New Frontiers in Nutrition for Pet Health: The role of Nutrigenomics

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Nutrigenomics

- How diet influences gene transcription, protein expression and metabolism, ultimately providing personalised nutrition for maintenance of health and prevention of disease.
- The science of Nutrigenomics seeks to provide a molecular understanding for how common dietary chemicals affect health by altering the expression and / or structure of individual's genetic makeup

The study of nutritional effects on gene expression

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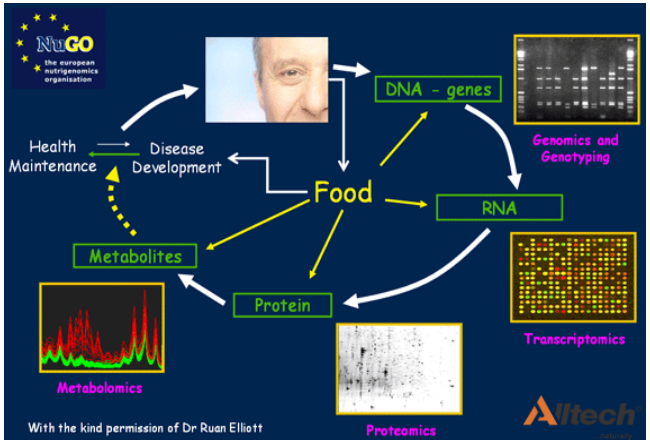

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Genome sequencing

- **1995** first bacterial genome sequenced
- **1996** first eukaryote to have its genome fully sequenced (yeast)
- **2000** Draft of human genome published
- **2003** Human genome fully sequenced
- **2005** Dog genome fully sequenced

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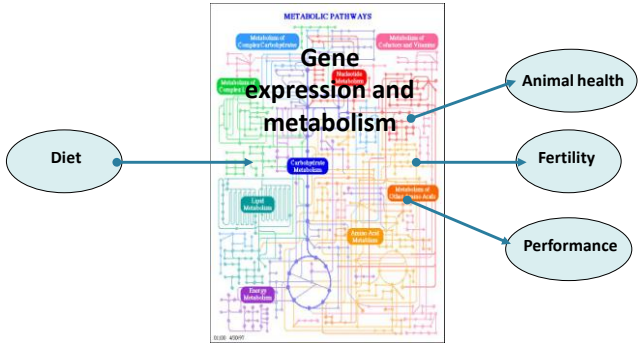
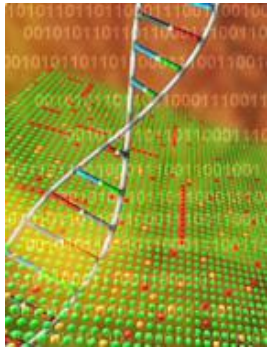
Traditional animal trial approach.



DNA Microarray Technology



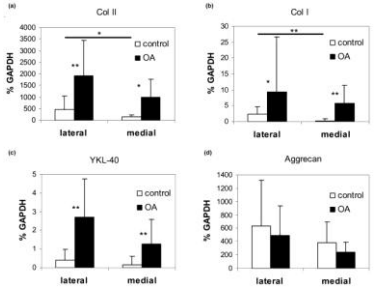
- Evaluates the expression profile of thousands of genes within a single experiment.
- Shows which genes are upregulated, downregulated or unchanged in response to a given treatment.

Advantages of microarray techniques to study gene expression

- less invasive
- more informative research
- Determination of:
  - **Minimal, optimal** and **toxic** concentration of nutrients
  - **Efficacy** and toxicity of **new ingredients**
  - Effect of nutrition on **development, prevention** and treatment of **complex diseases**

Joint Health / Cartilage degeneration

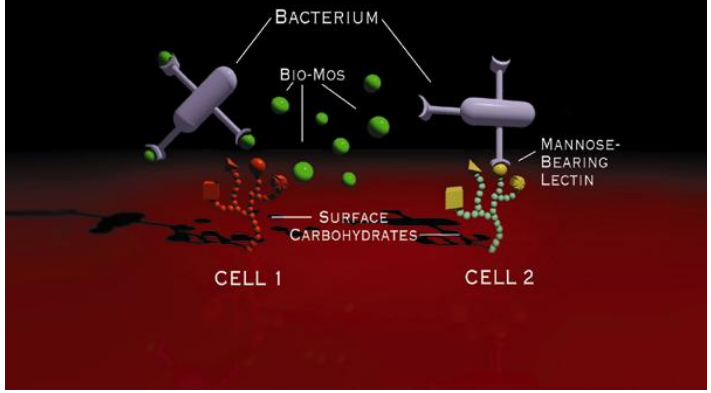
Local mRNA expression levels in cartilage of experimental osteoarthritis (OA): **(a)** collagen type (col) II, **(b)** col I, **(c)** YKL-40 and **(d)** aggrecan. Shown are mean relative expression levels of mRNA in lateral and medial tibial plateau summarized for 6, 12, 24 and 48 weeks. * $P < 0.05$, ** $P < 0.001$.



