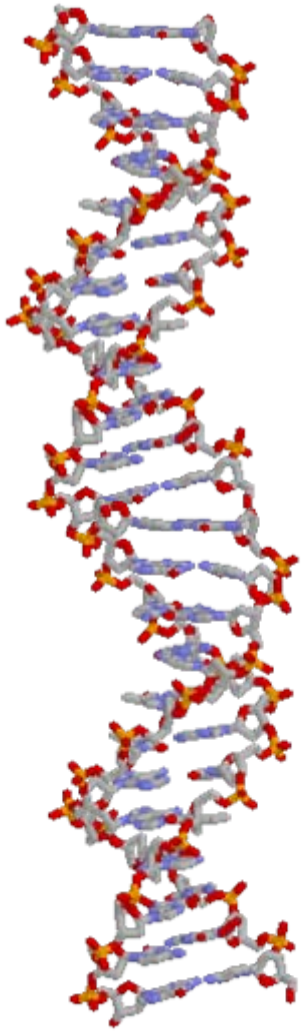


Nutrigenomics – Exploring the link between genetic expression and nutrition.

Dr. Keith Filer
Research Manger
AP Biosciences Centre

Gene chip

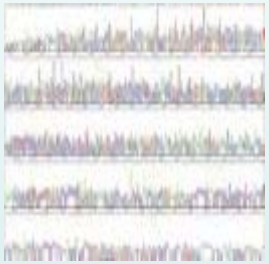
Nutrigenomics

- How diet influences gene transcription, protein expression and metabolism.
- 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

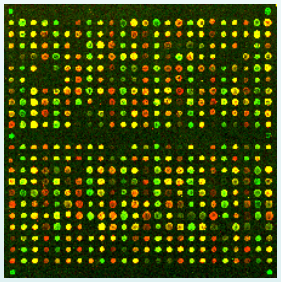
Alltech®

DNA → (Expression) → RNA → (Translation) → Protein → (Metabolism) → Metabolite

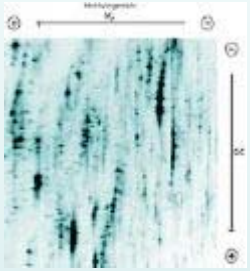
Genome | Transcriptome | Proteome | Metabolome



Sequencing
SNP analyses
QTL



Microarray
qRT-PCR

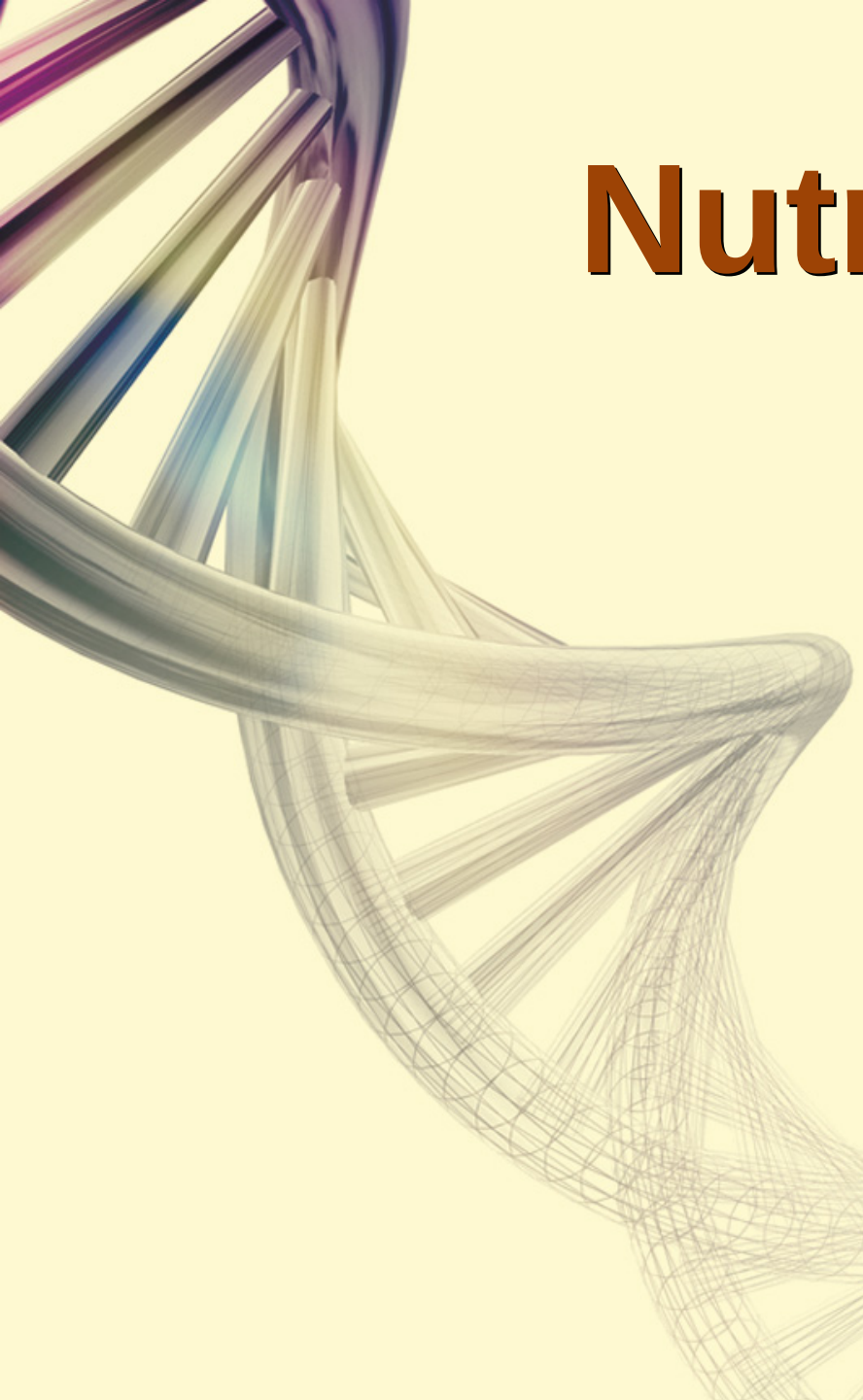


2D Gel
MALDI-TOF
ESI-MS/MS

GC-HPLC
LC/MS or
GC/MS
FTR, NMR

Bioinformatics
Biocomputation

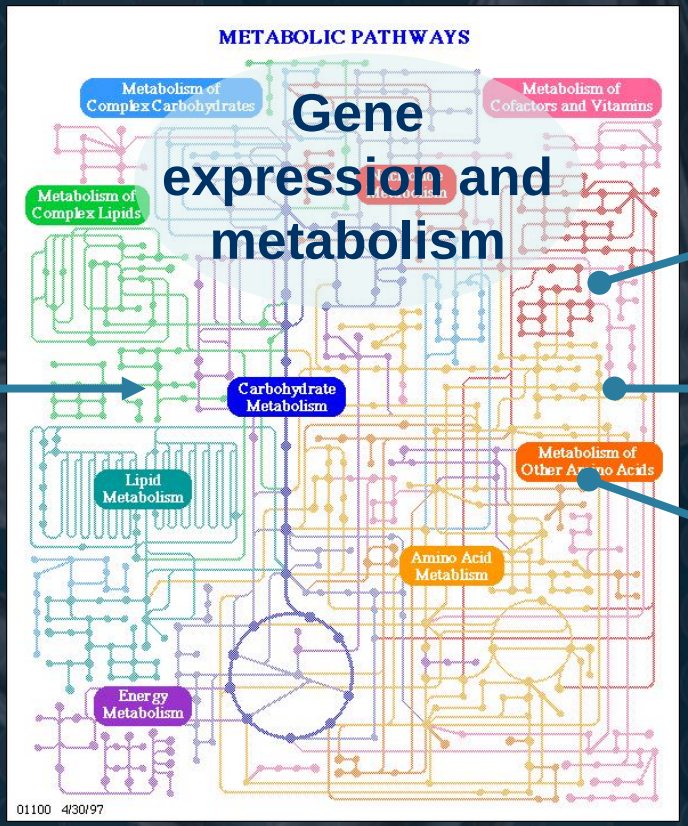
DIET + **FUNCTIONAL GENOMICS** = **NUTRITIONAL GENOMICS**



Nutrigenomics

How can we best assess the effects of a nutrient on an organism?

