Studying The Impact Of A Standardized Dry Grape Extract On Pullets' Metabolome

04/09/2024

***PhD* student** :
ABERKANE Fatima Zohra

The story of a laying hen

Growing | **Pre-laying** | **Laying**



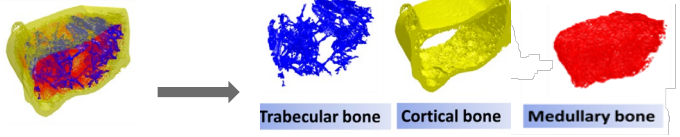
0 — 22

0-15 weeks — 18 weeks — From 19 weeks

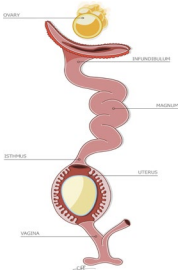
Skeletal and muscles developement

Sexual maturity
Estrogen pic

Egg formation



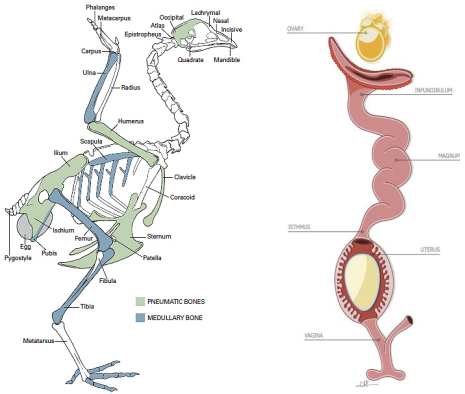
Hydroxyapatite
$Ca_5(PO_4)_3$



Sinclair-Black M. et al., *Frontiers in Physiology*, 2023

The story of a laying hen

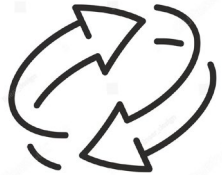
Laying and eggs formation



Skeletal system ⟷ Reproductive system

Hydroxyapatite
$Ca_5(PO_4)_3$

- ✓ 20%-40% of calcium from bones
- ✓ Bone resorption by osteoclastesis



Repeated process

High metabolism

Environnemental challenges

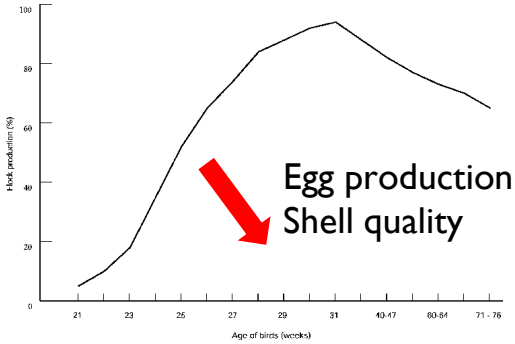
Vaccination

Oxidative stress

High frequency vaccination-induced immune stress reduces bone strength with the involvement of activated osteoclastogenesis in layer pullets

Mengze Song, Xiaoyan Lin, Jingpeng Zhao, Xiaojuan Wang, Hongchao Jiao, Haifang Li, Shuhong Sun, and Hai Lin[1]

Department of Animal Science, Shandong Agricultural University, Shandong Key Lab for Animal Biotechnology and Disease Control, Taian, Shandong, P. R. China 271018

