Choose the Diet Wisely:

Purified Diets vs. Chows in Lab Animal Research

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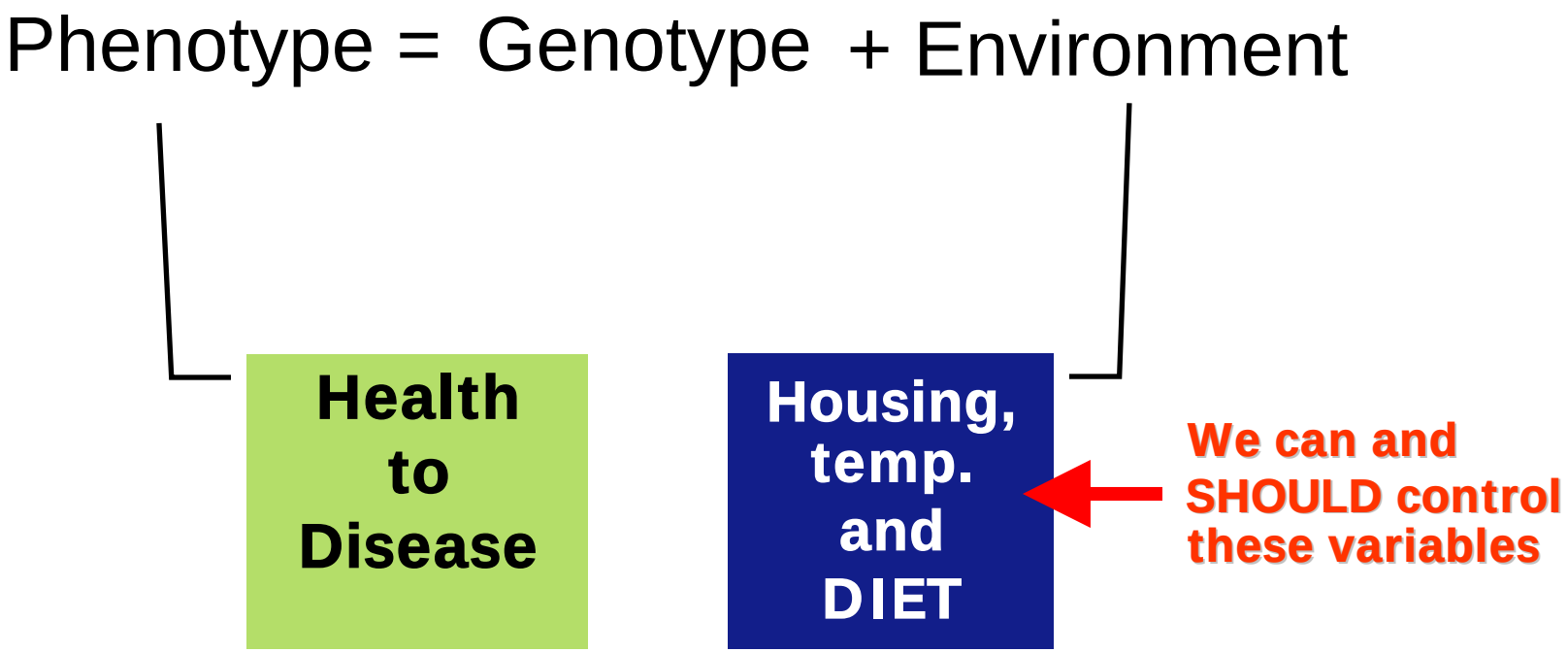
Research Diets, Inc.

Outline

- Laboratory Animal Diet Choices
- Effect of Diet on Nephrocalcinosis and Tissue Heavy Metals

Diet Affects Phenotype



"...a standard diet was used..."

"...rats were fed a normal diet ..."

"...a typical diet was fed ..."

THERE IS NO SUCH THING AS A STANDARD DIET

THERE IS NO SUCH THING AS A PERFECT DIET

- Depends on:
  - Intended use
    - Health, disease?
  - Species?
    - Rat vs. mouse vs. guinea pig vs...
  - How well does it produce desired data?
  - Report? Repeat? Revise?

Formulas:
Open, Closed, Variable and Fixed

- Open: complete formula available to researcher

- Closed: only know list of ingredients,

  not quantities

  - Variable: ingredient amounts are changed depending on nutrient content
  - Fixed: formula is made with same amounts of ingredient each time

We are all Nutritionists

- Report, Repeat and Revise
- Choose a diet
- Chow

vs. OpenSource
Purified Diet

A Rodent Chow

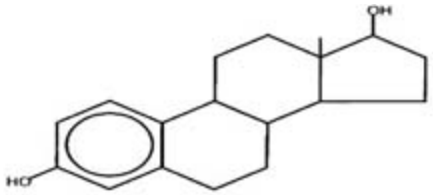
INGREDIENTS: Ground corn, dehulled soybean meal, dried beet pulp, fish meal, ground oats, brewers dried yeast, cane molasses, dehydrated alfalfa meal, dried whey, wheat germ, porcine meat meal, wheat middlings, animal fat preserved with BHA, salt, calcium carbonate, choline chloride, cholecalciferol, vitamin A acetate, folic acid, pyridoxine HCl, DL-methionine, thiamin mononitrate, calcium pathothenate, nicotinic acid, dl-alpha tocopherol acetate, cyanocobalamin, riboflavin, ferrous sulfate, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt chloride.

Ingredients can contain multiple nutrients and also NON-nutrients

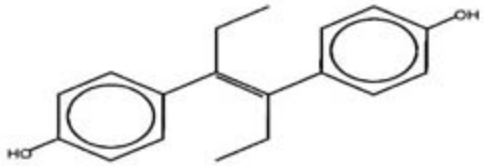
Dehulled soybean meal

- Protein
- Fat
- Carbs
- Fiber
- Minerals
- Vitamins
- **PHYTOESTROGENS**
- Xenobiotics

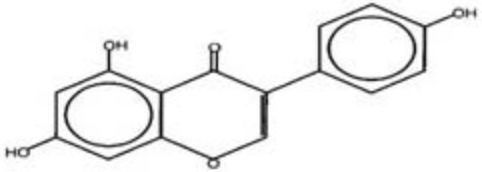
What are Phytoestrogens?