Traditional animal trial approach



Diet

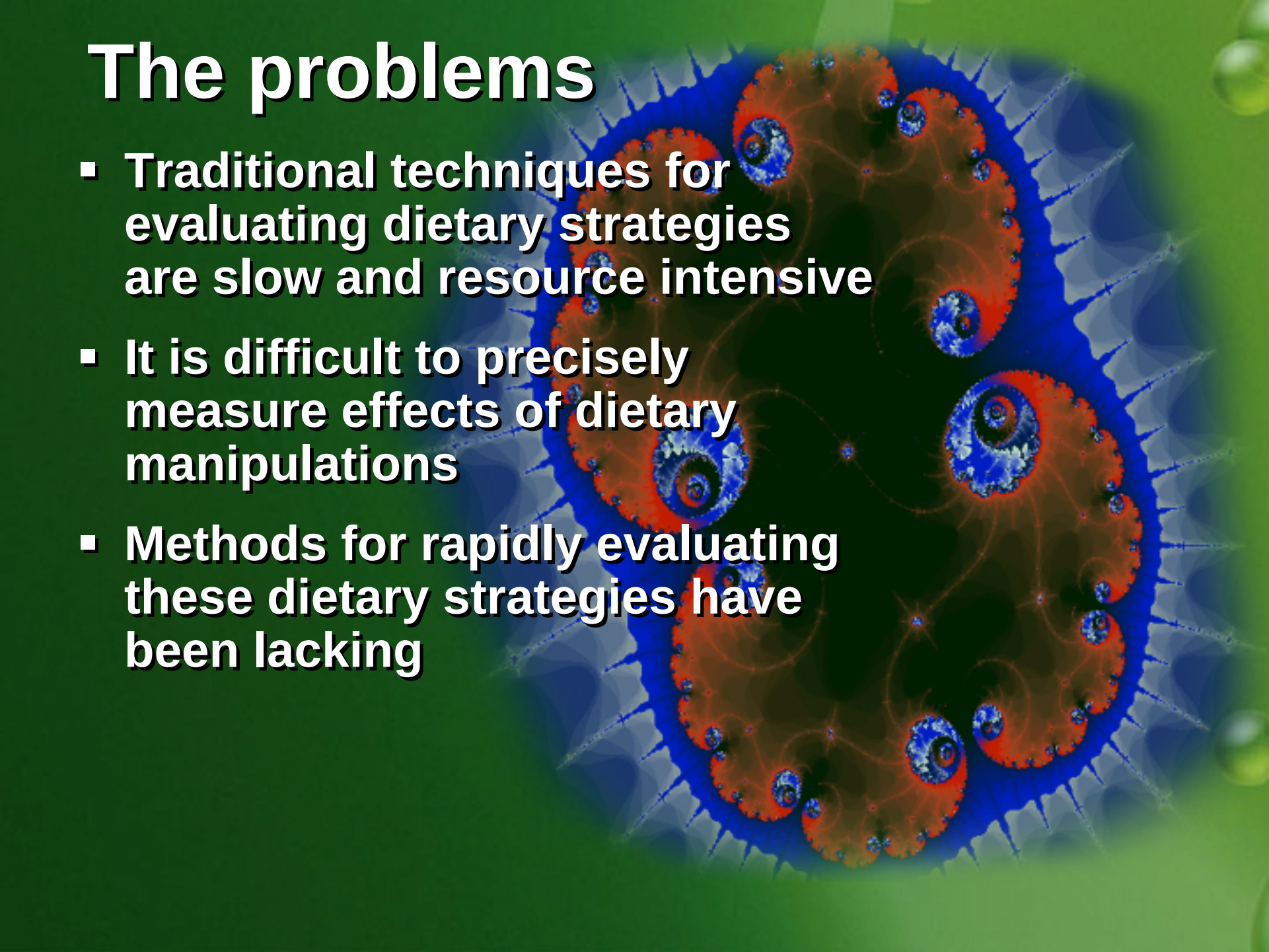
Animal health

Fertility

Performance

The problems

- Traditional techniques for evaluating dietary strategies are slow and resource intensive
- It is difficult to precisely measure effects of dietary manipulations
- Methods for rapidly evaluating these dietary strategies have been lacking



Transcriptomics research
- DNA microarray or GeneChip® System

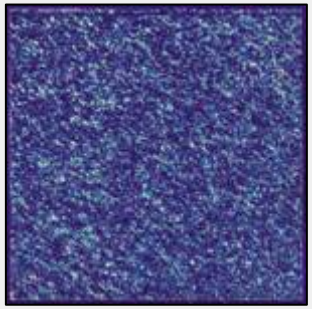


Image courtesy of Affymetrix

DNA Microarray Technology

- Evaluates the activity of thousands of genes in a single experiment.
- Tells us which genes are switched on, switched off or remain unchanged in response to a nutrient or diet.

Nutrigenomics

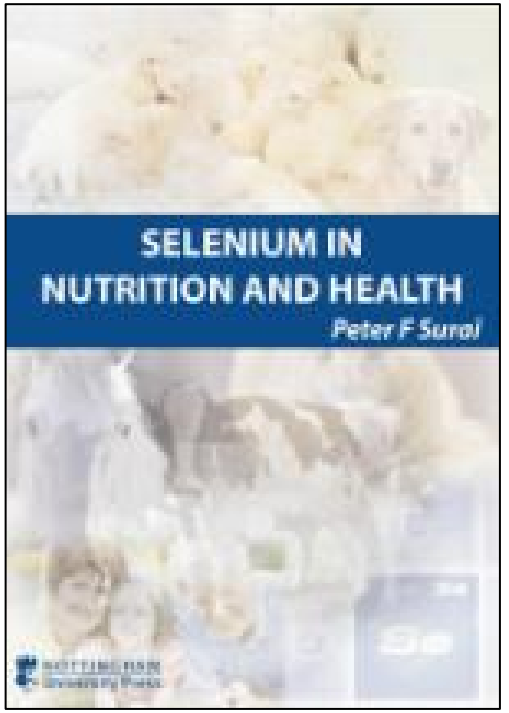
Alltech®

Many different areas!

- Fertility
- Embryonic development
- Muscle growth
- Meat quality
- Feed efficiency
- Immunobiology

Some practical examples

Nutrigenomic effects of selenium on fertility and production



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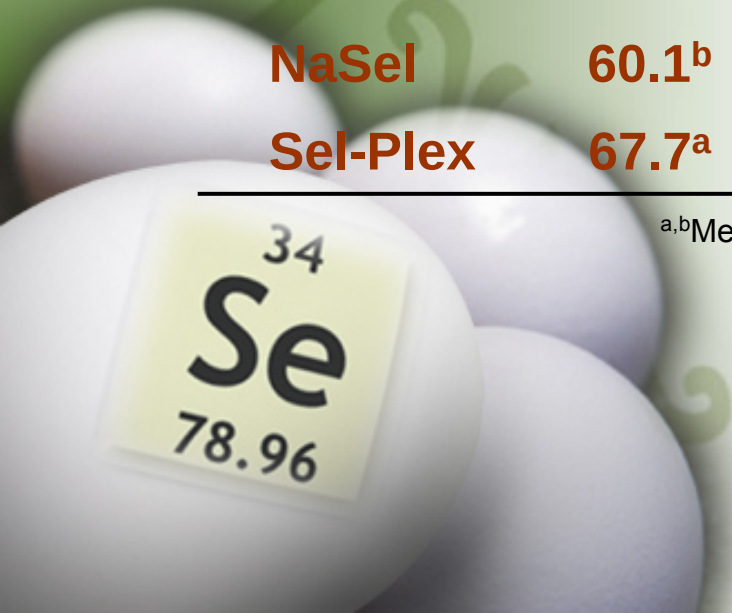
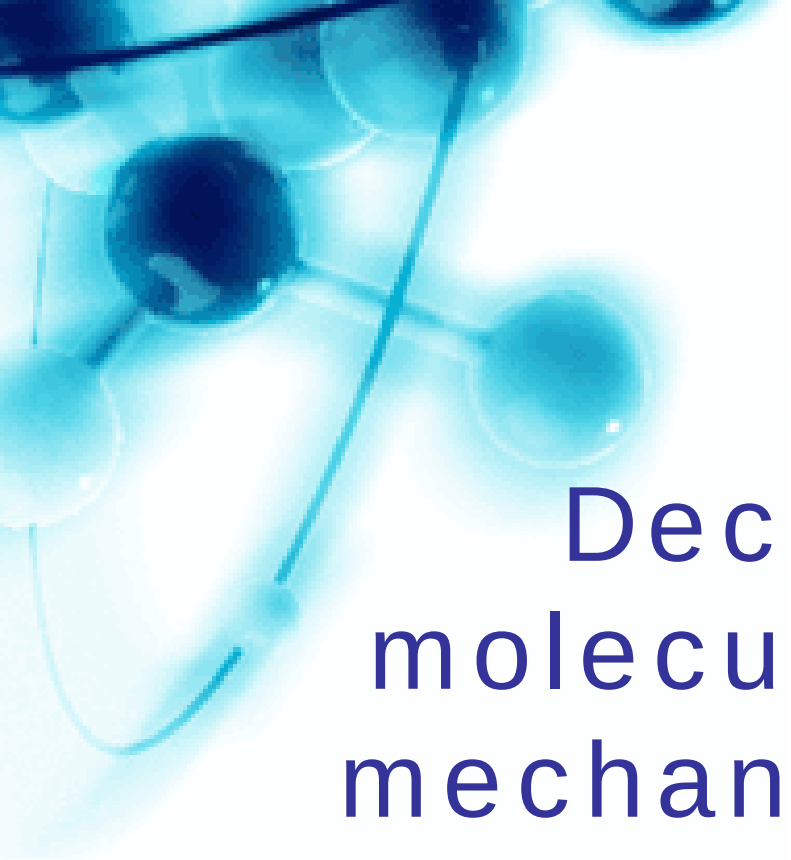
Effect of selenium inclusion and source on egg production traits of broiler breeders

	Hen-housed egg production, 49-58 wk (%)	Total egg production (#)	Settable egg production (#)	Unsettable egg production (% of total)	Chick production (No. 58 wk)
NEG	60.2[b]	174.5	168.5	3.49	131.3[b]
NaSel	60.1[b]	172.7	168.6	2.37	139.1[ab]
Sel-Plex	67.7[a]	177.7	174.6	1.90	145.8[a]

[a,b]Means within a column with no common superscript differ (P<0.05).

Renema, 2006





Deciphering the molecular/biochemical mechanisms underlying those effects

Effect of selenium nutrition on gene expression in broiler breeder hens

- Hens fed one of three diets:
  - Control (0.02 ppm)
  - Sodium selenite supplemented (0.3 ppm)
  - Sel-Plex supplemented (0.3 ppm)
- Collected muscle, liver, cerebellum, cortex, duodenum and oviduct after 58 weeks of Se supplementation
- Performed microarray analysis on the duodenum and oviduct
- Comparisons were made between selenium-treated and control (n=7 per group)