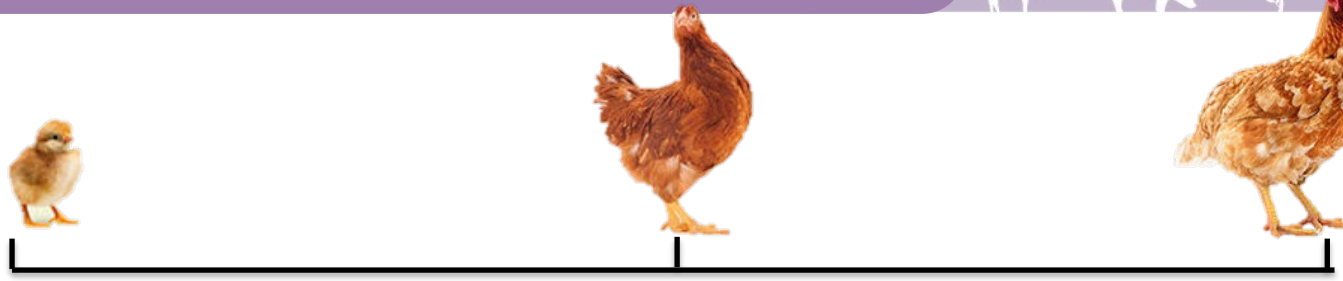


Egg production
Shell quality

Bone abnormalities

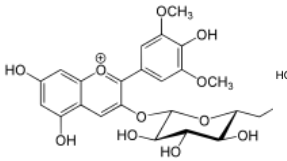
Osteoporosis

*The story of a laying hen : **giving hand ?***

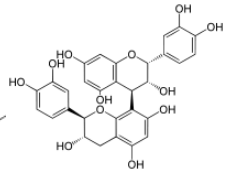


From an early stage

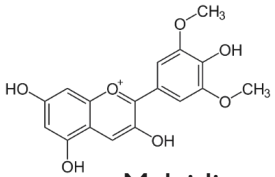
Standardisation on polyphenols :



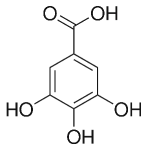
Anthocyanins



Procyanidins



Malvidin



Gallic acid

Nor-Grape®

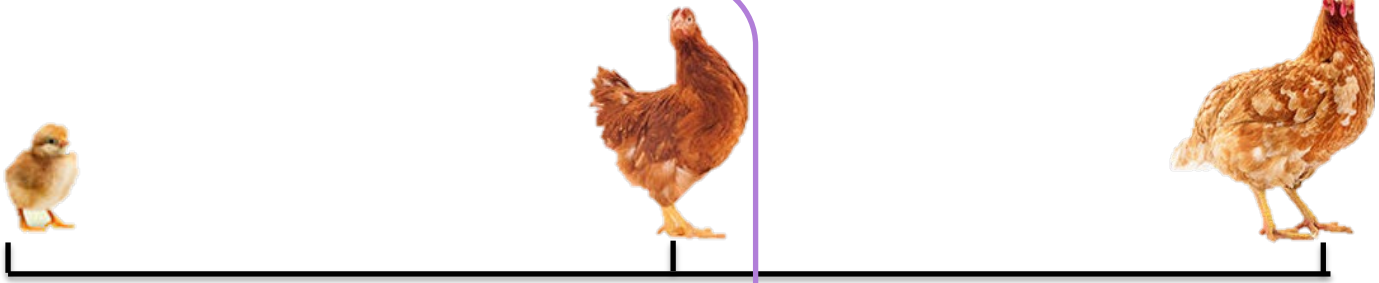
Standardized Dry Grape Extract (SDGE)

Increases production of antioxidant enzymes

Better immune response

Engler et al., 2021, published results

Objective : study of the impact of a Standardized Dry Grape Extract (SDGE) on pullets' bones and the contribution to the characterization of its mode of action



From an early stage

Nor-Grape®
SDGE

Positive impact on bone developement ?

Mechanisms or/and metabolic pathways behind it ?

Evaluation of Standardized Dry Grape Extract (SDGE) on pullets

Wk17

CTL (n=18150)

30mg/kg NG (n=18150)

Bone parameters analysis

✓ Bones sampling

Analysis	Parameter
Autopsy	Keel bone deformity
Bone quality	Bone flexibility, dry matter, mineral matter, Ca + P content