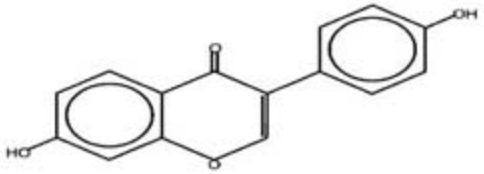
Estradiol



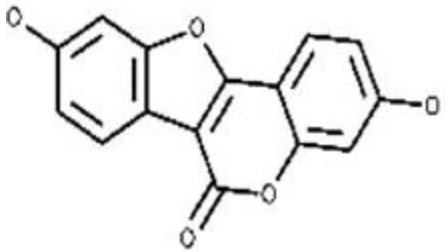
Diethylstilbesterol



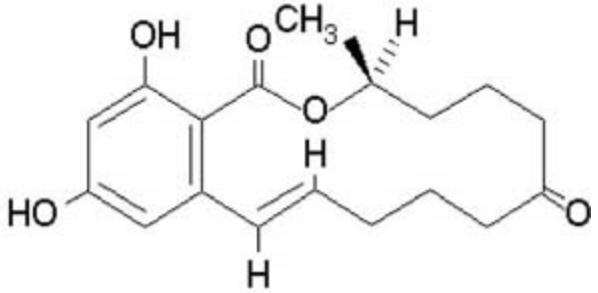
Genistein



Daidzein



Coumestrol



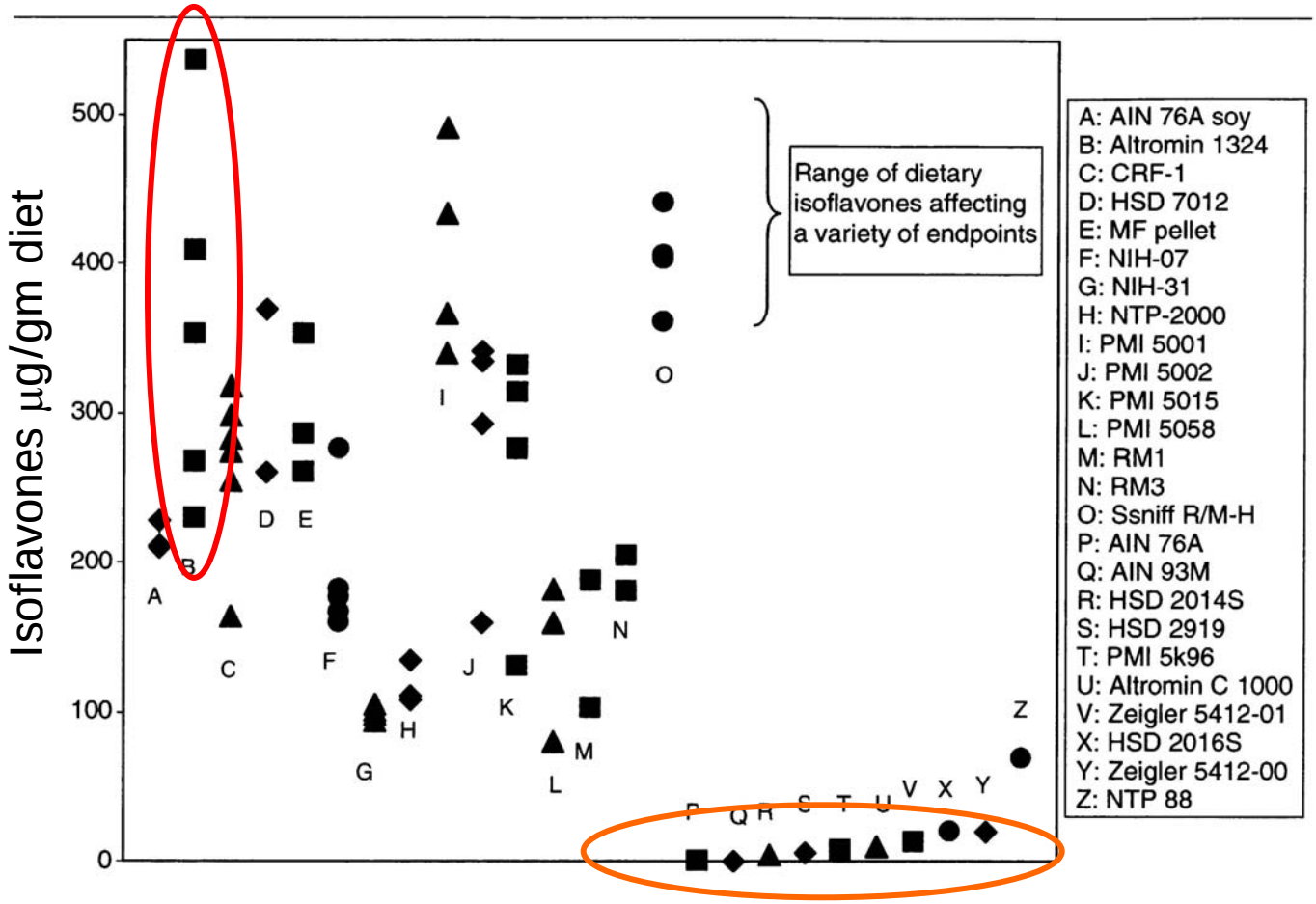
Zearalenone

What Are Effects of Dietary Phytoestrogens?

- Have pro- or anti-estrogenic activity
- Reduce mammary tumor rate
- Reduce serum cholesterol
- Increase bone density in older rats
- Affect the response to exogenous estrogen

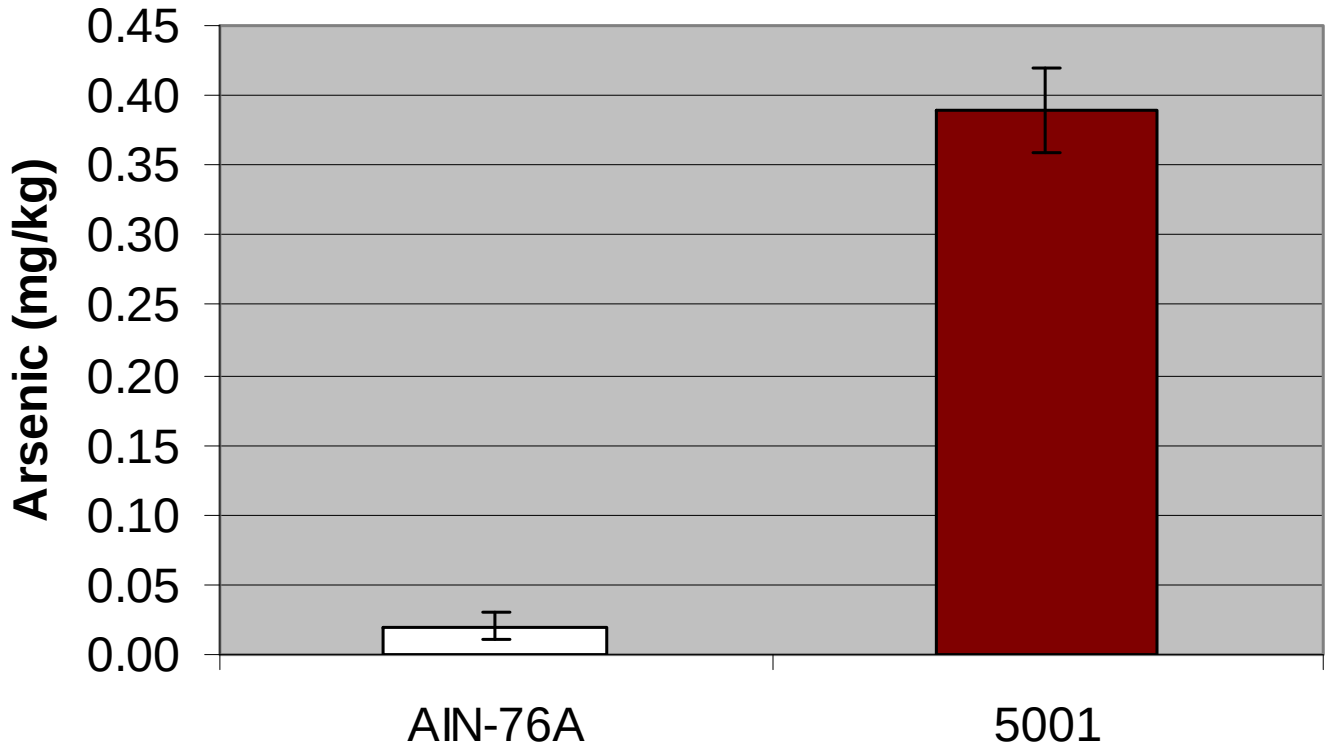
Thigpen et al., ILAR Journal 45: 401-416, 2004