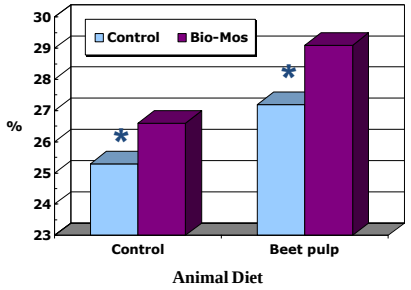
Lorenz et al. 2004

EPA, has proved to alter the action of a degenerative enzyme that causes **cartilage degradation**

Pathogen Agglutination through Bio-Mos



Improved Fibre Digestibility – Change in Fecal Microflora



*Significantly different (P ≤ 0.05)

Kappel 2005, Trial 565

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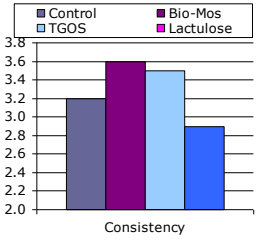
Stool Consistency and Quality

- Improved consistency with Bio-Mos
- Less unbound water
- Reduced ammonia



Ideal score between 3.5 and 4

Zentek 2002, Trial 450

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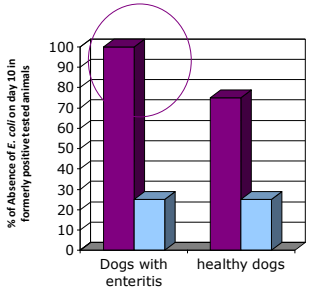
Use of Bio-Mos in Puppies with Enteritis: Fecal Culture Results



- 16 puppies (8 healthy, 8 with enteritis)
- 4 of each group fed Bio-Mos
- All ill puppies got medical treatment
- Dosing 2 g/day

Most puppies suffer from enteritis after weaning.
Bio-Mos could help in such a demanding life stage.

Gouveia 2005, Trial 668

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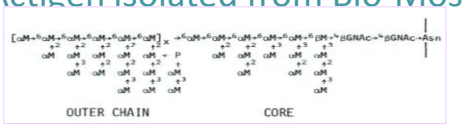
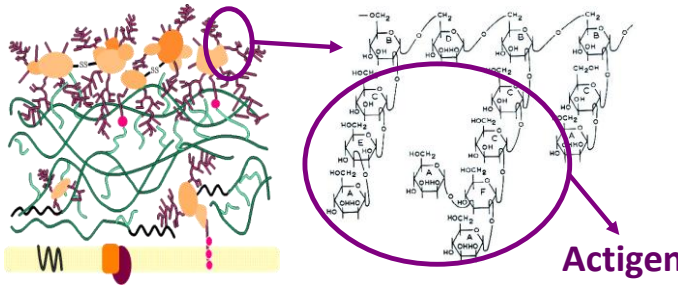
Bio-Mos isolated from Yeast cell wall, Actigen isolated from Bio-Mos





Some key observations from gene expression patterns in poultry

- Of the 4210 genes modified by Bio-Mos and Actigen supplementation of challenge diets 1592 (38%) were effect the same way by both supplements

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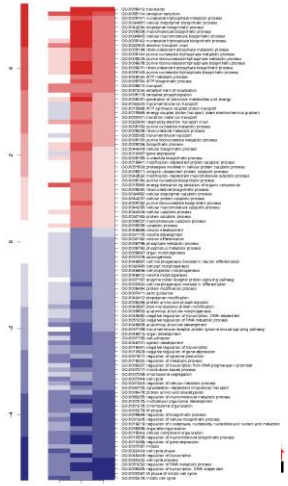
Key metabolic processes in the jejunum effected by wheat challenge, Bio-Mos and Actigen

PAGE Biology processes evaluation

(Corn as Control, Exp1, Wheat; Exp2, Bio-Mos; Exp3, Actigen. P<0.01, FDR<0.05)

- Up-regulated
  - Protein translation
  - Energy production (e-transport and ATP production)
  - Transport systems
- Down-regulated
  - Cell cycling and division
  - Chromosome and cell organization
  - Cell adherence