Influence of selenium nutrition on gene expression in broiler breeder hens

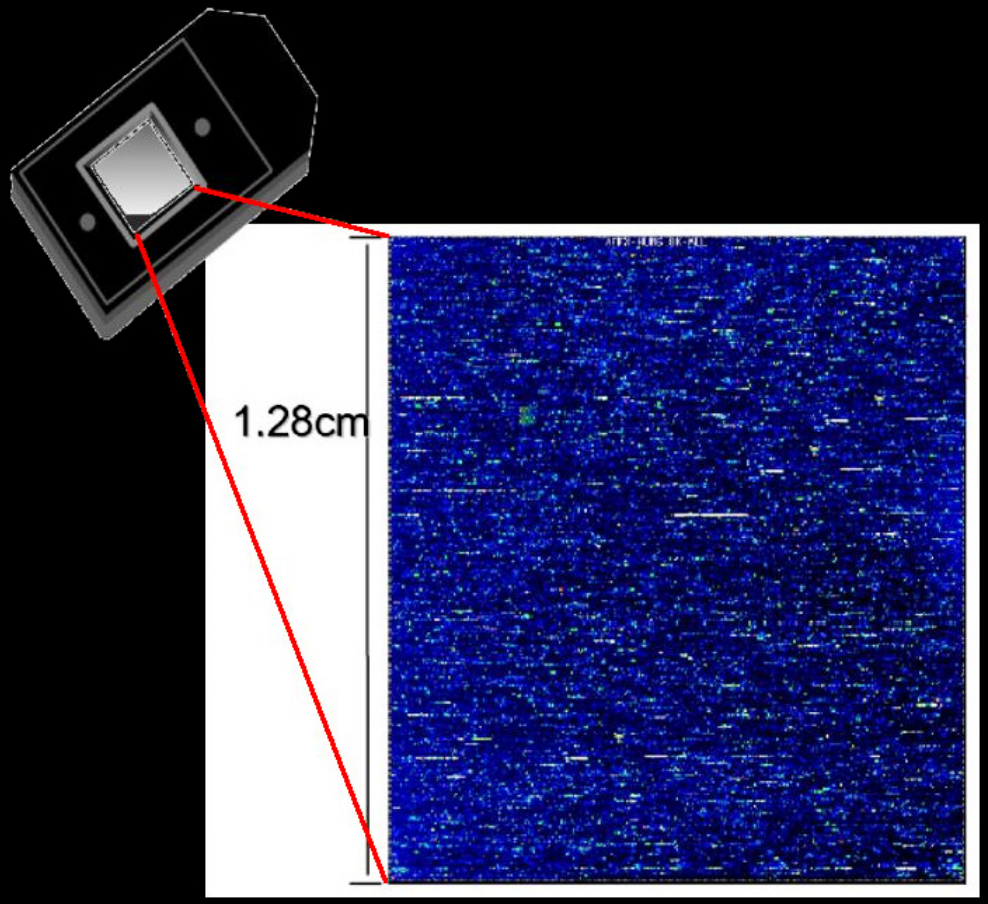
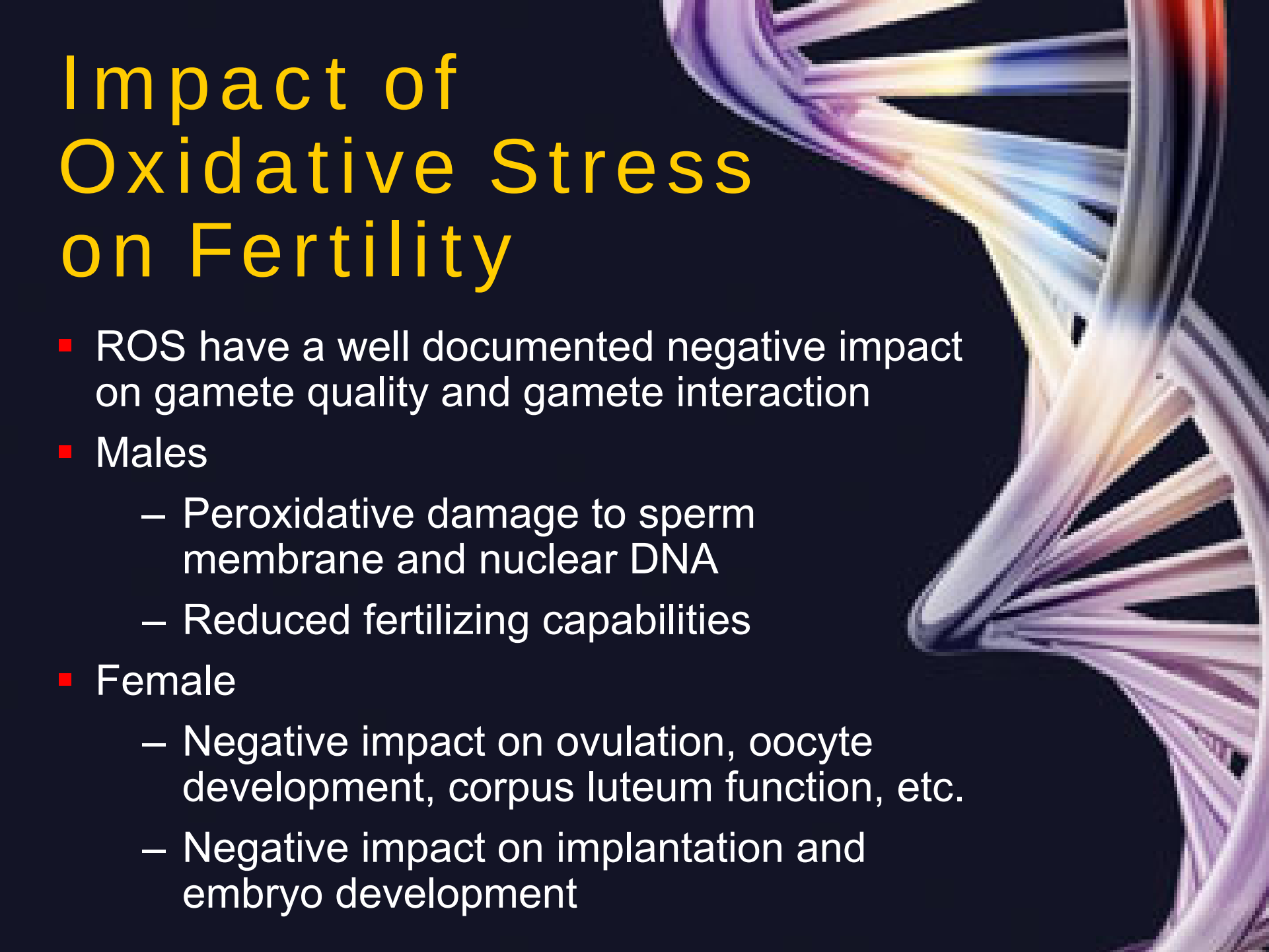


Image of hybridized array

Affymetrix Chicken Genome Array

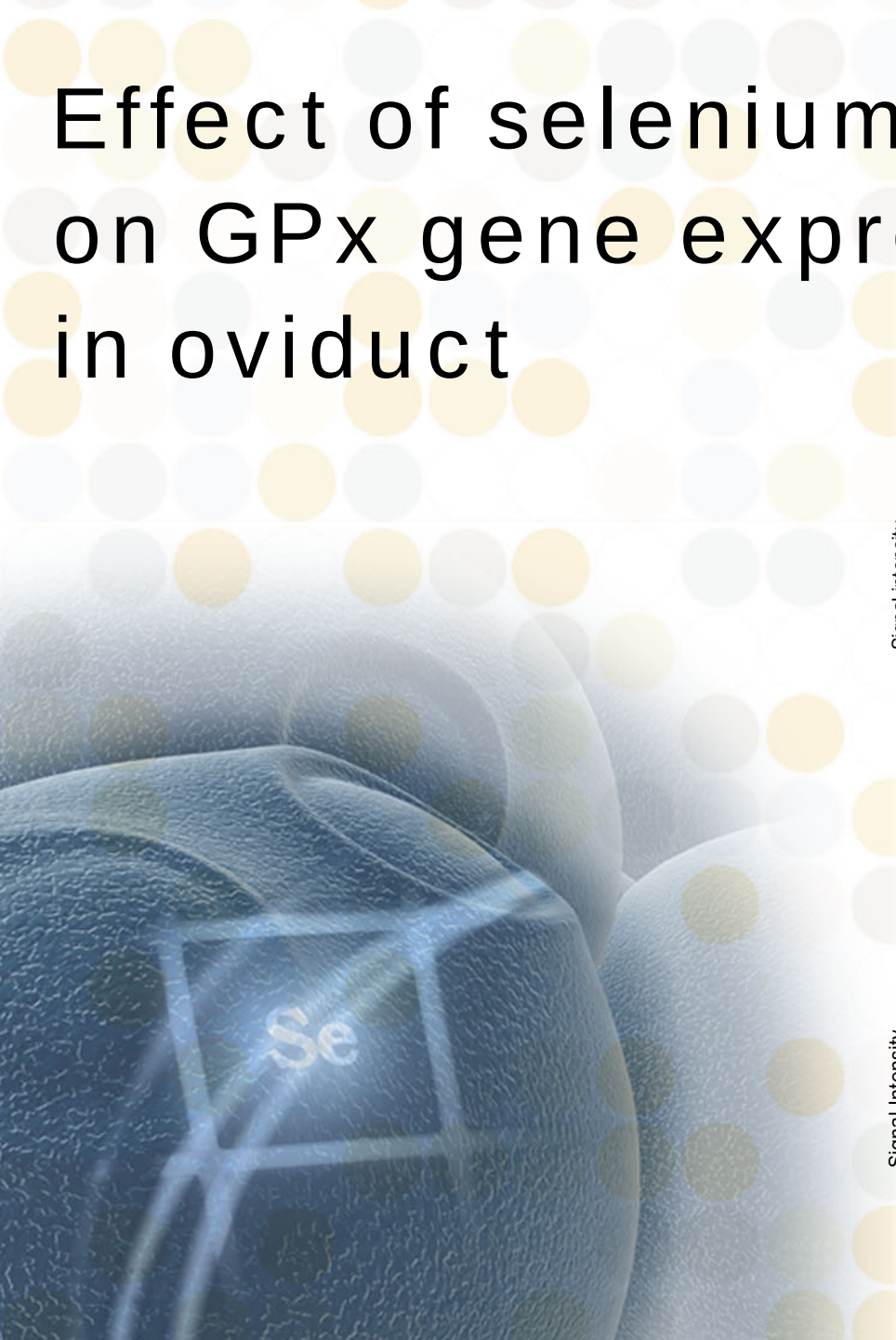
- ~38,000 probe sets on the array
- ~14,000 unique transcripts on the array
- Removed duplicates and probe sets without a known Entrez Gene ID
- 1,304 transcripts significantly affected by at least one treatment in intestine @ $P \leq 0.05$
- 5,105 transcripts significantly affected by at least one treatment in oviduct @ $P \leq 0.01$