Isoflavone Levels in Lab Animal Diets Do Vary



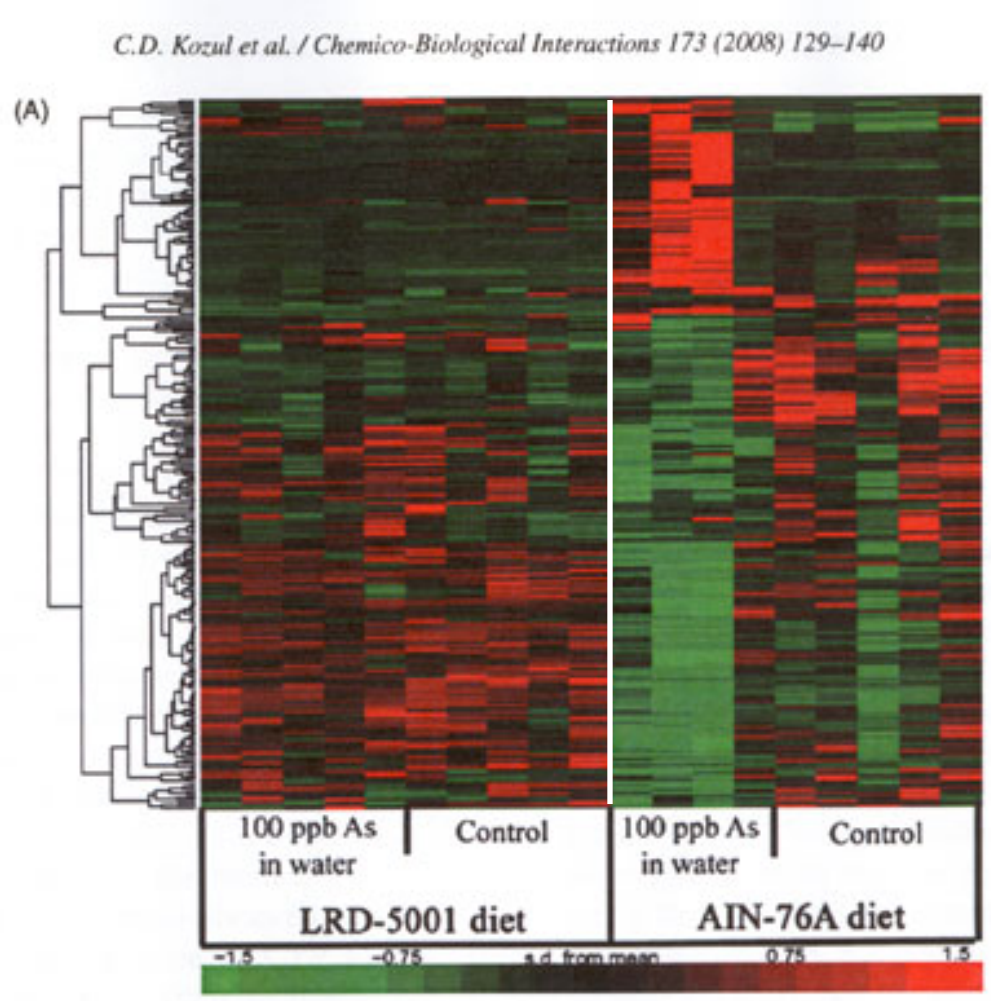
Jensen et al., *Laboratory Animals* 2007, 41:1-18

Arsenic present in chow



Kozul et al., Chemico-Biol Interact 173:129-140, 2008

Arsenic in chow masks effect of arsenic in water on liver gene expression



Kozul et al., Chemico-Biol Interact 173:129-140, 2008

OpenSource Purified Diets

Definition:

Lab animal diets in which each nutrient is supplied by a separate, purified ingredient.

AIN-76A Rodent Diet

Report, Repeat, Revise

A Typical Purified Diet Formula

Ingredient	gm	*kcal*
Casein	200	*800*
DL-Methionine	3	*12*
Corn Starch	150	*600*
Sucrose	500	*2000*
Cellulose	50	*0*
Corn Oil	50	*450*
Mineral Mix S10001	35	*0*
Vitamin Mix V10001	10	*40*
Choline Bitartrate	2	*0*
Total	**1000**	***3902***

J. Nutr. 107:1340-1348, 1977
J. Nutr. 110:1726, 1980

Physiologic Fuel Values

Protein =	4 kcal/gm
CHO =	4 kcal/gm
Fat =	9 kcal/gm
Fiber =	0 kcal/gm

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www.ResearchDiets.com

Diet Comparisons

CHOWS

Advantages

- Cheap
- Long use history

Disadvantages

- Variable, often unknown formula (“closed”)
- Inflexible, difficult/impossible to modify
- Possible xenobiotic, nonnutrient chemicals

PURIFIED DIETS

Advantages

- Open, reportable and repeatable formula
- Formulas easier to modify
- Low or no xenobiotic, nonnutrient ingredients