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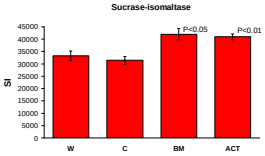
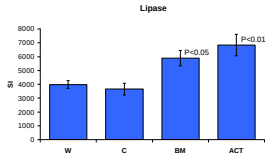
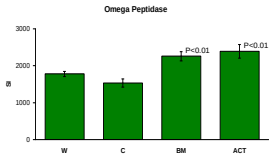
Effects of Bio-Mos & Actigen on gene expression: Increased expression of genes for digestive enzymes in the small intestines







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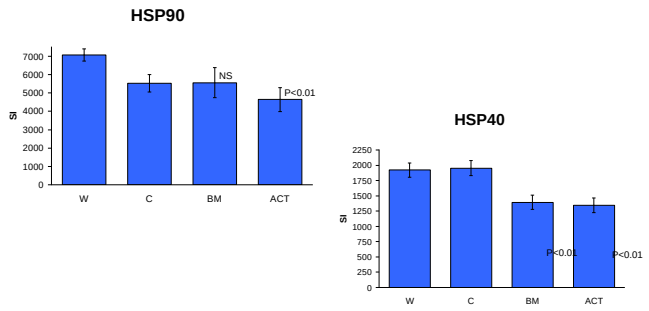
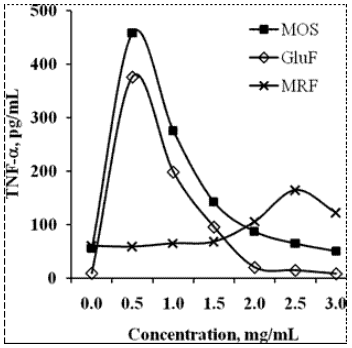
Effects of Bio-Mos & Actigen on gene expression: Decreased expression of stress protein genes



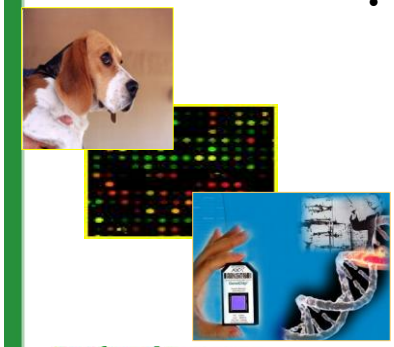


Tumor necrosis factor-a (TNF-a) production by alveolar macrophages activated with increasing concentrations of mannan oligosaccharide (Bio-Mos), glucan fraction (GluF), or Actigen (MRF). TNF-a response of AMf peaked at 0.5 mg/mL Bio-Mos ($P < 0.01$), 0.5 mg/mL GluF ($P < 0.01$), and 2.5 mg/mL Actigen ($P < 0.01$).
(Data were means of 4 replicates.)

University of Illinois

Microarray Beagle Studies

Swanson, Schook, and Fahey Labs.



- **University of Illinois:**
  - 2 diets fed for 12 months
    - High fat and low fiber
    - Low fat and high fiber
  - 2 age groups
    - 1 yr old = 20 yr old human
    - 12 yr old = 77 yr old human
    - Cerebral Cortex & Hepatic samples taken.

Cerebral Cortex sample:

- **Gene expression changes**
  - Age-induced changes: 567 transcripts
  - Diet-induced changes: 38 transcripts
  - Diet-induced (high fat/low fibre):
  - Young dogs
    - **Genes associated with neurogenerative diseases ↑ in humans**
    - **Transthyretin ↓**
  - Geriatric dogs
    - **Genes associated with brain cell injury, inflammation and Alzheimer's diseases ↑**

The Form of Selenium is so Important…

Conventional:

"Feed selenium to prevent deficiency problems"

Conventional source:

Inorganic sodium selenite

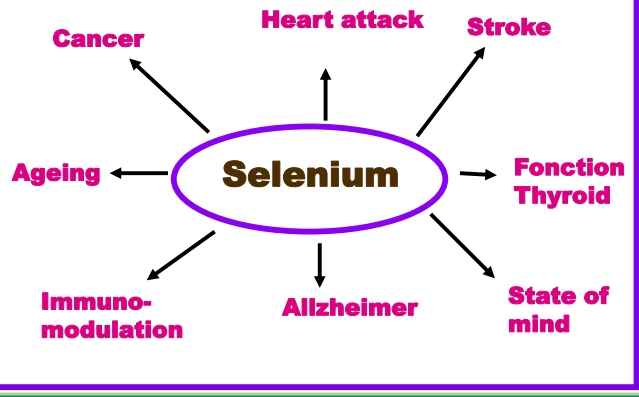
Modern:

"Provide All the Tools for Optimum Health, Performance and Well Being"

Modern source:

Sel-Plex organic selenium

Effect of Se on the human health status



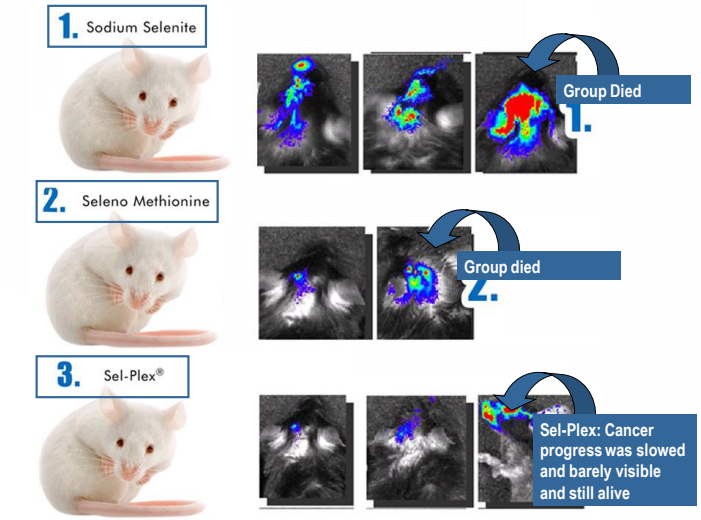
Cancer Statistics

- Cancer is the major cause of death in pets greater than 10 years old
- 45% of all dogs older than 10 years of age die of cancer
- 23% of all dogs die of cancer

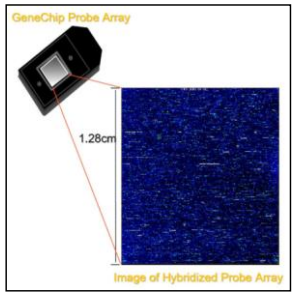
Incidence of cancer in dogs

Dogs		Bitches	
Type of Cancer	% of Total Cancers	Type of Cancer	% of Total Cancers
Connective Tissue	17	Breast	51
Testis	16	Connective Tissue	9
Skin (melanoma)	14	Skin (melanoma)	8
Mouth and throat	10	Lymphoma	6
Lymphoma	10	Mouth and Throat	5
Bone	4	Liver and bile tracts	2
Stomach and intestines	3	Bone	2

Bessinger, Todd, 2004 Review of the Epidemiology of Cancer in Dogs, as appeared in: Kelsey, J.L. et al., 1998, Epidemiologic studies of risk factors for cancer in pet dogs. Epidemiologic Reviews 20 (2): 204-217.



Influence of Se nutrition on gene expression in mice



- Affymetrix® MOE430 2.0 array
- ~21,000 "known genes" on the array
- Filter @ $P \leq 0.01$ (treatment versus selenium deficient)
- 1,804 genes significantly affected by at least one treatment in liver
- 3,316 genes significantly affected by at least one treatment in muscle
- 2,425 genes significantly affected by at least one treatment in cerebral cortex

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Key Changes at a Molecular Level.

- Enhanced Antioxidant Status
- Reduced Cellular Stress
- Improved Cellular Performance

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Thioredoxin/Thioredoxin Reductase System

- Key system in maintaining intracellular redox balance
- Reduced thioredoxin activates enzymes important in antioxidant function and DNA synthesis as well as a variety of gene transcription factors
- Thioredoxin knockout mice die shortly after implantation

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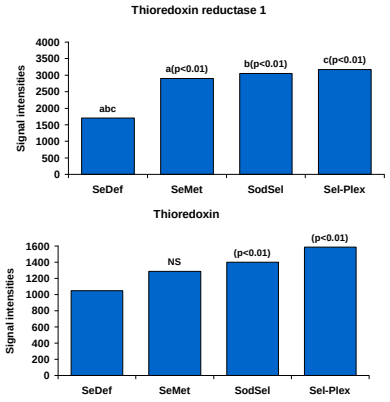
- Effects of selenium on TRX/TRXr gene expression



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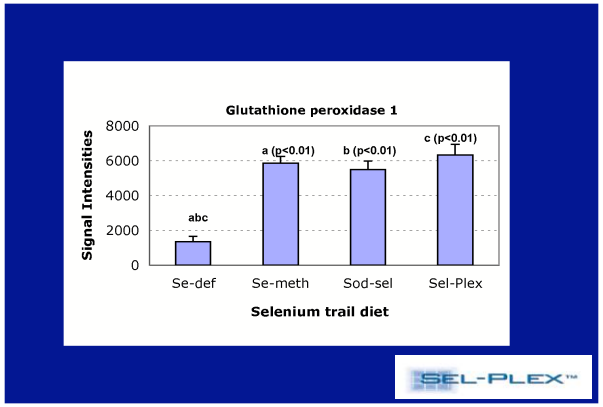