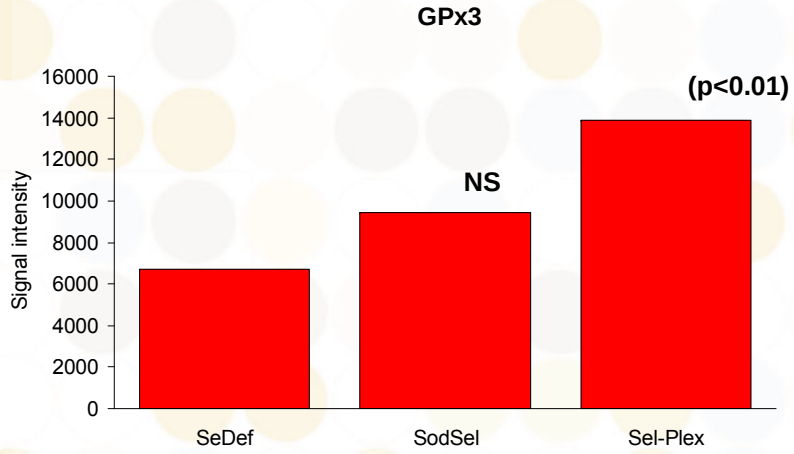
Impact of Oxidative Stress on Fertility

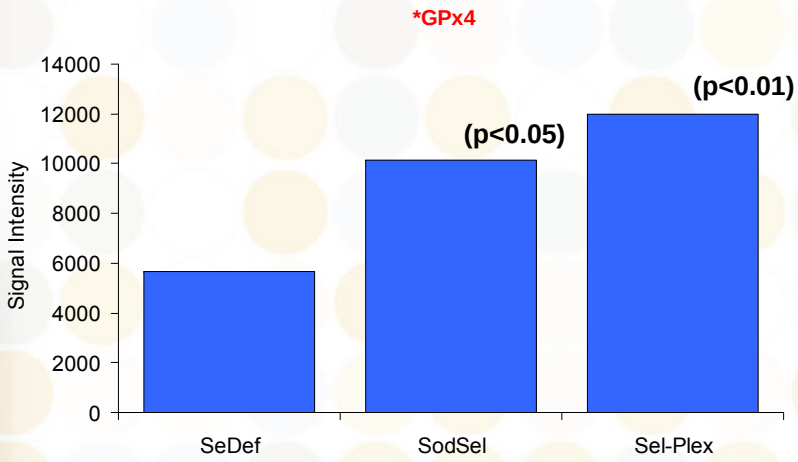


- ROS have a well documented negative impact on gamete quality and gamete interaction
- Males
  - Peroxidative damage to sperm membrane and nuclear DNA
  - Reduced fertilizing capabilities
- Female
  - Negative impact on ovulation, oocyte development, corpus luteum function, etc.
  - Negative impact on implantation and embryo development

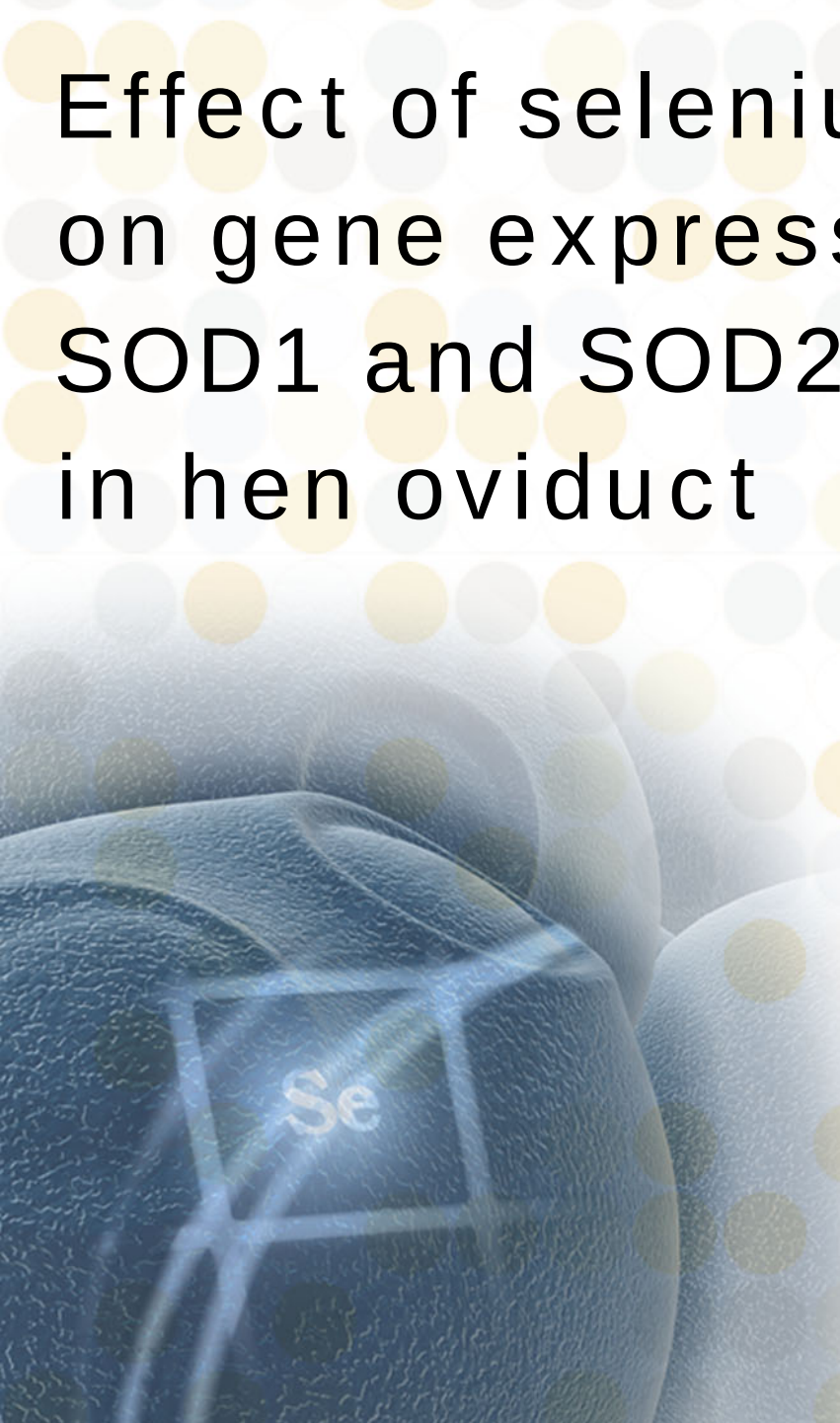
Effect of selenium supplementation on GPx gene expression profiles in oviduct

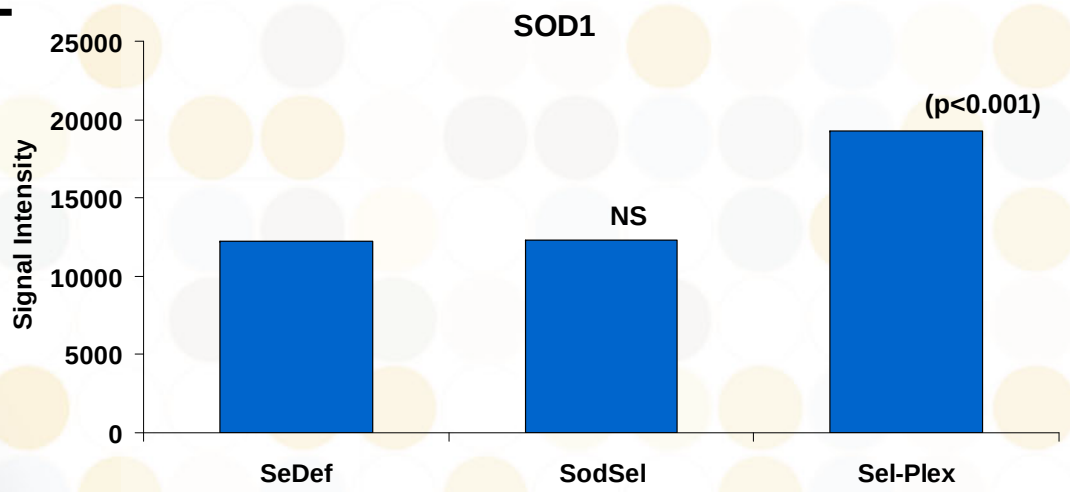


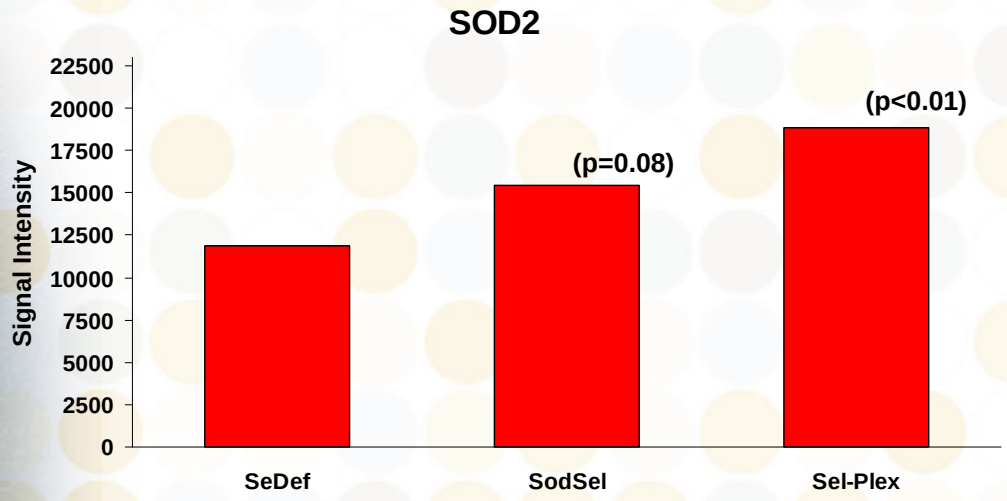




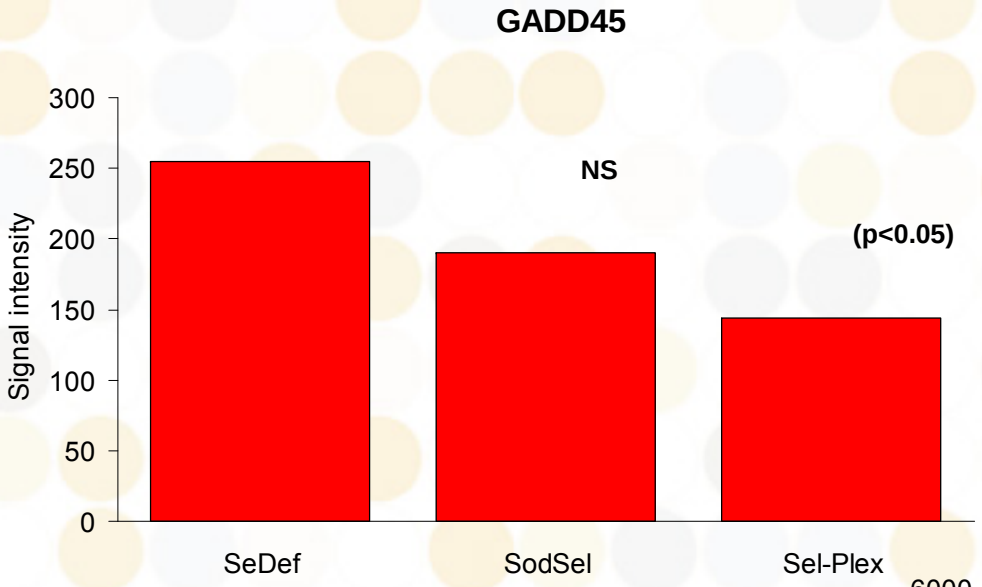
Effect of selenium supplementation on gene expression profiling of SOD1 and SOD2 in hen oviduct