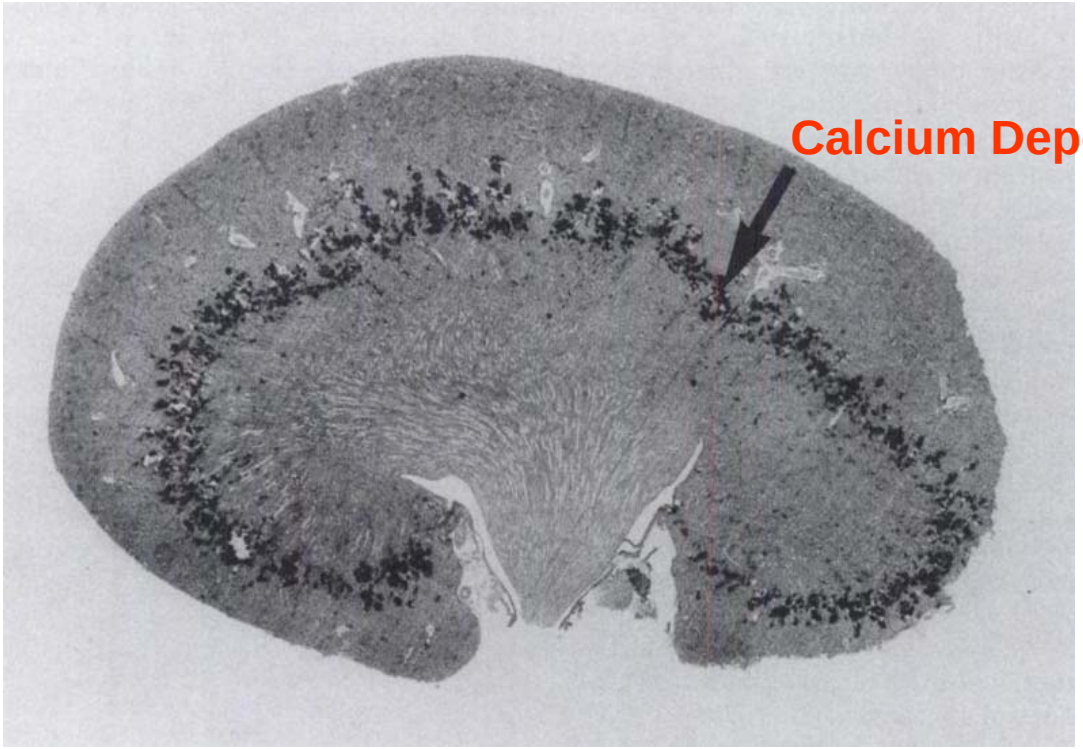
Disadvantages

- Cost more BUT…
- Longevity issues

Purified ingredient diets can induce unintended phenotypes

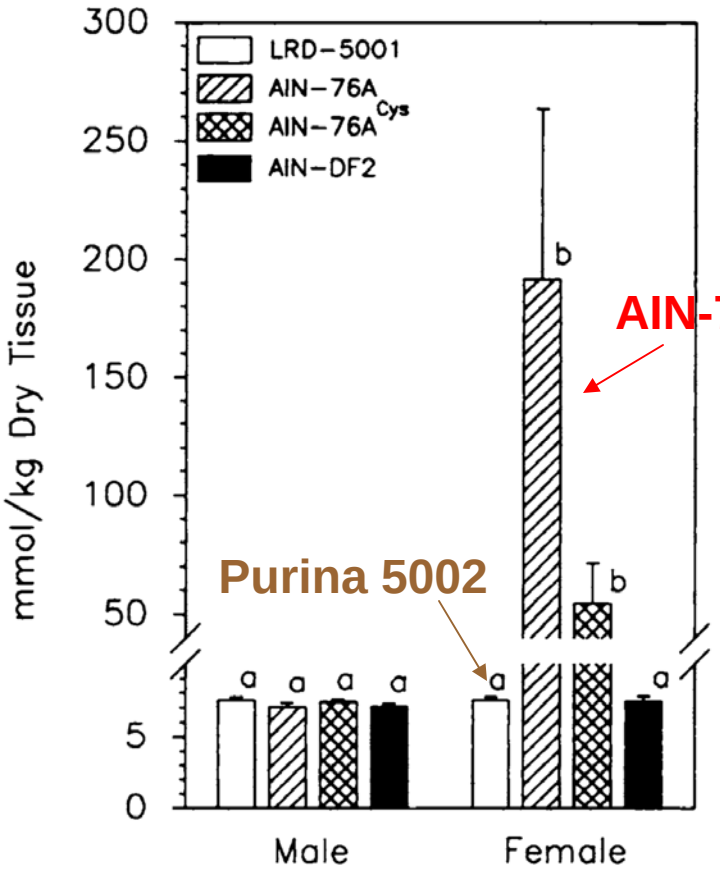
- Elevated Plasma Triglycerides / Insulin
- Elevated Blood Pressure
- Accelerated Pubertal Onset
- Reduced Lifespan
- Kidney Calcinosis (KC)

KC in Weanling, Female Wistar Rat



Ritskes-Hoitinga et al, J Nutr 119:1423 – 1431, 1989

AIN-76A Increases Kidney Calcium Compared to Purina 5002



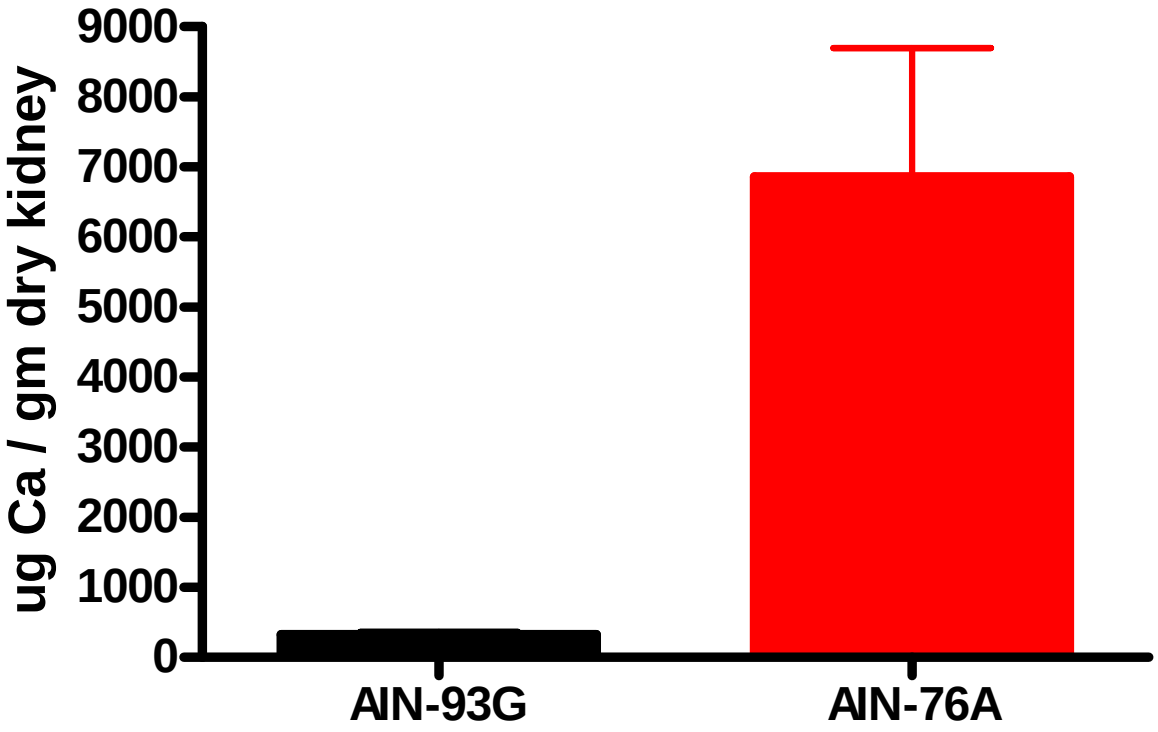
AIN-76A

Ca/P Molar Ratio = 0.75

Purina 5002

Reeves et al, J Nutr 123: 1923-1931, 1993

AIN-93G Diet Minimizes KC…



…but at the expense of a P deficient mineral mix.

Open Standard Diet to Further Improve Existing Formulas

- Mineral Mix – Adequate P
- Carbohydrate – Minimize fructose
- Fiber – Increase, Add soluble fiber
- Pellet Quality – Softer pellets

Objectives for Study 1

1. The effect of replacing the AIN-76A mineral mix with RDI mineral mix containing adequate P on kidney calcium in weanling, female SD rats.