Metabolomic analysis

✓ Plasma sampling

Evaluation of SDGE on pullets : metabolomics analysis

I Metabolites extraction

Hydrophobic extracts

Hydrophilic extracts

2 groups ; n=2x8

Control group

NG group

Zhang et al., *analytical chemistry*, 2019

Evaluation of SDGE on pullets : metabolomics analysis

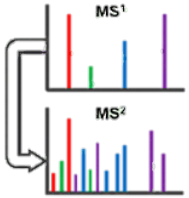
1 Metabolites extraction

Hydrophobic extracts

Hydrophilic extracts

2 UHPLC-QTOF analysis

- ✓ Separation
- ✓ Ionisation (ESI- and ESI+)
- ✓ Fragmentation



2 groups ; n=2x8

Control group

NG group

Evaluation of SDGE on pullets : metabolomics analysis

1 Metabolites extraction

Hydrophobic extracts

Hydrophilic extracts

2 UHPLC-QTOF analysis

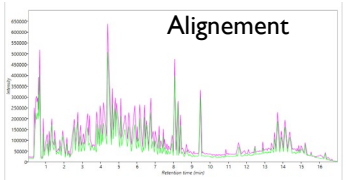
2 groups ; n=2x8

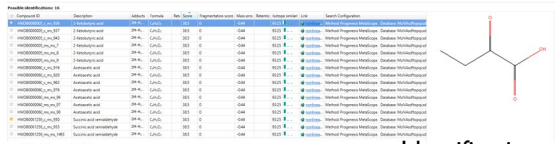
Control group

NG group

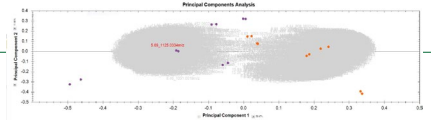
3 Data processing

Progenesis QI





Identification



Statistics

Evaluation of SDGE on pullets : metabolomics analysis

1 Metabolites extraction

Hydrophobic extracts

Hydrophilic extracts

2 UHPLC-QTOF analysis

2 groups ; n=2x8

Control group

NG group

3 Data processing

Progenesis QI

4 Pathway analysis

KEGG Kyoto Encyclopedia of Genes and Genomes

IMPaLA

Small Molecule Pathway Database

reactome

Evaluation of SDGE on pullets

Wk17

CTL (n=18150)

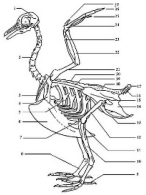
30ppm NG (n=18150)