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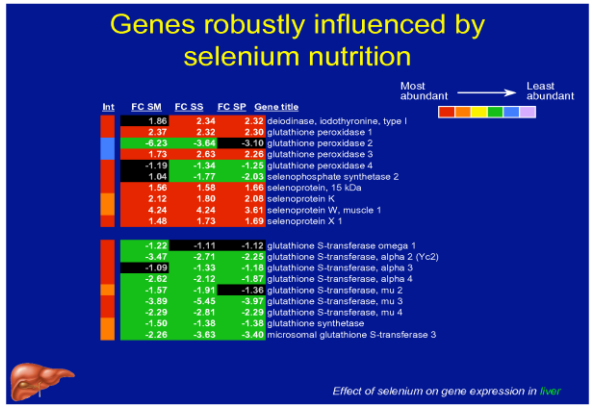

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Genes robustly influenced by selenium nutrition

Int	FC SM	FC SS	FC SP	Gene title
	2.98	3.55	3.30	glutathione peroxidase 1
	1.18	-2.14	-2.06	glutathione peroxidase 3
	-1.05	-1.41	-1.27	glutathione peroxidase 4
	-1.79	-2.21	-2.57	Selenophosphate synthetase 2
	1.42	1.74	1.72	selenoprotein, 15 kDa
	1.15	1.42	1.42	selenoprotein K
	1.66	1.60	1.54	selenoprotein M
	1.18	-1.80	-2.07	selenoprotein N, 1
	1.11	1.32	1.31	selenoprotein P, plasma, 1
	3.28	2.21	2.10	selenoprotein W, muscle 1
	-1.13	-1.65	-1.85	thioredoxin reductase 2
	-3.94	-3.40	-1.14	glutathione S-transferase, alpha 3
	-1.33	-1.16	-1.13	glutathione S-transferase, alpha 4
	-1.09	-1.21	-1.29	glutathione S-transferase, mu 2
	1.02	1.36	1.20	glutathione S-transferase, mu 5
	1.02	-1.77	-1.17	glutathione S-transferase, mu 7
	1.03	-1.16	-1.21	glutathione S-transferase, pi 1
	-1.02	-1.55	-2.21	glutathione S-transferase, theta 3
	-1.20	2.16	2.02	microsomal glutathione S-transferase 3

Fewer genes affected than in liver, less abundantly expressed, and less consistent among diets

Effect of selenium on gene expression in muscle

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Decreased MAPK signaling suggests lowered cellular stress in SodSel and SelPlex

Int.	FC_SM	FC_SS	FC_SP	Gene_title
	1.22	-2.58	-1.99	MAP kinase-activated protein kinase 2
	1.01	-1.60	-1.57	MAP kinase-activated protein kinase 5
	1.00	-1.59	-1.57	MAP kinase-interacting serine/threonine kinase 2
	1.07	-1.77	-1.59	mitogen activated protein kinase 3
	-1.06	-2.05	-1.58	mitogen activated protein kinase binding protein 1
	1.06	-3.63	-3.03	mitogen activated protein kinase kinase 2
	1.06	-1.54	-1.47	mitogen activated protein kinase kinase 3
	1.03	-1.35	-1.29	mitogen activated protein kinase kinase 5
	-1.05	-3.95	-4.62	mitogen activated protein kinase kinase kinase 10
	1.06	-2.47	-2.53	mitogen activated protein kinase kinase kinase 3
	1.32	-1.67	-1.71	mitogen activated protein kinase kinase kinase 7
	-1.13	-1.31	-1.20	mitogen-activated protein kinase 6
	-1.06	1.36	1.27	mitogen-activated protein kinase kinase 1 interacting protein 1
	-1.24	-1.89	-1.26	mitogen-activated protein kinase kinase kinase kinase 4

Effect of selenium on gene expression in muscle

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Glutathione-S-Transferases: A superfamily of stress-inducible detoxifying enzymes

Gene name	Symbol	FC SM	FC SS	FC SP
Glutathione S-transferase, alpha 3	Gsta3	NS	-2.3	-2.5
Glutathione S-transferase, alpha 4	Gsta4	NS	NS	-2.5
Glutathione S-transferase, mu 1	Gstm1	NS	-2.4	NS
Glutathione S-transferase, mu 2	Gstm2	NS	-2.1	-2.1
Glutathione S-transferase, mu 3	Gstm3	NS	-2.7	-2.3
Glutathione S-transferase, theta 1	Gstt1	NS	NS	-1.4
Glutathione S-transferase, theta 2	Gstt2	NS	-1.3	NS

- Promoter regions of GST genes contain antioxidant response elements
- They are switched on in response to oxidative stress
- Lower expression levels => lower oxidative stress

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Identifying genetic markers for oxidative stress and DNA damage

- GADD45β (Growth Arrest and DNA Damage-Inducible) gene.
- Regulation of cell cycle and apoptosis (programmed cell death).
- Induced in response to oxidative stress and, in particular, DNA damage.
- Now recognized as an excellent marker gene for these stressors.