2. The effect of OpenStandard Diet (OSD) developed by RDI on kidney calcium.

Diets

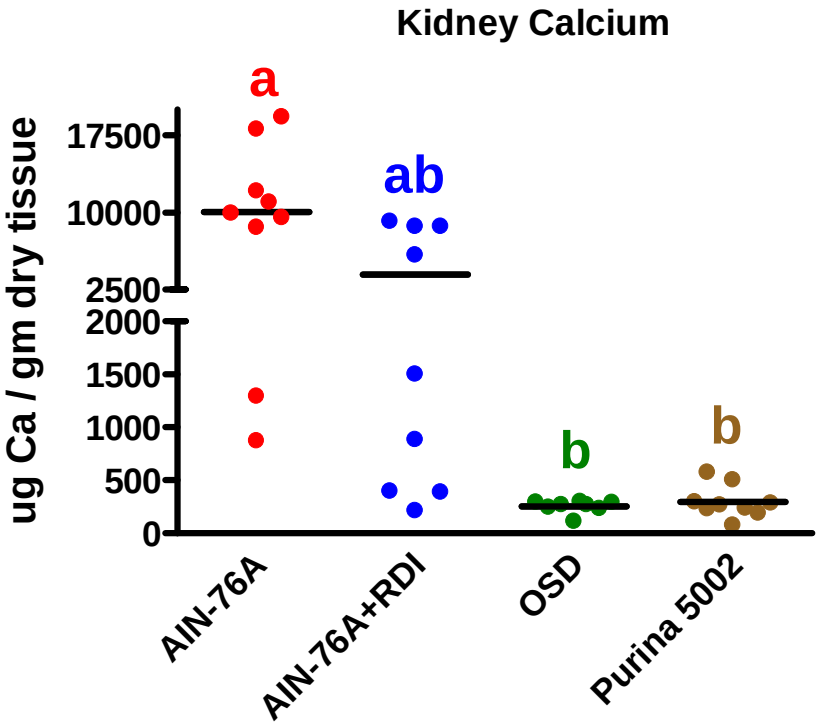
1. AIN-76A
2. AIN-76A + RDI Mineral Mix
3. OSD
4. Purina 5002

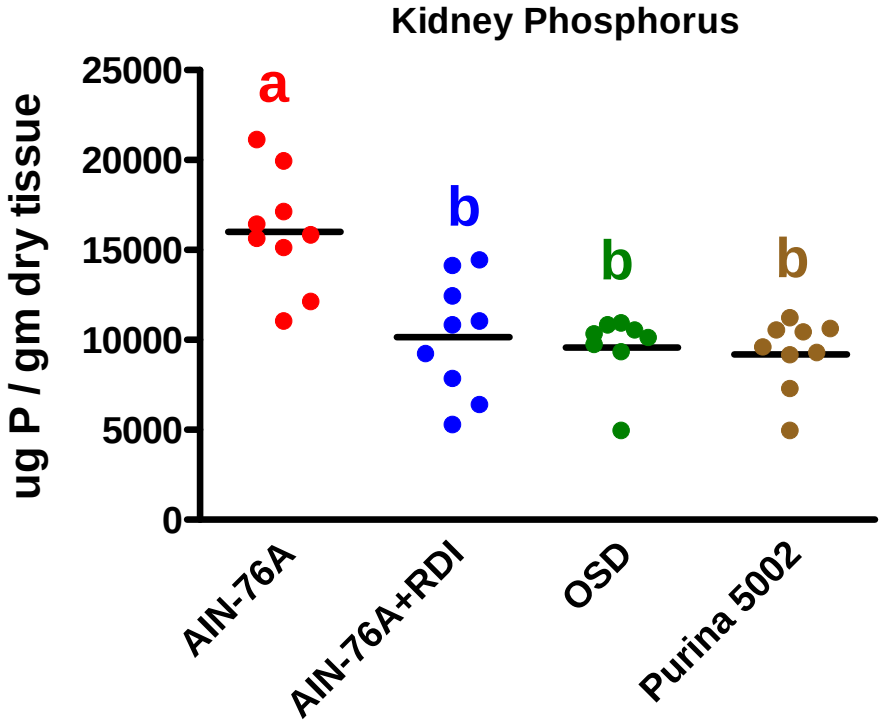
AIN-76A vs. RDI Mineral Mix

- AIN-76A Mineral Mix
  - Ca = 5.2 gm / kg
  - P = 4 gm / kg
  - Ca / P Molar Ratio = 0.75
  - K = 3.6 gm / kg
  - F & Mo = 0

- RDI Mineral Mix
  - Ca = 6 gm / kg
  - P = 3 gm / kg
  - Ca / P Molar Ratio = 1.07
  - K = 6 gm / kg
  - F = 0.9 mg / kg
  - Mo = 1.6 mg / kg

RDI minerals lowered kidney Ca and P but not to the same extent as OSD and Purina 5002





Groups with different letters within each graph are significantly different from one another ($p<0.05$).

What other nutrients in OSD could be influencing kidney Ca?

1. The digestible carbohydrate source (i.e. fructose or glucose).
   - *Bergstra et al, J Nutr 123: 1320-1327, 1993*

2. The fiber source (soluble or insoluble fiber).
   - *Anderson et al, Nutr Report Int, 1985*

3. The supplemental sulfur containing amino acid (i.e. DL-methionine or L-cystine).
   - *Reeves et al, J Nutr 123: 1923-1931, 1993*

Study 2 Objectives

- Influence of modifying carbohydrate, sulfur AA or fiber on KC.

- Influence of different grain-based chow diets on KC and their Ca / P molar ratios.

Diet Design

	OSD	OSD-C	OSD-M	OSD-S	OSD-CMS
L-Cys	√	√		√	
Starch/ Dextrose	√	√	√		
Cellulose/ Inulin	√		√	√	
Cellulose		√			√
DL-Meth			√		√
Sucrose				√	√

