A NEW LOOK AT NITRATE TOXICITY

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Nitrate (NO_3^-) Background

Major form of N in most soils.
Often first factor limiting plant growth.
(Fertilizer N = ammonium nitrate, urea)
Assimilated rapidly by growing plants.

Problems:

Plant or fertilizer nitrate toxic for cattle, sheep, other livestock.
(acute-collapse, death: chronic-abortion)
High nitrate water supply - infant deaths.
("Blue Baby" effect)
Yields toxic fermentation gases. (N_20)
Environmental accumulation.

Nitrate (NO_3^-) History

Discovery of Toxicity:

1894 - Chile saltpeter is toxic to cattle.

1895 - Corn plant toxicity related to KNO_3 crystals in corn leaf axils, cut surfaces, inside stalks. Blamed on *potash* poisoning! Burned like "firecracker fuses." (26,000-35,000 ppm NO_3-N).

1939 - Oat hay poisoning. When nitrate was precipitated out, sap longer toxic! Methemoglobin & nitrite in bile and urine. (5,800 ppm NO_3-N)

Nitrate (NO_3^-)

Toxicity types:

- **Acute - Collapse, death**
- **Chronic? - Long-term, sub-lethal.**
  - **No appetite, low production, abortion?**

Toxic dose varies widely:

- **Plant vs chemical (KNO_3) added.**
- **Water vs Feed (water more toxic).**
- **Dry vs Wet feed. (Dry hay releases nitrate 3 times faster than wet forage.)**
- **Species differ - Hemoglobin sensitivity.**
- **Animals in a species differ- adaptation, intake patterns.**

Nitrate (NO_3^-)

Acute Poisoning - Signs of anoxia:

1. Bluish/chocolate brown mucous membranes.
2. Rapid, difficult, noisy breathing.
3. Rapid pulse (150+/minute)
4. Salivation, bloat, tremors, staggering.
5. Weakness, coma.

Confirmatory diagnosis:

Dark, “chocolate-colored” blood.
Methemoglobinemia.
High blood nitrate.
Residual oxygen combining capacity.

Stoltenow 1998

Nitrate (NO_3^-)

Basis of expressing nitrate concentration

Basis:	*Ratio*	*Toxic level in forage*
NO_3-N	1.0	1,000ppm = .10%
NO_3	4.4	4,400ppm = .44%
KNO_3	7.3	7,300ppm = .73%

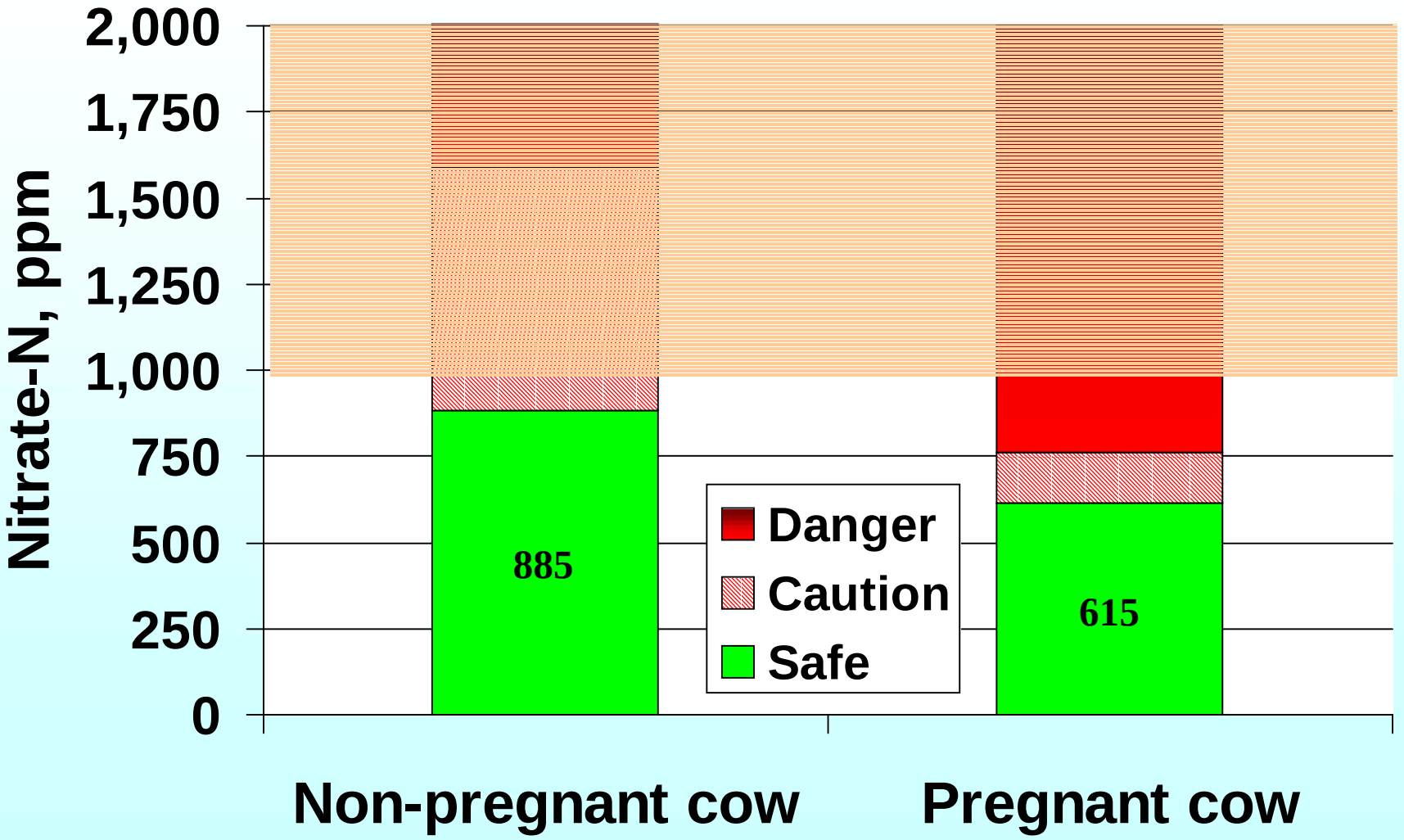
All these are equal! Just different basis.