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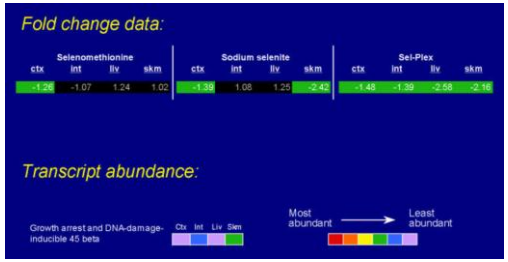
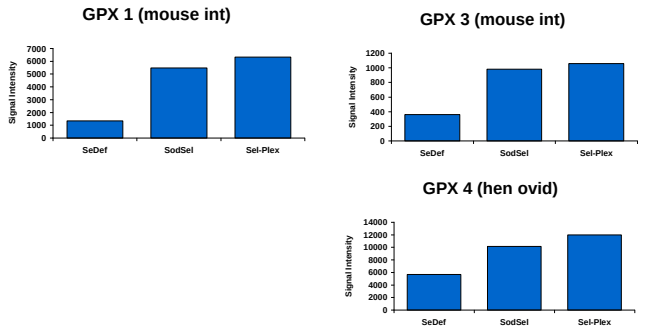
Cross-tissue analysis: GADD45β

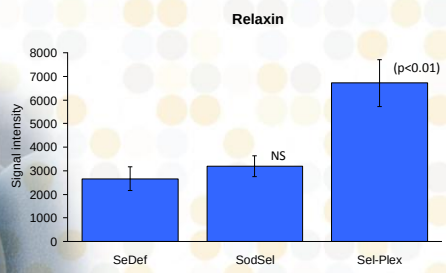


- GADD45β expression significantly decreased across all tissues tested by Sel-Plex® only
- Indicates lower endogenous oxidative stress and DNA damage throughout the entire animal

Effect of selenium supplementation on GPX gene expression profiling (mouse and hen)

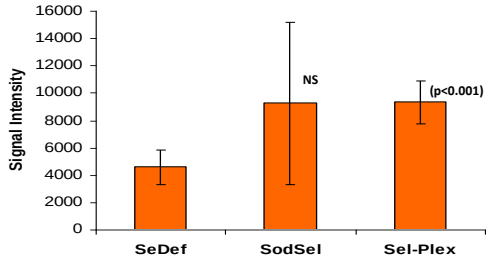


Effect of selenium supplementation on relaxin gene expression in hen oviduct



Unexpected upregulation by Sel-Plex of the gene encoding this important pregnancy-associated hormone.

Seleno Protein P gene expression profiling in hen oviduct



Spermatozoal Abnormalities Found in Semen from Roosters (0.2 mg/kg Se)

Spermatozoal Form	Basal %	Selenite %	Sel-Plex %
Normal	57.9[c]	89.4[b]	98.7[a]
Bent Midpiece	18.7[a]	6.2[b]	0.7[c]
Swollen Midpiece	1.6[a]	0.4[b]	0.1[c]
Ruptured Midpiece	0.9[a]	0.1[b]	0.0[b]
Swollen Head	1.3[a]	0.2[b]	0.2[b]
Cork Screw Head	15.4[a]	1.8[b]	0.2[c]
Coiled	3.2[a]	0.8[b]	0.0[c]
Fragment/Other	1.0[a]	1.1[a]	0.1[b]

Edens et al., 2002

[a,b] in row sign. different (P< 0.05)

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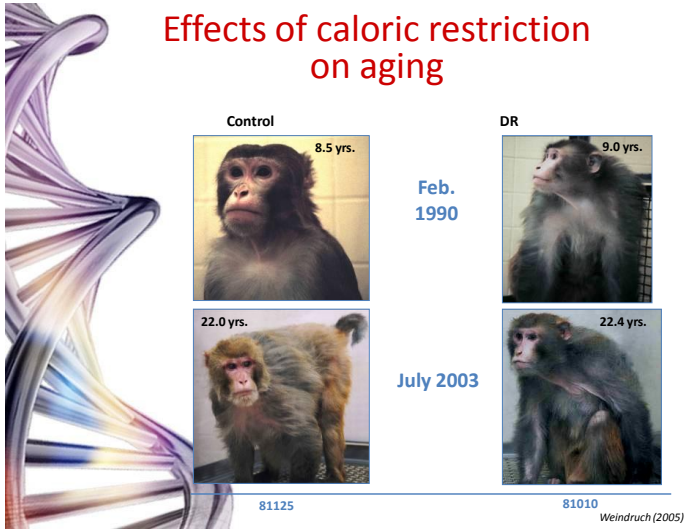
Effects of caloric restriction on aging