Bone parameters analysis

✓ Bones sampling

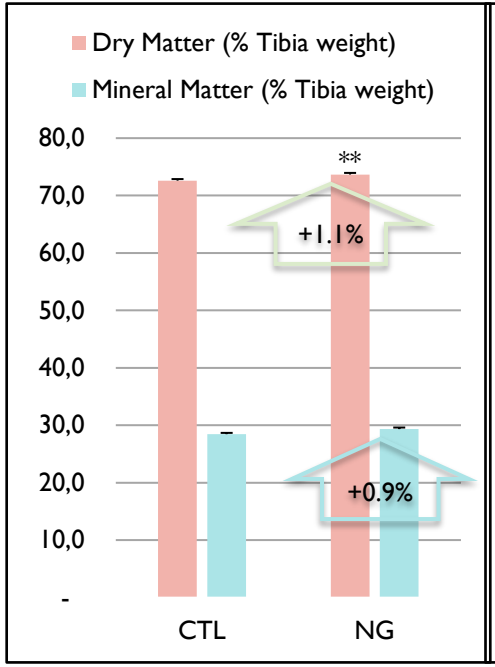
Metabolomic analysis

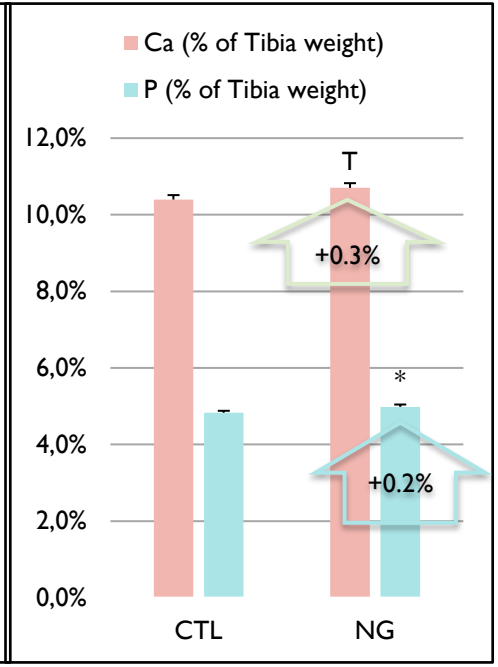
✓ Plasma sampling

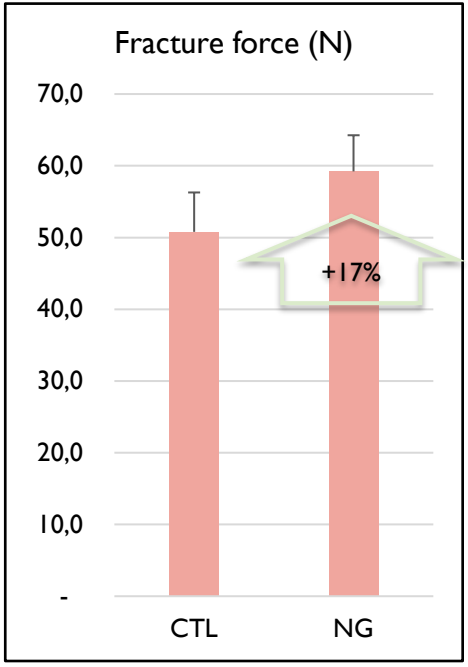


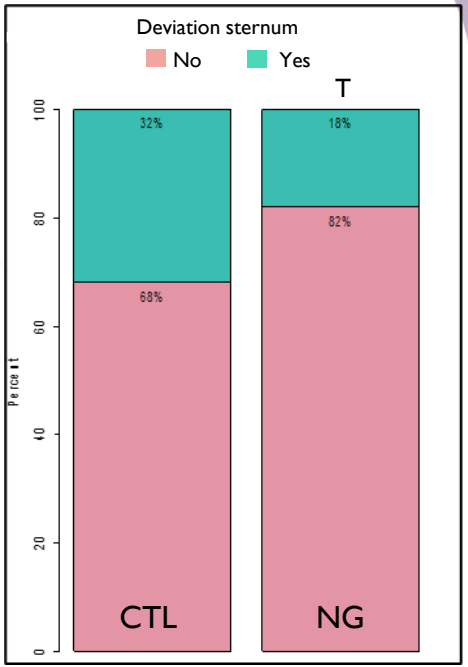
Evaluation of SDGE on pullets : bones parameters analysis

Results









SDGE increases mineral content and strengthens pullet's bones

T: p<0.10; *:p<0.05, **:p<0.01

Evaluation of a SDGE on pullets

Wk17

CTL (n=18150)

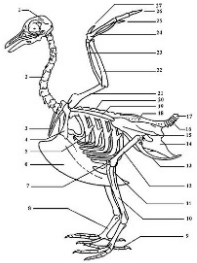
30ppm NG (n=18150)

Metabolomic analysis

✓ Plasma sampling

Bone parameters analysis

✓ Bones sampling



Evaluation of SDGE on pullets : metabolomic analysis

Results

Plasma

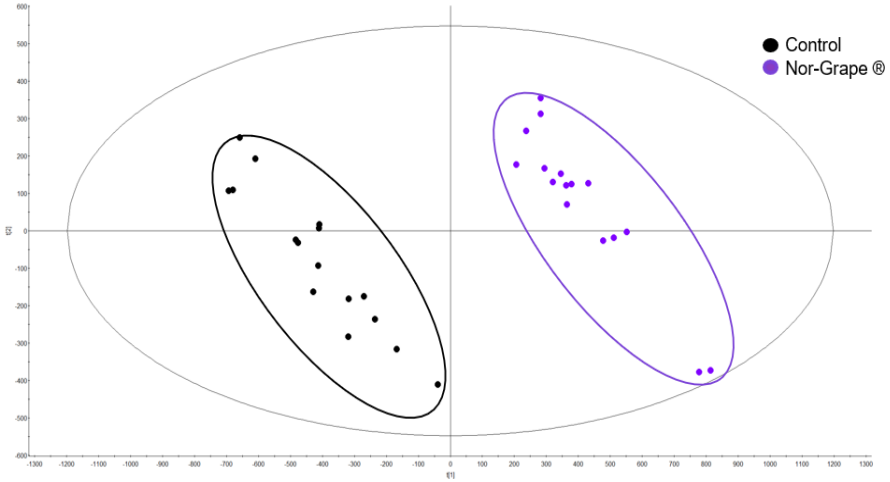
Hydrophobic extracts in negative ionisation : PLS-DA