Other Diets as Controls for KC

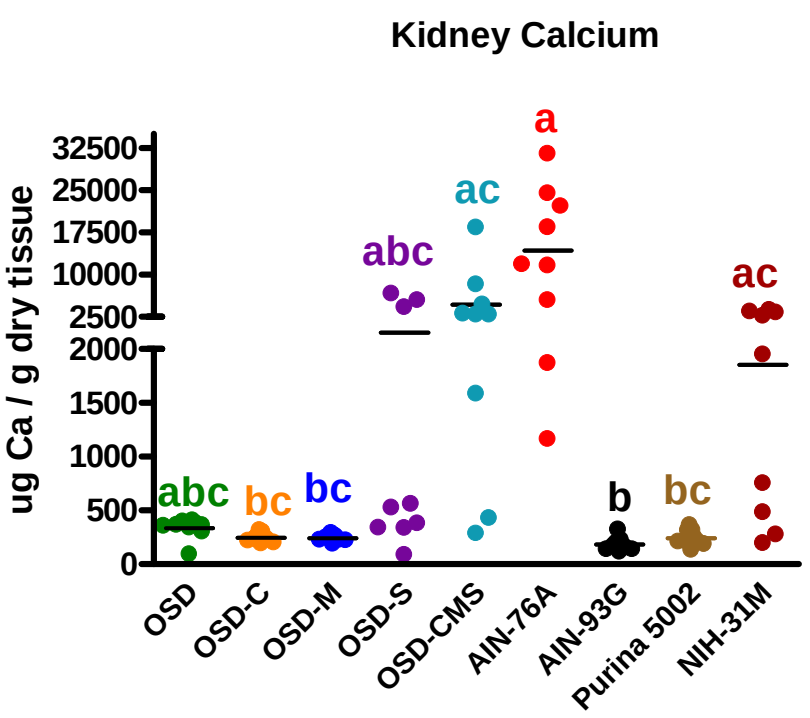
6. **AIN-93G (- KC)**

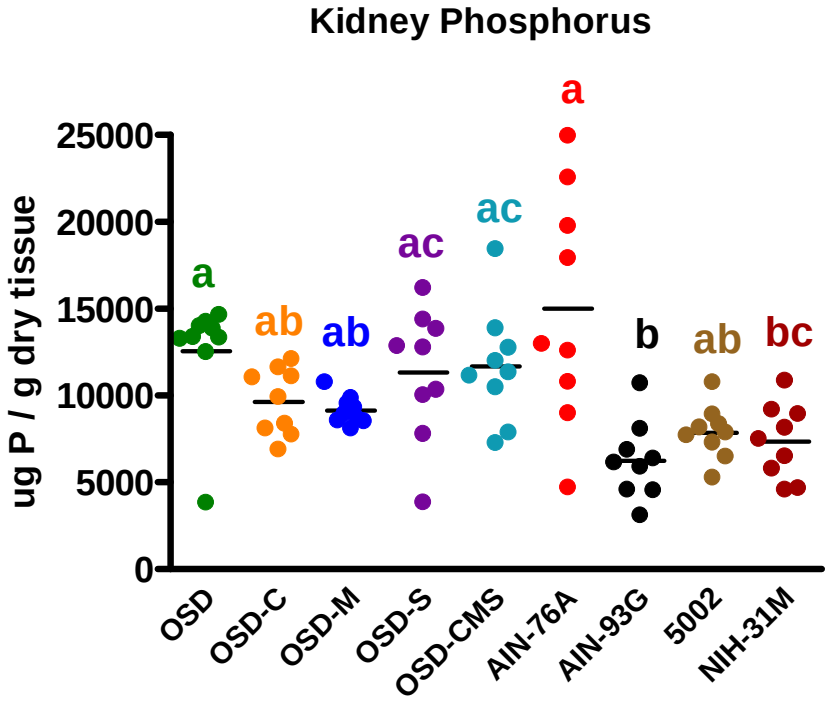
7. **AIN-76A (+ KC)**

8. **Purina 5002 (- KC)**

9. **NIH-31M (+ or - ?)**

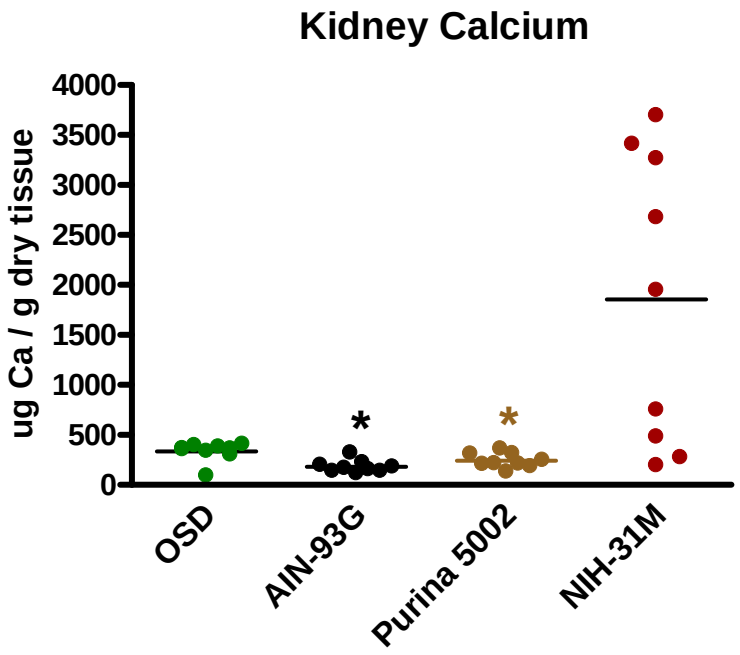
Replacement of sucrose with corn starch/dextrose reduces kidney Ca

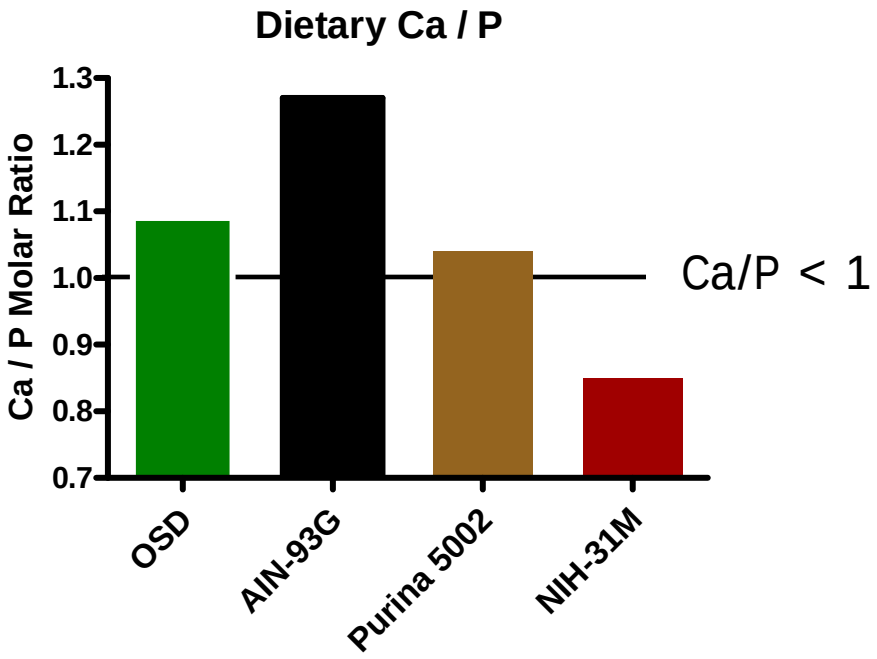




Groups with different letters within each graph are significantly different from one another ($p<0.05$).