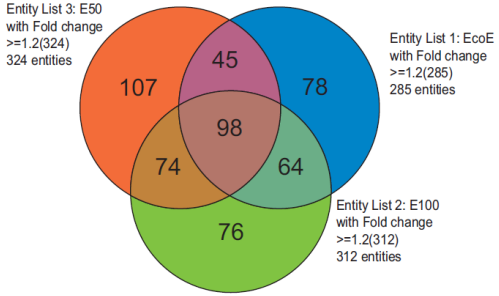
Weindruch (2005)

Δ Age (fold)%	Encoded Protein	Class	CR prevention	Se change (FC Se Def)
↑ 4.9	Complement C4	Inflammation	52%	N
↑ 1.8	C1qb	Complement/Inflammation	100%	↓ 1.5*
↑ 1.8	C1qc	Complement/Inflammation	75%	↓ 1.5*
↑ 1.7	C1qa	Complement/Inflammation	100%	↓ 1.6*
↑ 2.0	Casein Kinase 1-delta	Genotoxicity/Stress	N	↓ 1.3
↑ 1.9	Cathepsin Z	Protease/Stress	70%	↓ 1.48*
↑ 1.8	Cathepsin D	Protease/Stress	64%	↓ 1.3*
↑ 1.7	Cathepsin S	Protease/Stress	56%	N
↑ 1.8	Jun b	Neural injury/Stress	N	↓ 2.0*
↑ 1.8	Gadd	DNA damage/Stress	N	↓ 1.5*
↓ 2.0	Hox-1.4	Growth/Trophic factor	N	HoxA-9 ↑ 4.58
↓ 1.7	Hox-3.5	Growth/Trophic factor	37%	HoxA-2 ↑ 6.72
↓ 1.7	Ubiquitin thiolesterase	Protein degradation	49%	↑ 1.79*

* Denotes effects unique to Sel-Plex

Inflammatory Response | Stress Response | Neocortical growth factors | Protein turnover

Figure 2. Venn diagram of genes differentially regulated by E50, E100 or EcoE in breast muscle of broilers when compared to control birds.



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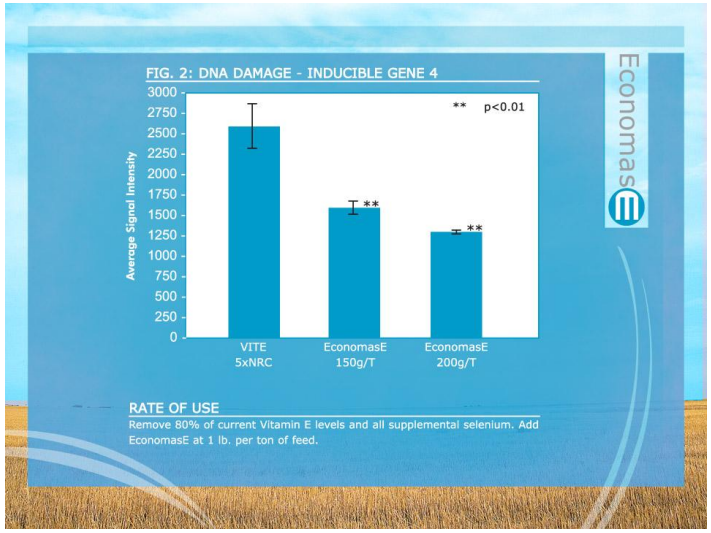
FIG. 2: DNA DAMAGE - INDUCIBLE GENE 4
Average Signal Intensity
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
** p<0.01
**
**
VITE 5xNRC
EconomasE 150g/T
EconomasE 200g/T
Economas
RATE OF USE
Remove 80% of current Vitamin E levels and all supplemental selenium. Add EconomasE at 1 lb. per ton of feed.