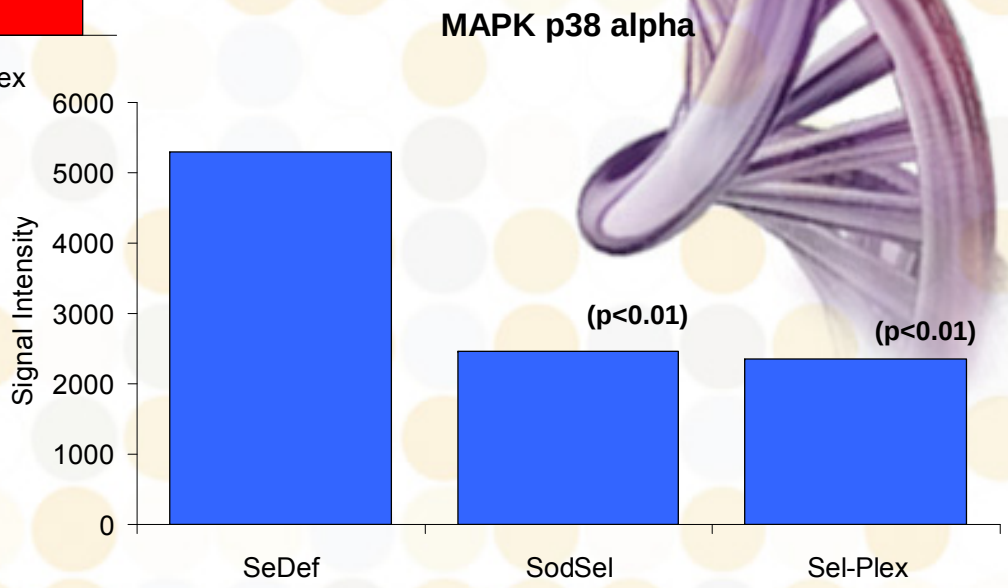




DNA damage checkpoint genes



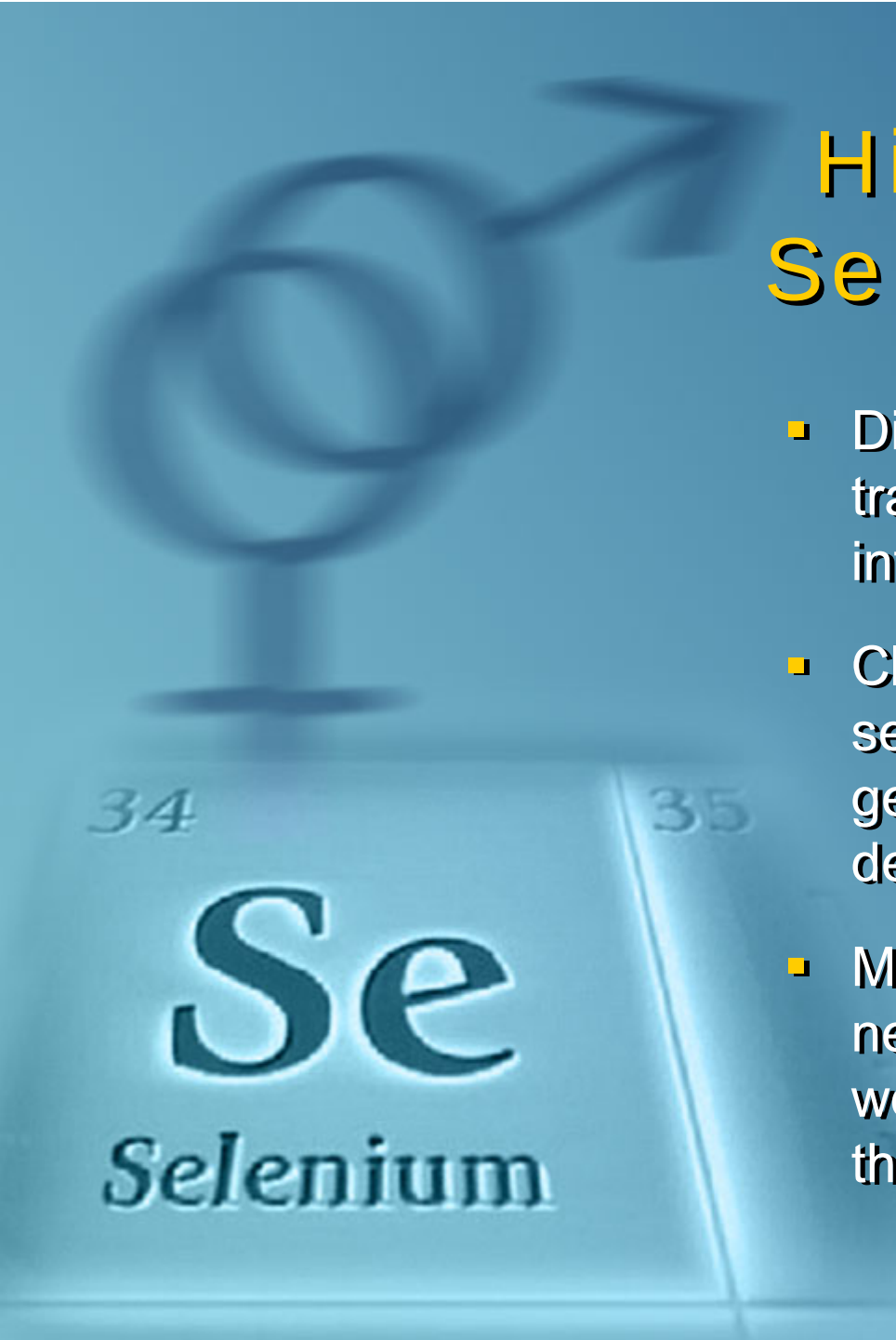
- Induced by genotoxic stress
- Trigger apoptosis (programmed cell death)
- Increased expression = increased DNA damage



Unexpected transcriptional shifts noted in response to selenium supplementation in oviduct

Effect of selenium supplementation on transcription/growth factors in hen oviduct

Gene Title	Biological Process	FC SelDef SodSel
Fibroblast growth factor 1 (acidic) (FGF1)	Angiogenesis, cell proliferation/differentiation, embryonic development, organogenesis, tissue repair	+1.02
Fibroblast growth factor 2 (basic) (FGF2)	Limb and nervous system development, cell cycle regulation, wound healing	+1.75
SNF1-like kinase	Regulation of insulin receptor signaling, cell cycle regulation, muscle growth/differentiation	+1.81
Platelet-derived growth factor alpha (PDGFA)	Growth factor activity, regulation of progression through the cell cycle, cell proliferation	+1.54
Cyclin D1 (CCND1)	Cell cycle, cell division regulation	+1.51
Activin A receptor, type 1 (ACVR1)	Activin signaling, FSH production, follicular development in ovary	+1.19



Hidden effects of Sel-Plex on fertility

- Dietary selenium source elicited transcriptional shifts that are intimately linked with fertility
- Clear differences in the effects of selenium sources on fertility related gene expression patterns have been demonstrated
- Many of the effects noted have never been linked to selenium and would have remained hidden without the use of microarray techniques

Another example

These molecular approaches explain the effects of selenium on ***production-energy metabolism***

Reported changes in milk production associated with Sel-Plex supplementations: *The observations*