- **After 17 weeks of supplementation :**

  Control (CTL) VS SDGE (Nor-Grape®)

8 plasma samples of laying hens (n=8)

➢ 2 analytical replicates for each extract



✓ **PLS-DA of 1568/9671 differently expressed *features*** (p <0,05)

Evaluation of SDGE on pullets : metabolomic analysis

Results

Hydrophobic extracts in negative ionisation : identification

Plasma

1568 differently expressed features

P <0.05

+max fold change > 2

574 differently expressed features with a difference of >2

MoNA hmdb

Appropriate databases

420 annotated features

Mass similarity (ppm) < 5
Isotope similarity > 70
score > 35

122 accepted identifications

51 overexpressed metabolites ▲

71 under-expressed metabolites ▼

Evaluation of SDGE on pullets : metabolomic analysis

Results

Hydrophobic extracts in negative ionisation : pathway analysis

Plasma

pathway name	pathway source	overlapping metabolites	all metabolites	P-value
Free fatty acid receptors (FFAR)	Reactome	2	23	<0.01
γ-glutamyl cycle	HumanCyc	2	29	<0.01
Proton/oligopeptide cotransporters	Reactome	1	2	<0.01
histamine biosynthesis	HumanCyc	1	4	<0.05
Methylhistidine Metabolism	SMPDB	1	4	<0.05
G alpha (q) signalling events	Reactome	2	56	<0.05
Gamma-glutamyl cycle for the biosynthesis and degradation of glutathione	Wikipathways	1	6	<0.05
Deadenylation-dependent mRNA decay	Reactome	1	8	<0.05
Organic anion transport	Reactome	1	8	<0.05
Miscellaneous transport and binding events	Reactome	1	9	<0.05
Fatty Acids bound to GPR40 (FFAR1) regulate insulin secretion	Reactome	1	9	<0.05
Arachidonate Epoxygenase - Epoxide Hydrolase	Wikipathways	1	11	<0.05

Antioxidant metabolism

Free Fatty Acids Receptors

SDGE impacts pathways related to G-proteins-coupled receptors (GPCR)

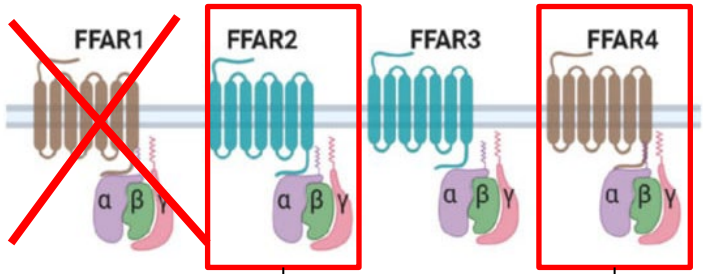
IMPaLA

Discussion

Metabolic pathways impacted by grape extract

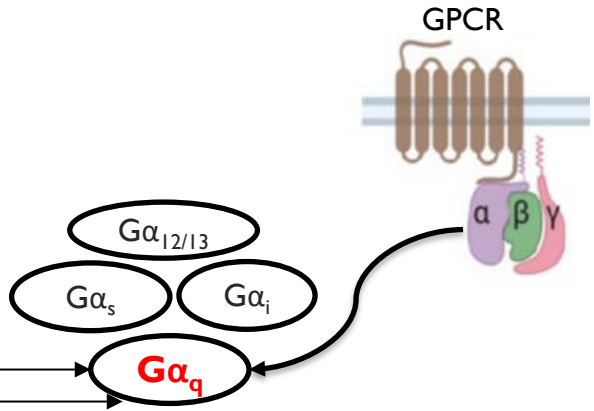
In *Gallus gallus*

Free Fatty Acid Receptors (FFAR)