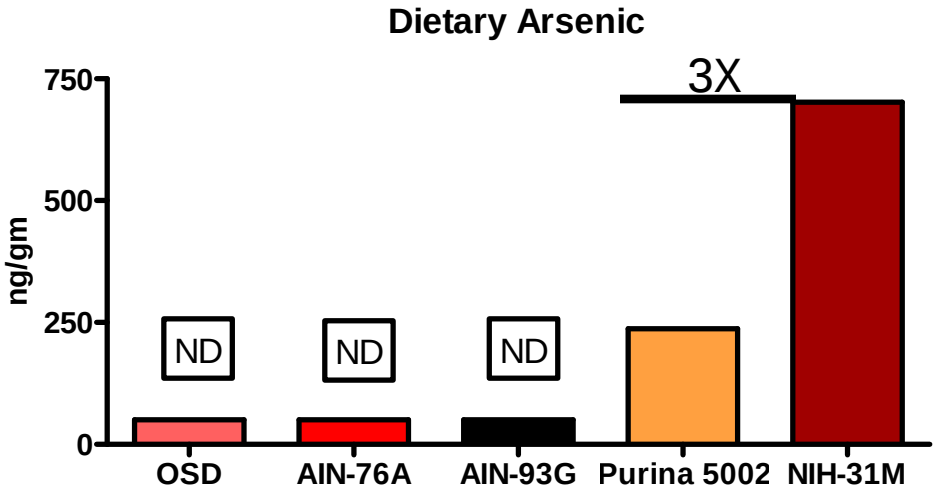
High kidney Ca in rats fed NIH-31M may be due to low Ca/P Molar Ratio

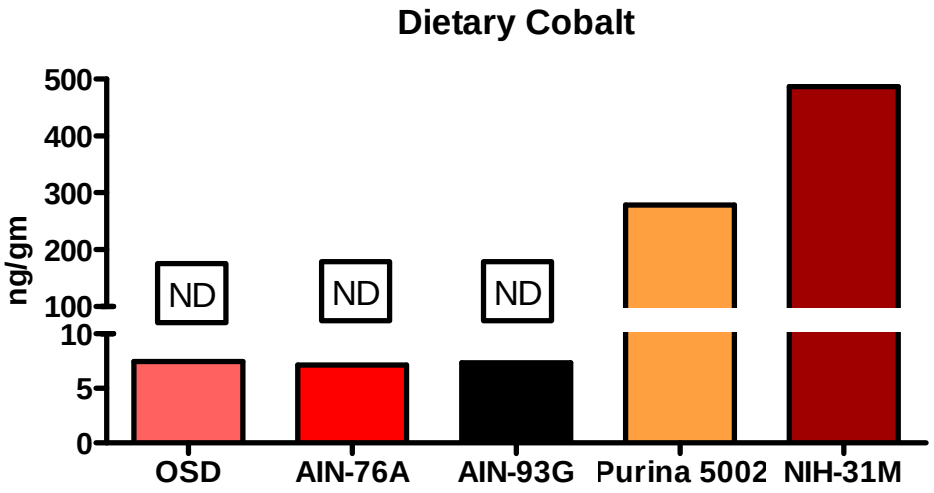




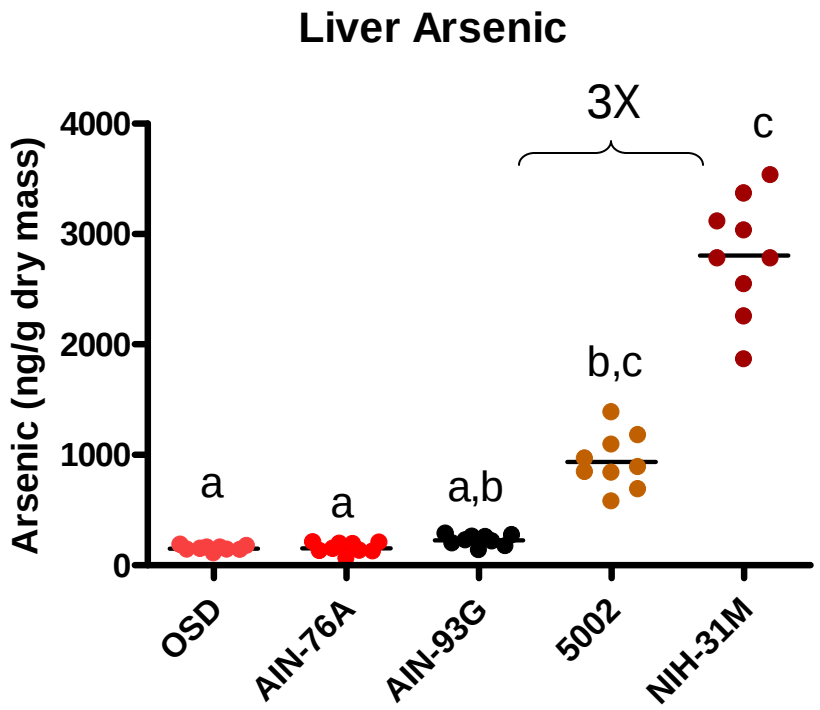
Groups with asterisk are significantly different from NIH-31M, p<0.05

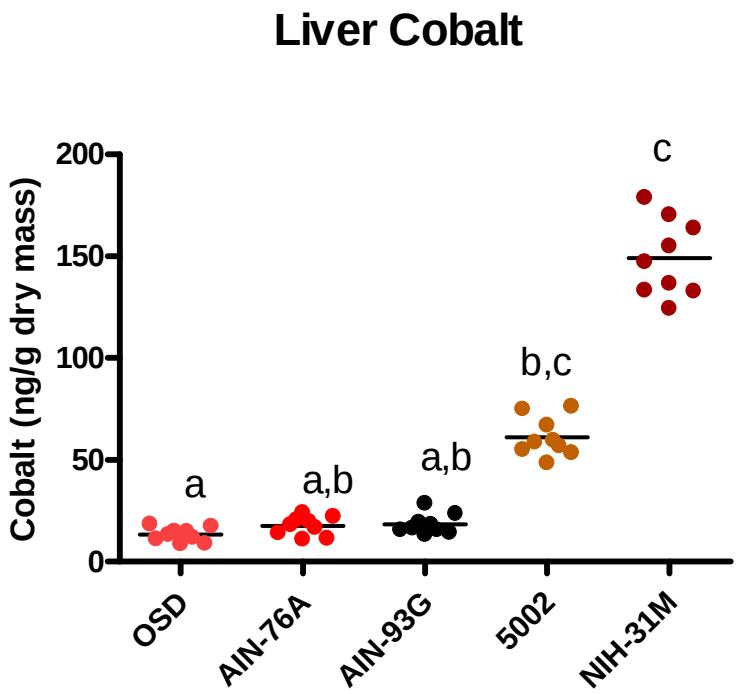
NIH-31M had higher levels of As and Co than Purina 5002