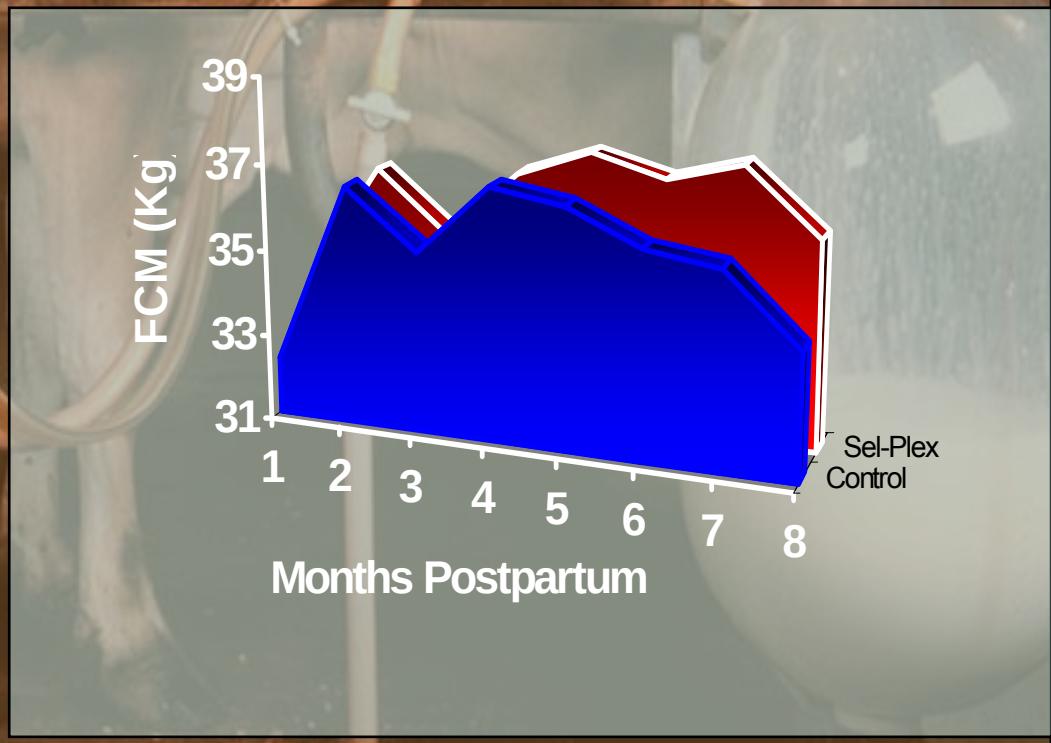
Controlled research studies

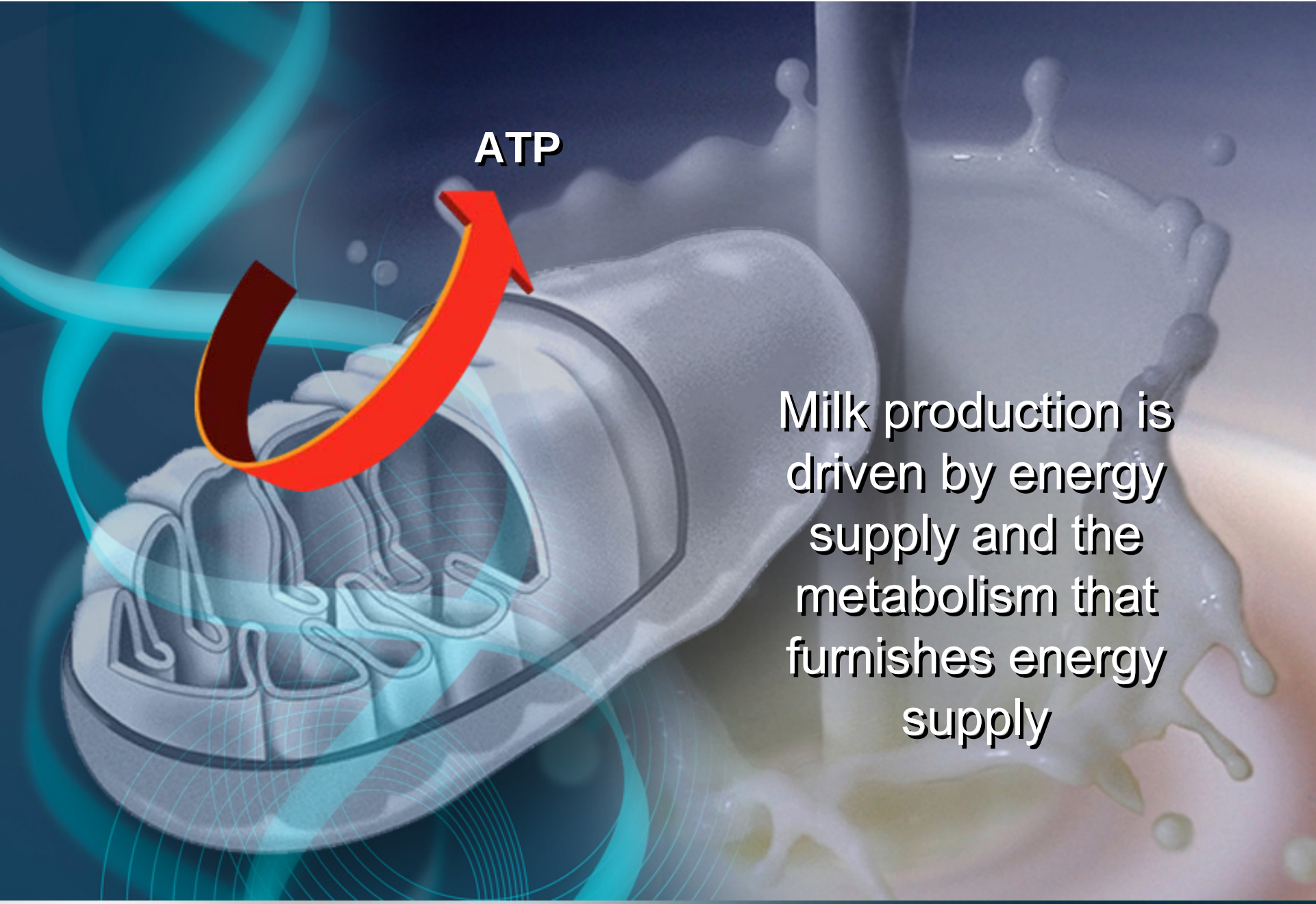
Florida

- **Fat corrected milk production was 0.9 kg greater in Sel-Plex supplemented cows**

California

- **Fat corrected milk production was 1.9 kg greater in Sel-Plex supplemented cows**





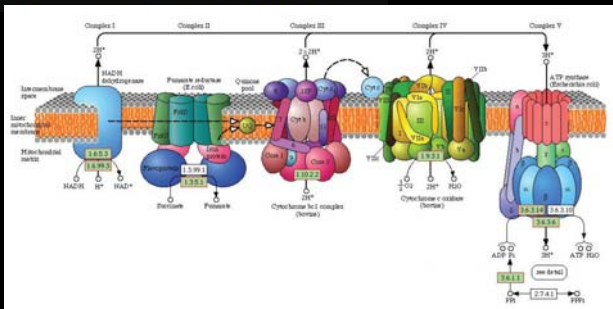
Milk production is driven by energy supply and the metabolism that furnishes energy supply

Alltech®

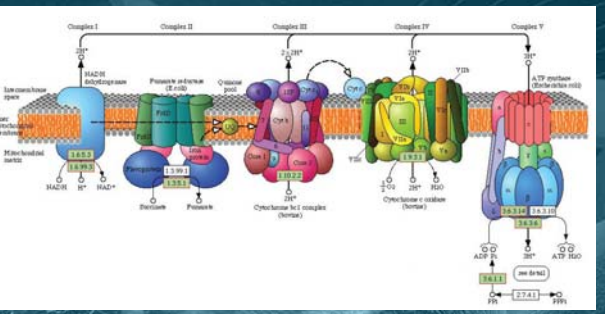
Energy production genes remarkably affected by selenium nutrition

Oxidative phosphorylation (energy production)

Gene Symbol	FC_SodSel	Gene Title
NDUFA5	**1.94**	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa
NDUFA7	1.68	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7, 14.5kDa
NDUFB3	1.91	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa
NDUFB4	**-3.10**	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa
NDUFB5	-1.12	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa
NDUFB6	**3.07**	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6, 17kDa
NDUFC2	1.90	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 2, 14.5kD
NDUFS3	1.41	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa (NADH-coe
NDUFS4	1.44	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coe
NDUFS6	**1.60**	NADH dehydrogenase (ubiquinone) Fe-S protein 6, 13kDa (NADH-coe
RCJMB04_33n14	**-4.55**	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa
UQCRFS1	**-1.80**	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1
COX6A1	1.91	cytochrome c oxidase subunit VIa polypeptide 1
COX6C	**3.69**	cytochrome c oxidase subunit VIc
CYCS	1.43	cytochrome c, somatic
ATP5G1	2.08	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit C1
ATP5H	**2.47**	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d
---	**3.30**	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, is
ATP5S	1.11	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (f
ATP5C1	1.82	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma pol



Increased ATP synthesis → **More efficient diet utilization** → **More energy for production**



Other applications for Nutrigenomics

- New ingredient X
- Will it enhance production?
- Will it improve fertility?
- Will it affect product quality?

These questions can now be rapidly addressed using Nutrigenomics

Alltech®

Application in product development

Nutritional strategies that provide for alternatives to vitamin E supplementation

The problem

- Antioxidants are required for optimal growth, immunity, and reproduction

- Historically, nutritionists have added vitamin E 5 to 10 times in excess of NRC requirements for swine and poultry

Supplies of vitamin E are limiting and are expensive

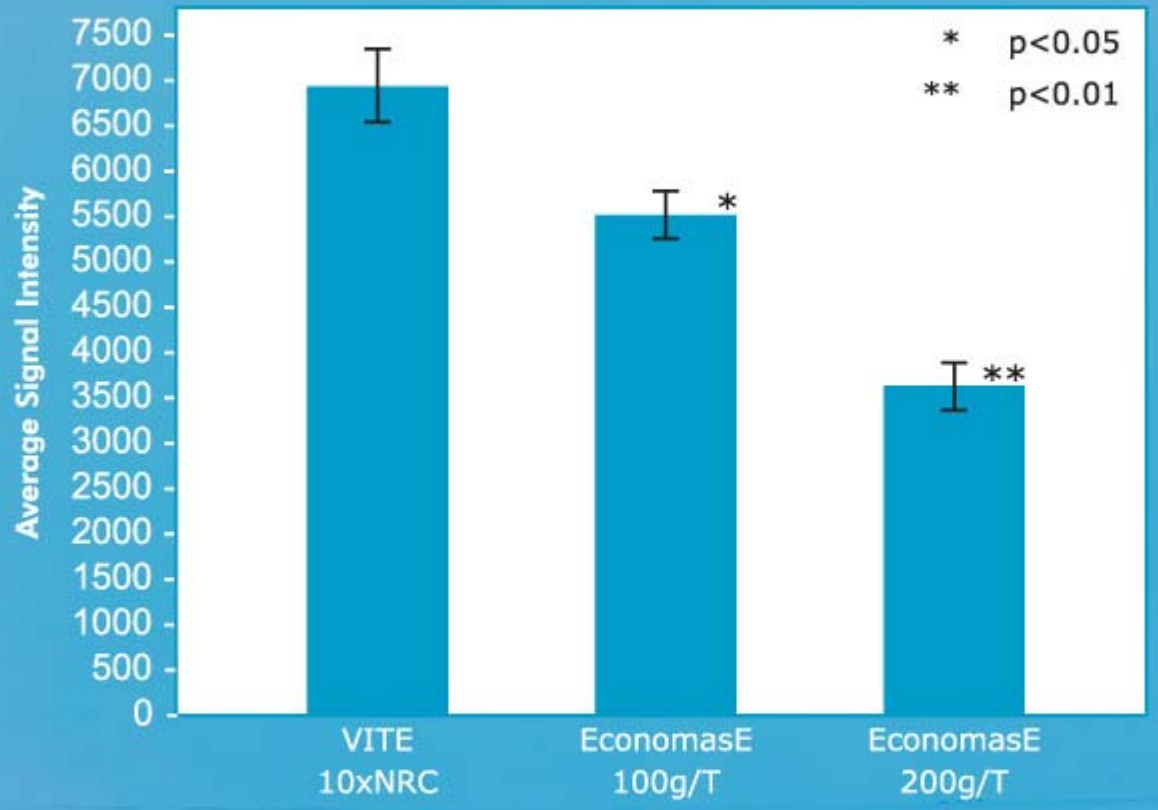
The alternative strategy:

- EconomasE™ is a proprietary blend of ingredients that maximizes the antioxidant status of the animal.