FFARs are G protein-coupled **receptors activated by free fatty acids**

G alpha (q) signalling events



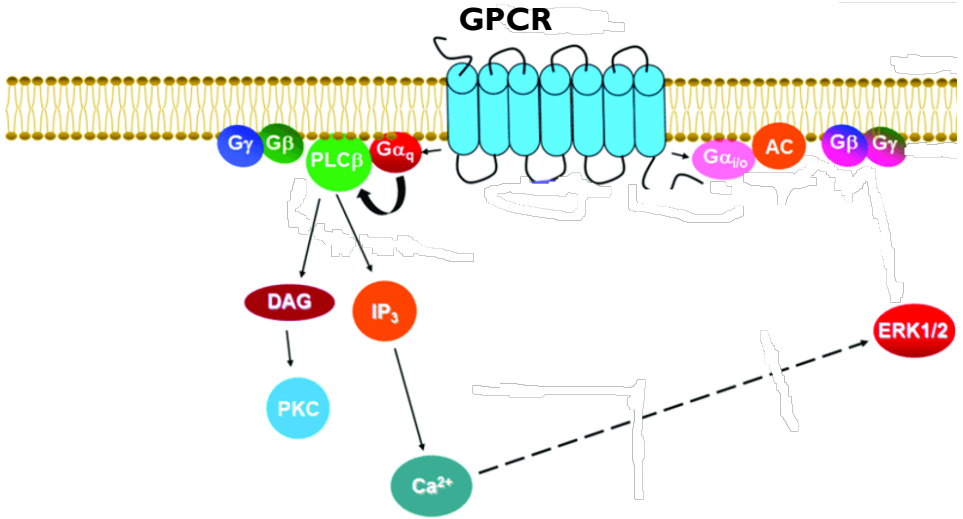
Both **FFAR2** and **FFAR4** are present in chicken and possess an α_q subunit

Evaluation of SDGE on pullets

Discussion

Metabolic pathways impacted by grape extract

What's important about α_q subunit?



Regulation of osteoblast differentiation

Calcium signaling

α_q subunit regulates calcium mobilization and osteoblast differentiation

Evaluation of SDGE on pullets

Discussion

Metabolic pathways impacted by grape extract

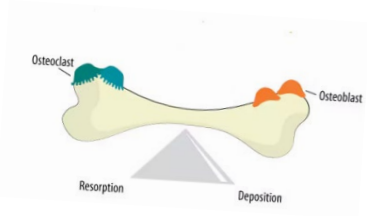
What is the exact role of FFAR in bone development ?

Endocrinology. 2016 Jul;157(7):2621-35. doi: 10.1210/en.2015-1855. Epub 2016 May 4.

Free Fatty Acid Receptor 4 (GPR120) Stimulates Bone Formation and Suppresses Bone Resorption in the Presence of Elevated n−3 Fatty Acid Levels

Seong Hee Ahn [1], Sook-Young Park [1], Ji-Eun Baek [1], Su-Youn Lee [1], Wook-Young Baek [1], Sun-Young Lee [1], Young-Sun Lee [1], Hyun Ju Yoo [1], Hyeonmok Kim [1], Seung Hun Lee [1], Dong-Soon Im [1], Sun-Kyeong Lee [1], Beom-Jun Kim [1], Jung-Min Koh [1]

FFAR4 increases osteoblastic bone formation and decreases osteoclastic bone resorption



FFA receptors regulates bone developement

Evaluation of SDGE on pullets

Discussion

Metabolic pathways impacted by grape extract

What ligands activate these receptors ?

Food Sci Biotechnol (2020) 29(4):579–584
https://doi.org/10.1007/s10068-019-00688-4

Check for updates

Effect of propolis phenolic compounds on free fatty acid receptor 4 activation

Hyunnho Cho[1] · Kyong Kim[1] · Nayeon Kim[1] · Minji Woo[1] · Hye Young Kim[1]

Several polyphenols, including caffeic acid, activates FFAR4

PLoS One. 2018 Jul 11;13(7):e0200449. doi: 10.1371/journal.pone.0200449. eCollection 2018.

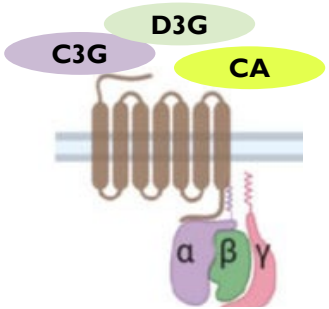
Anthocyanins from purple corn activate free fatty acid-receptor 1 and glucokinase enhancing in vitro insulin secretion and hepatic glucose uptake

Diego A Luna-Vital [1], Elvira Gonzalez de Mejia [1]

Affiliations + expand

PMID: 29995924 PMCID: PMC6040766 DOI: 10.1371/journal.pone.0200449

Free PMC article



C3G, D3G and other anthocyanins activate FFAR1 receptor.

