soluble TNFR1 level and nicotinamide adenine dinucleotide phosphate NADPH) oxidase activity are determined by Elisa kits; superoxide is measured by dihydroethidium staining. Only V-F treatment inhibits the expression of VCAM-1 and COX2, via suppressing NF-kB and p38 MAPK signaling, respectively, while reduced oxidative stress via the inhibition of NADPH oxidase activity; V-F treatment attenuates both gene and protein expressions of TNFR1.

APPLICATION

V-F treatment ameliorates TNFα-induced endothelial inflammation and oxidative stress likely via the inhibition of TNFR1 signaling, suggesting its potential as a functional food ingredient or nutraceutical in the prevention and treatment of hypertension and cardiovascular diseases.

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CHICKEN MUSCLE-DERIVED ACE2 UPREGULATING PEPTIDE VVH-PKESF INHIBITS ANGIOTENSIN II-STIMULATED INFLAMMATION IN VASCULAR SMOOTH MUSCLE CELLS VIA THE ACE2/ANG (1–7)/MASR AXIS

H. Fan, J. Wu

KEYWORDS: chicken, bioactive peptides, inflammation, metabolism, signaling pathways, vascular smooth muscle cells

ABOUT

Hypertension is a major risk factor for cardiovascular diseases, leading to millions of deaths every year worldwide. Pharmacological interventions are the major therapy for hypertension treatment, including ACE inhibitors and AT_1R blockers. However, side effects are often accompanied with the administration of synthetic drugs; many cases of hypertension are still not under control. Hence, there has been considerable interest over the past years in developing alternatives from natural sources for combating hypertension. One of the promising compounds are food-derived bioactive peptides. This study aimed to evaluate the modulatory effects of four spent hen muscle-derived peptides [VRP, LKY, VRY, and VVHPKESF (V–F)] on angiotensin II (Ang II)-induced inflammation in rat vascular smooth muscle A7r5 cells.

APPROACH

A7r5 cells were treated with 50 μM peptides for 1 h followed by 1 μM Ang II treatment for up to 24 h for the detection of iNOS and COX2. In some experiments, the antagonist of AT_1R (losartan potassium; 50 μM), Ang II type 2 receptor (AT_2R) (PD123319; 1 μM), or MasR (A779; 1 μM) was added with peptides. To study the involvement of the intracellular signaling pathway, A7r5 cells were treated with 50 μM peptides for 1 h before 1 μM Ang II treatment for 15 min for the detection of signaling pathways including nuclear factor kappa B (NF-kB) p65 and mitogen-activated protein kinases (MAPKs) members including p38, c-Jun N-terminal kinases (JNK), and extracellular signal-regulated kinases 1/2 (ERK1/2).

ANALYSIS OF RESULTS

Only V–F could significantly attenuate Ang II-stimulated inflammation via the inhibition of NF-kB and p38 MAPK signaling, being dependent on the Mas receptor (MasR) not on the Ang II type 1 or type 2 receptor (AT_1R or AT_2R). V–F accelerated Ang II degradation by enhancing cellular ACE2 activity, which was due to ACE2 upregulation other than a direct ACE2 activation. These findings demonstrated that V–F ameliorated Ang II-induced inflammation in A7r5 cells via the ACE2/Ang (1–7)/MasR axis. Three peptide metabolites of V–F–VHPKESF, PKESF, and SF–were identified but were not considered major contributors to V–F's bioactivity. The regulation of peptide V–F on vascular inflammation supported its functional food or nutraceutical application in the prevention and treatment of hypertension and cardiovascular diseases.

APPLICATION

In summary, among four chicken muscle-derived peptides (VRP, LKY, VRY, and V–F), only V–F significantly inhibited Ang II-induced inflammatory responses in A7r5 cells. The protective effect of V–F in ameliorating Ang II-stimulated vascular inflammation indicated its potential in regulating blood pressure in vivo, which awaits to be explored in animals in the near future. Prior to that, its gastrointestinal fate and transepithelial transport are warranted to be investigated soon.

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