- Gene expression models have shown that EconomasE™ helps maximize the synthesis, recycling, and response of the animal's antioxidant system, especially vitamin E.

	ctx	int	liv	skm	ctx	int	liv	skm	ctx	int	liv	skm
Rpl12	-1.09	1.47	-1.11	1.07	1.24	-1.38	-1.34	1.46	1.14	-1.47	-1.21	1.39
Rpl13a	-1.06	1.17	-1.04	1.01	1.13	-1.31	-1.23	1.16	-1.08	-1.26	-1.27	1.18
		12	-1.02	1.02	1.16	-1.66	-1.18	1.27	1.02	-1.51	-1.10	1.27
		00	*-1.04*	*1.03*	*1.14*	*-1.68*	*-1.20*	*1.23*	*-1.08*	*-1.53*	*-1.16*	*1.22*
		23	*1.02*	**1.16**	*1.24*	*-1.38*	*-1.09*	*1.23*	**1.18**	*-1.18*	*-1.06*	*1.19*
		04	-1.04	1.20	1.22	-1.42	-1.10	1.23	1.06	-1.17	-1.07	1.27
		06	-1.31	-1.05	1.17	1.47	-1.34	1.09	1.13	1.43	-1.31	1.27
		03	-1.10	1.08	-1.23	1.22	1.76	-2.25	-1.48	1.20	1.54	-2.31
		22	-1.07	1.04	-1.15	1.25	-1.19	-1.37	-1.20	1.13	-1.28	-1.28
		19	-1.02	-1.02	1.03	-1.28	-1.13	1.82	-1.26	-1.30	-1.11	1.81
		30	-1.04	-1.01	1.21	-1.41	-1.20	1.32	1.11	-1.12	-1.24	1.32

FIG. 1: ROS DAMAGE - INDUCIBLE GENE 1

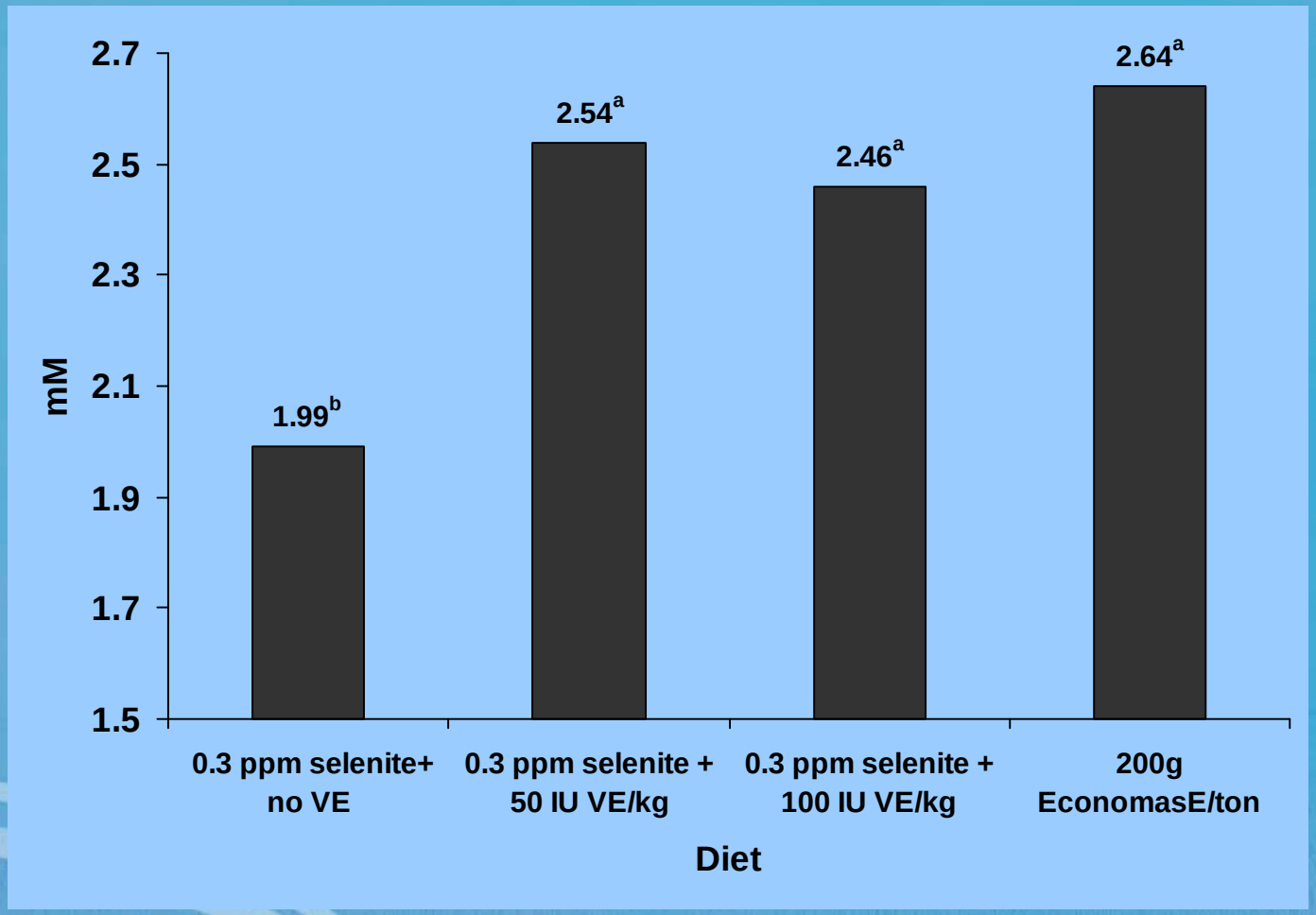


Economas E

Validation:

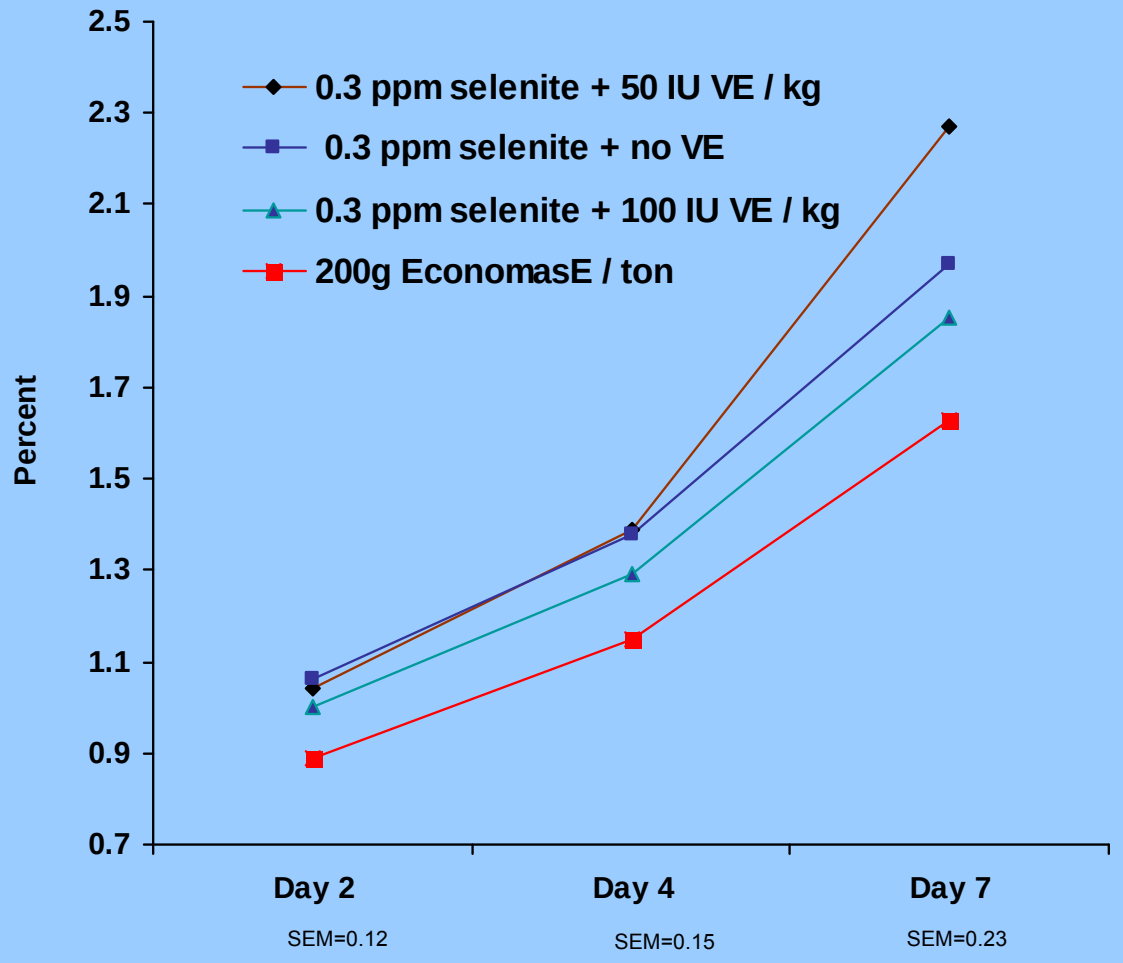
- Studies in poultry validate what we are seeing when we add EconomasE to a premix at 200g/ton in place of supplemental Vitamin E and Se