Polyphenols can also activate FFAR receptors.

C3G : cyanidin-3-*O*-glucoside
D3G : Delphinidin-3-*O*-glucoside
CA : caffeic acid

Evaluation of SDGE on pullets : metabolomic analysis

Results

Hydrophobic extracts in negative ionisation : pathway analysis

Plasma

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Antioxidant metabolism

IMPaLA

Evaluation of SDGE on pullets

Discussion

Metabolic pathways impacted by grape extract

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 13, pp. 8864–8870, March 31, 2006

Activation of Family C G-protein-coupled Receptors by the Tripeptide Glutathione*[S]

Received for publication, December 1, 2005, and in revised form, January 26, 2006 Published, JBC Papers in Press, February 2, 2006, DOI 10.1074/jbc.M512865200

Minghua Wang[1], Yi Yao[1], Donghui Kuang, and David R. Hampson[2]

From the Departments of Pharmaceutical Sciences and Pharmacology, University of Toronto, Toronto, Ontario M5S 2S2, Canada

In vitro **tests showed that CaSR receptors could be activated by gluthathione**

Molecular distribution and localization of extracellular calcium-sensing receptor (CaSR) and vitamin D receptor (VDR) at three different laying stages in laying hens (*Gallus gallus domesticus*)

Qianru Hui,* Xiaoya Zhao,* Peng Lu,* Shangxi Liu,* Martin Nyachoti,* Karmin O,*,† and Chengbo Yang*,1

**Department of Animal Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada; and †CCARM, St. Boniface Hospital Research Centre, Winnipeg, Manitoba R2H 2A6, Canada*

- **CaSR is a member of the GPCR family.**
- **The CaSR gene is overexpressed at the beginning of the W19. Localization in different tissues including the tibia.**

CaSR receptor could be responsible for calcium mobilization in laying hens

Evaluation of SDGE on pullets

Discussion

Hypothesis : how can these results be explained ?

Nor-Grape ®

Glutathione

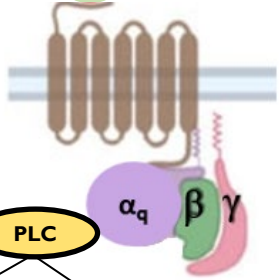
Fatty acids

Ligands

Glutathione

Fatty acid

Polyphenol



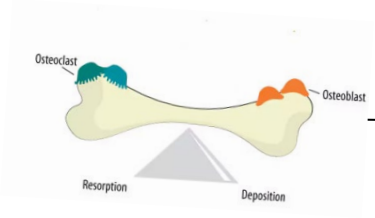
PLC

DAG

IP3

PKC

Calcium sensing and mobilization → → → → ERK1/2 pathway



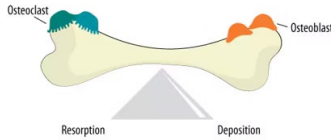
NOR-FEED
Botanicals • You know why

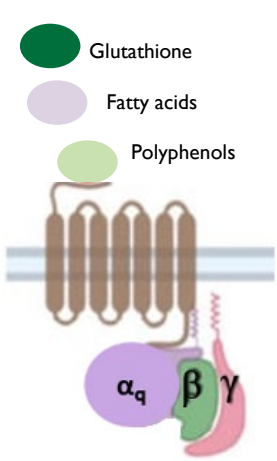
A synergistic effect of Nor-Grape® compounds may activate directly or indirectly several GPCR receptors and ensures bones strength in pullets

Evaluation of SDGE on pullets

Conclusion and perspectives

Nor-Grape®





- Supplementing pullets with Nor-Grape® strengthens their bones and prepares them for laying cycles.
- A synergistic effect of Nor-Grape® compounds may be responsible for the activation of GPCR receptors to ensure this effect.
- Further analysis are necessary to test these hypotheses in order to valide Nor-Grape® mechanism of action.

Thank you for your attention