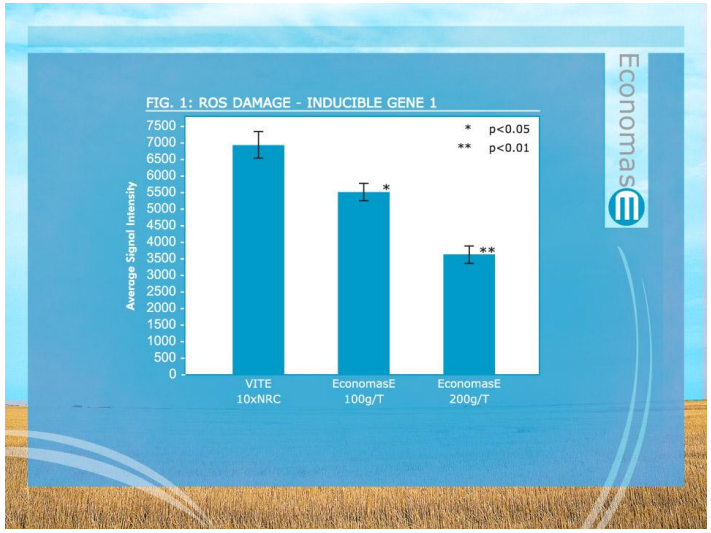
FIG. 1: ROS DAMAGE - INDUCIBLE GENE 1
Average Signal Intensity
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
* p<0.05
** p<0.01
*
**
VITE 10xNRC
EconomasE 100g/T
EconomasE 200g/T
Economas

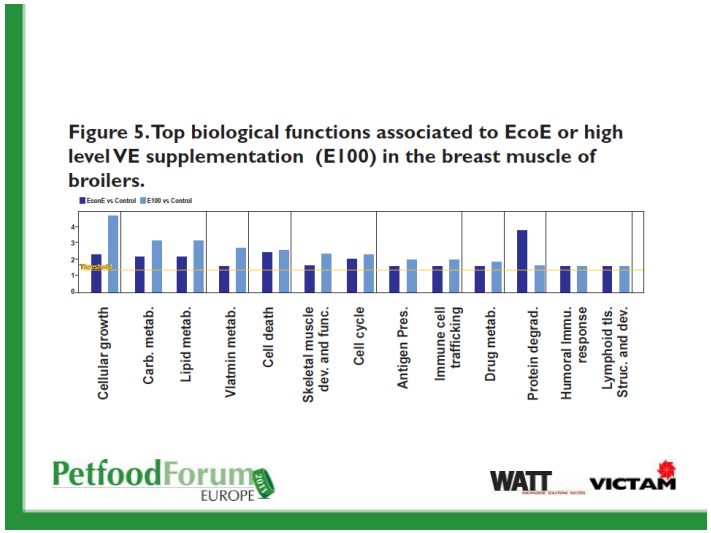
Figure 5. Top biological functions associated to EcoE or high level VE supplementation (E100) in the breast muscle of broilers.
EcoE vs Control
E100 vs Control
Cellular growth
Carb. metab.
Lipid metab.
Vitamin metab.
Cell death
Skeletal muscle dev. and func.
Cell cycle
Antigen Pres.
Immune cell trafficking
Drug metab.
Protein degrad.
Humoral immu. response
Lymphoid tis. Struc. and dev.
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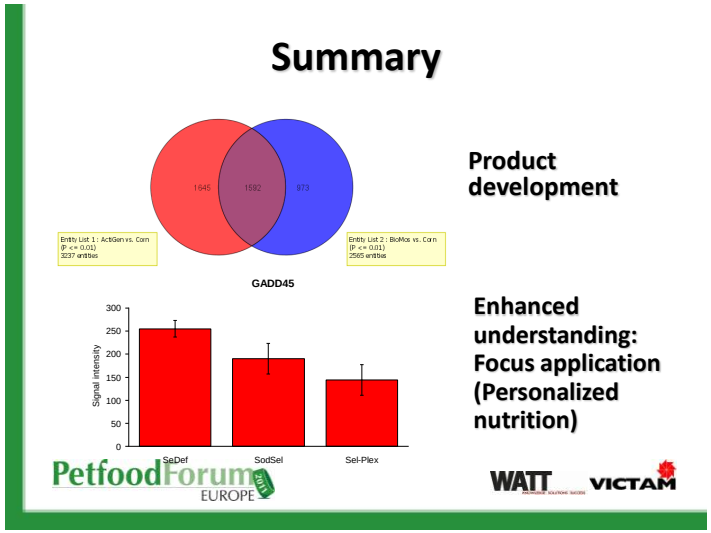
Summary
1646
1592
973
GADD45
Signal intensity
300
250
200
150
100
50
0
SeDef
SodSel
Sel-Plex
Product development
Enhanced understanding: Focus application (Personalized nutrition)
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