Total Antioxidant Capacity of Serum, mM

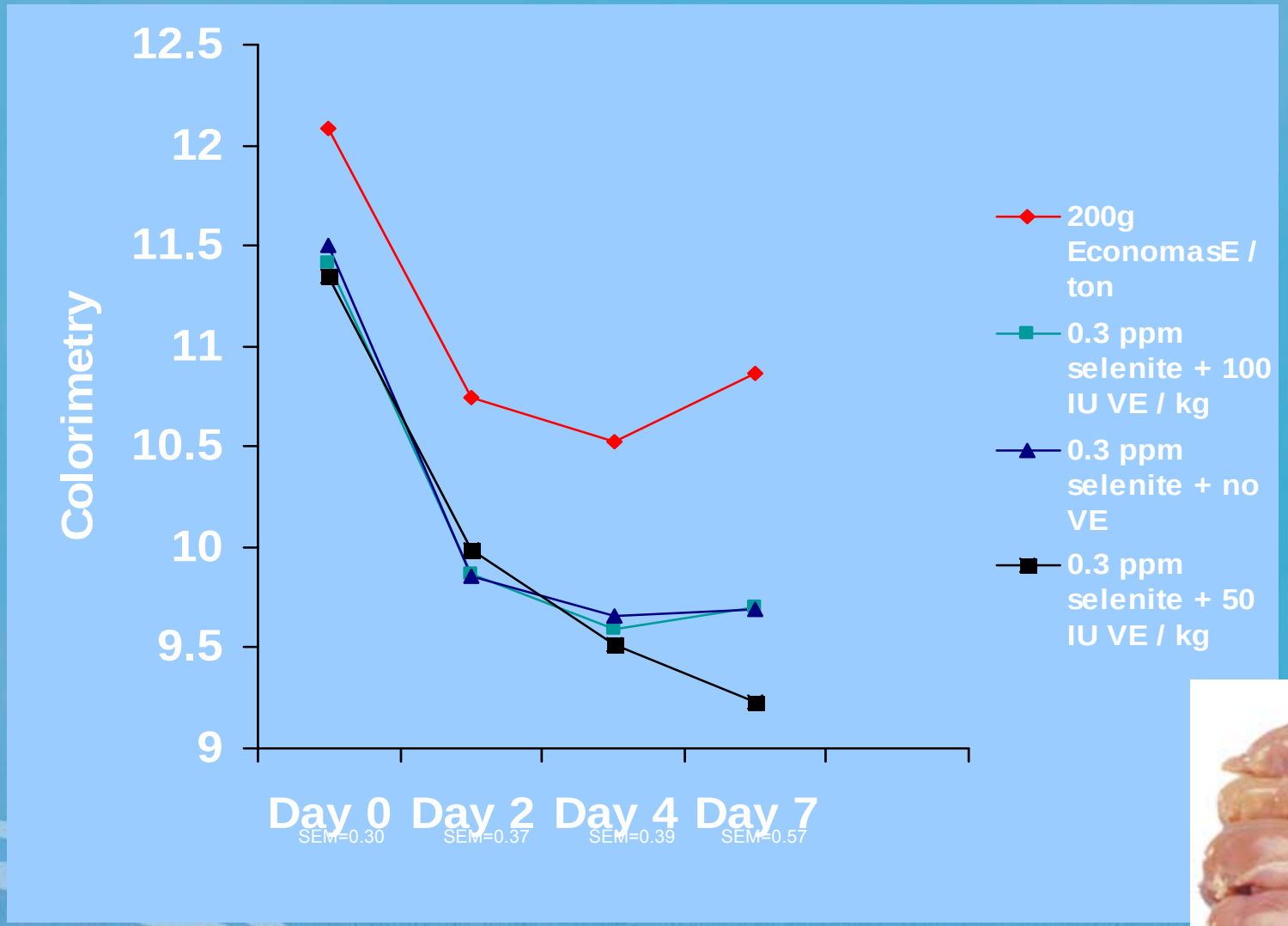


SEM=0.12

Chicken Breast Drip Loss, d 42



Colorimetry of Chicken Breast Filets a* (redness)



Nutrigenomics

- Rapid and effective evaluation of nutritional strategies is becoming a reality.
- Previously hidden effects of nutrition on animal performance can be revealed using this powerful technology.
- Thousands of data observations generated per experiment.
- Provides a valuable and novel approach for improving the efficiency of animal production.

promoting research
rewarding talent

Alltech's
Post Graduate Degree Programs

Alltech®

What is the Post Graduate Degree Program ?

The post graduate degree program sponsored by Alltech allows students to seek Masters and PhD degrees in a wide variety of research areas that benefit the animal nutrition industry.

What is the Post Graduate Degree Program ?

- In 2007 Alltech sponsored 38 PhD and M. Sc. research programs around the world.
- Sponsorship is in the form of scholarships and research funding.
- Sponsorship is renewed yearly for up to 3 years.
- The goal for Asia-Pacific is to add 15 new students this year.

How to Participate

- Fill out the forms and return to Asia-Pacific Biosciences Centre.
- Identify research area of interest.
- Identify a professor
- Prepare a proposal.

The Proposal

- The proposal addresses an important issue for the animal nutrition industry as well as Alltech and is consistent with the expertise of the professor.
- The proposal consists of background information on the topic, justification of the project, experimental design and budget.
- The final proposal is forwarded to the Asia-Pacific Biosciences Centre and reviewed.

The Review

- The proposal is reviewed based on scientific merit and importance by a regional review committee.
- Comments are forwarded to Alltech Central R&D for final approval.
- Administrative details are coordinated at the Asia-Pacific Biosciences Centre.

Completion

- Upon completion of the project a copy of the thesis and publications are provided to Alltech.
- Employment possibilities are then discussed with the student.

Contact information

Alltech Asia-Pacific

Biosciences Centre

APBioscience@alltech.com

promoting research
rewarding talent

promoting research
rewarding talent

Alltech's Internship Programs

Alltech®

What is the Internship Program ?

- Our undergraduate research internship program students an opportunity to spend time working on a research project in an industrial research laboratory at one of Alltech's three Bioscience Centers. The research you will carry out may be either an independent project or in conjunction with a university learning program.

Requirements

- Strong science based education
- Excellent communication skills
- Highly motivated
- Ability to work together in a team atmosphere
- Willingness to travel.

Possible Research Areas

- Enzyme production and applications
- Industrial fermentation processes
- Alcohol production
- Development and application of direct-fed microbial products
- Animal feed supplementation strategies
- Animal nutrition
- Animal health
- Environmental odor and pathogen reduction
- Neutralization of feed associates toxins
- Immune system modulation
- Process biochemistry, microbiology and engineering

Details

- Time frame is 3 – 6 months.
- Obtain enrollment forms for the program.
- Education background and interests will be matched with a scientist in the lab to develop a project.
- Assistance is provided to arrange travel.
- Final report and presentation required.

Contact information

Alltech Asia-Pacific

Biosciences Centre

APBioscience@alltech.com

or

www.alltech.com under careers

PROMOTING RESEARCH
REWARDING TALENT

WHAT IS THE ALLTECH YOUNG SCIENTIST AWARD?

- The Alltech Young Scientist Award program was created by Alltech, emphasizing the company's commitment to science and education, in order to promote research and develop talent.

- The program provides a unique opportunity for students to interact with one of the industry's leading players in the animal feed industry.

- The winners of the undergraduate and graduate Young Scientist Awards will receive a scholarship of $5,000 USD and $10,000 USD respectively, as well as a trophy of recognition.

WHO CAN PARTICIPATE?

- Alltech invites Undergraduate, Masters and PhD students of any Animal Science field currently enrolled in an accredited college or university to participate.

- Participating students must submit a paper chosen from a list of topics relating to natural solutions to animal health challenges.

- The winners of the regional phase progress to the international phase, where each candidate is asked to give a presentation to a panel of judges.

HOW TO PARTICIPATE

- Students must submit a scientific paper based on a topic involving animal feed technologies. Topics are suggested on our website.
- Undergraduate students' papers must be 3000 to 3500 words in length and graduate students' papers must be 3000 to 5000 words in length.
- The program will include two phases, and the winners from the regional phase will move on to the final international phase.
- There are two different categories of participation: Undergraduate and Graduate.
- There are four competing regions: North America, Latin America, Asia-Pacific and Europe and Africa.

PHASE 1 - REGIONAL LEVEL REQUIREMENTS

- Entrants must write a scientific paper or literature review based on a pre-approved topic.
- Topics are provided at www.alltechyoungscientist.com. Additional topics may be submitted for approval to youngscientist@alltech.com.
- The paper submitted must be an original and unpublished work of the entrant only.
- The paper must include a summary with a maximum of 250 words.
- In the regional phase, papers in languages other than English will be accepted.

PHASE 1 – AWARDS

Undergraduate

- 1st Place: $1,000 USD and one round trip ticket, lodging, and registration at Alltech's International Animal Health and Nutrition Symposium in Lexington, KY in May 2009.
- 2nd Place: $1,000 USD
- 3rd Place: $500 USD

Graduate

- 1st Place: $2,000 USD and one round trip ticket, lodging, and registration at Alltech's International Animal Health and Nutrition Symposium in Lexington, KY in May 2009.
- 2nd Place: $2,000 USD
- 3rd Place: $1,000 USD

PHASE 2 - INTERNATIONAL LEVEL REQUIREMENTS

(Undergraduate and Graduate students)

Posters

- Simplicity and clarity are essential.
- Use of photos and/or flow charts is encouraged.

Presentations

- Each regional winner must appear before a judging panel to present his/her work and participate in a question and answer session.
- Presentations should be based on the original paper submitted for the competition.
- Sound experimental design and interpretation are essential. PowerPoint slides are required.
- Entrants must be prepared to present their research in English.

PHASE 2 – AWARDS

Undergraduate Category

- 1st Place: $5,000 USD and Trophy
- 2nd Place: Trophy
- 3rd Place: Trophy
- Finalists: Certificate of Recognition

Graduate Category

- 1st Place: $10,000 USD and Trophy
- 2nd Place: Trophy
- 3rd Place: Trophy
- Finalists: Certificate of Recognition

PHASE 3 - NATIONAL LEVEL

(Undergraduate and Graduate students)

Papers

Papers ranked by country

Top three graduate and undergraduate papers awarded a prize

Minimum required per country 5

PHASE 3 – AWARDS

Undergraduate Category

- 1st Place: RMB 2,000
- 2nd Place: RMB 1,500
- 3rd Place: RMB 1,000

Graduate Category

- 1st Place: RMB 4,000
- 2nd Place: RMB 3,000
- 3rd Place: RMB 2,000

DEADLINES

- **Jan 29, 2010** - Deadline for registration at www.alltechyoungscientist.com
- **Feb 27, 2010** - Regional phase deadline for submitting papers
- **Apr 10, 2010** - Results of regional phase will be published on our website
- **May 08, 2010** - International phase deadline for submitting presentations
- **May 17, 2010** - Final presentations and awards given during Alltech's 26th International Animal Health and Nutrition Symposium in Lexington, KY

READY TO REGISTER?

- If you are ready to register, log on to www.alltechyoungscientist.com and click on the Register Now button.

- Do you have questions, comments or suggestions about the Alltech Young Scientist Program? If so, please send an email to youngscientist@alltech.com

Alltech Young Scientist

THANK YOU

www.alltechyoungscientist.com