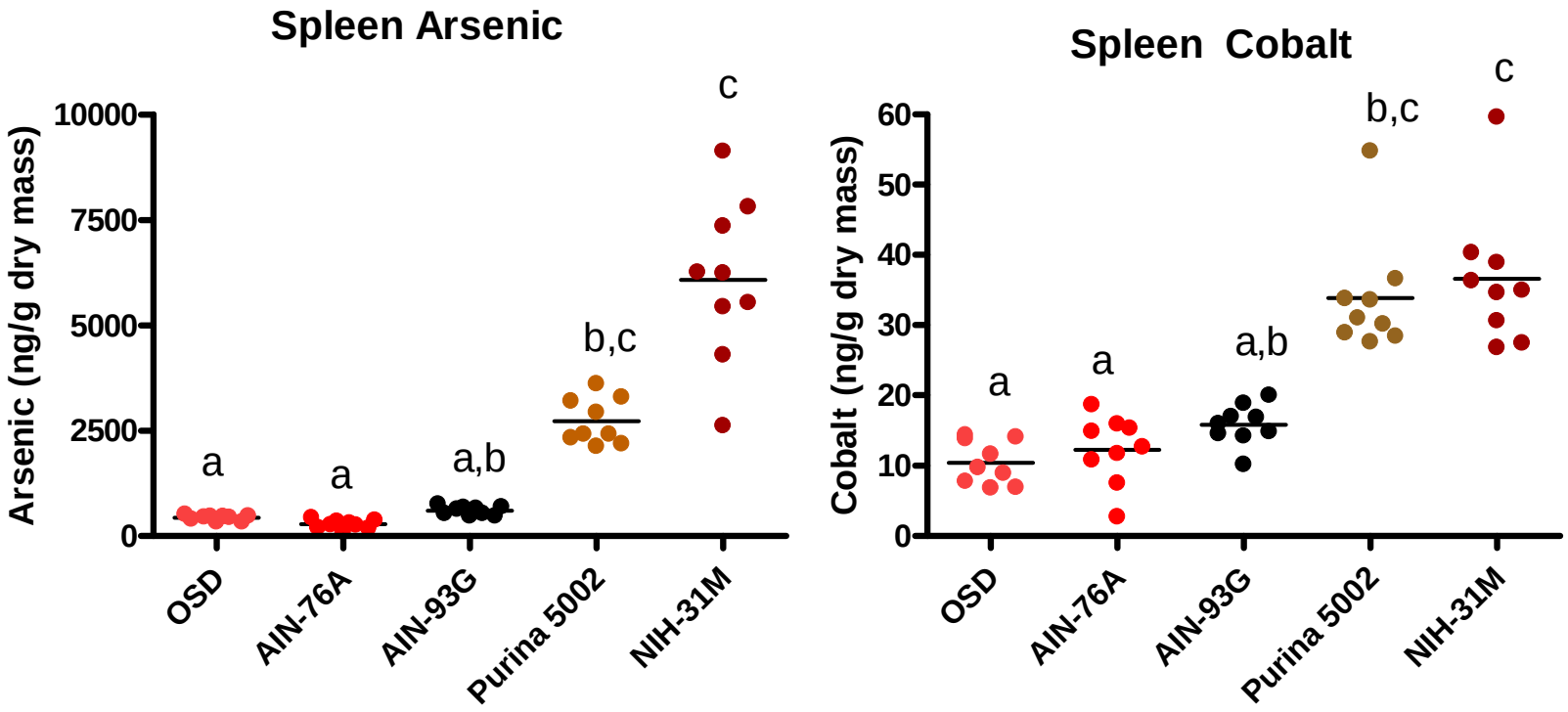
Liver As and Co reflected levels of these dietary metals





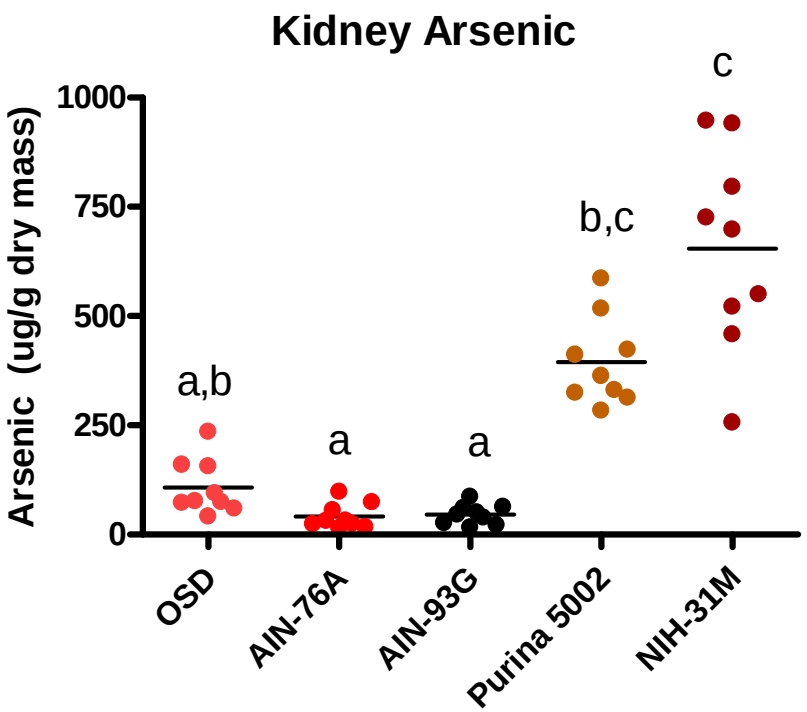
Groups with different letters within each variable are significantly different from each other ($p<0.05$)

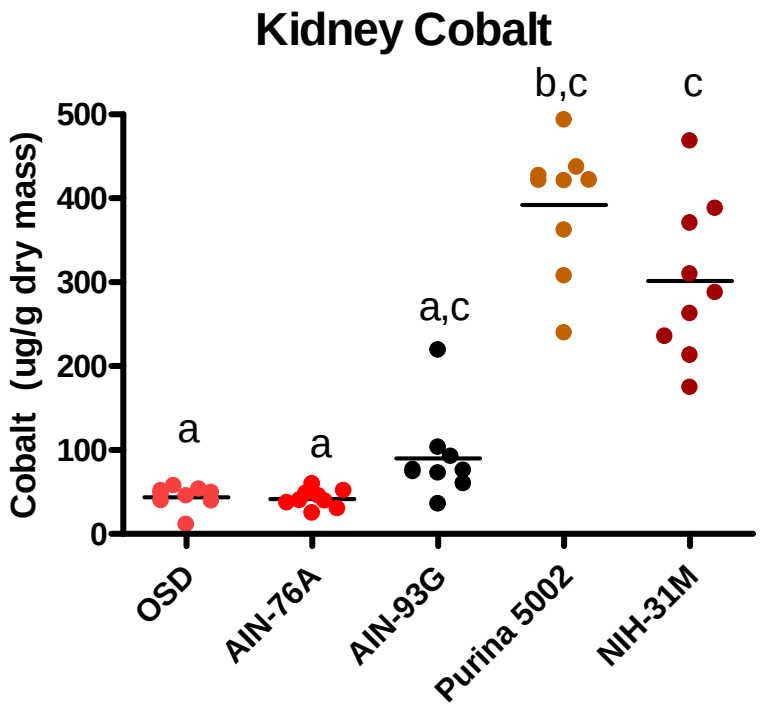
Chow Diets elevated spleen As and Co relative to purified diets



Groups with different letters within each variable are significantly different from each other ($p<0.05$)

Chow diets promoted higher kidney As and Co than purified diets





Groups with different letters within each variable are significantly different from each other ($p<0.05$)

Conclusions

- KC of rats fed OSD = AIN-93G
- Carbohydrate type influences KC
- Grain-based diets differentially influence KC
- HM tissue levels directly reflect dietary concentrations
- Purified diets can be used to limit tissue HM accumulation

Acknowledgements

- Taconic Biotechnology, Contract Research Solutions
  - John Couse, Kevin Riley, Elizabeth Aluck
- Michigan State University Diagnostic Center for Population and Animal Health
  - Justin Zyskowski