Methods for measuring nitrate:

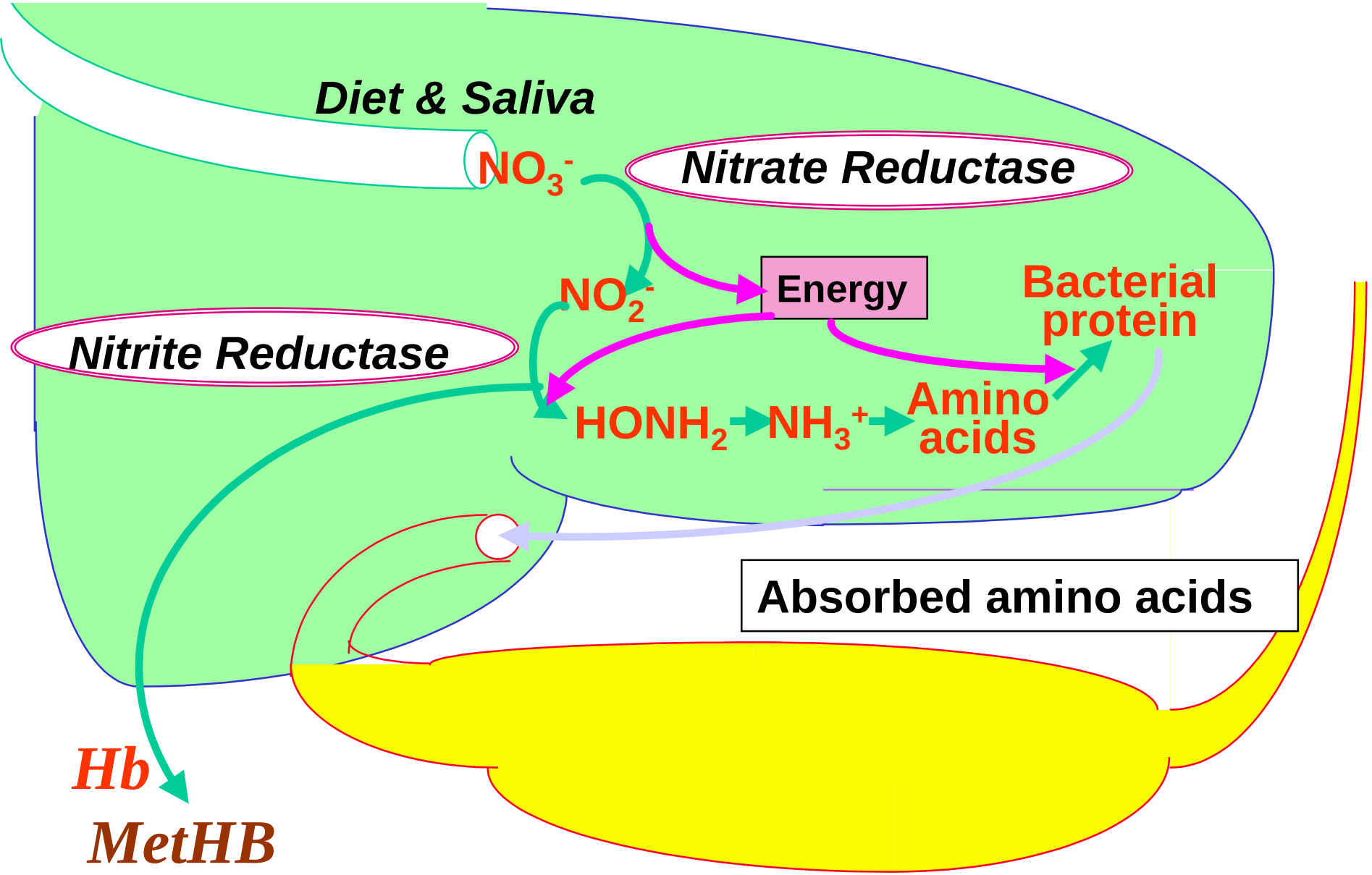
Qualitative - Field forage stain test

Quantitative - Colorimetric, Electrode

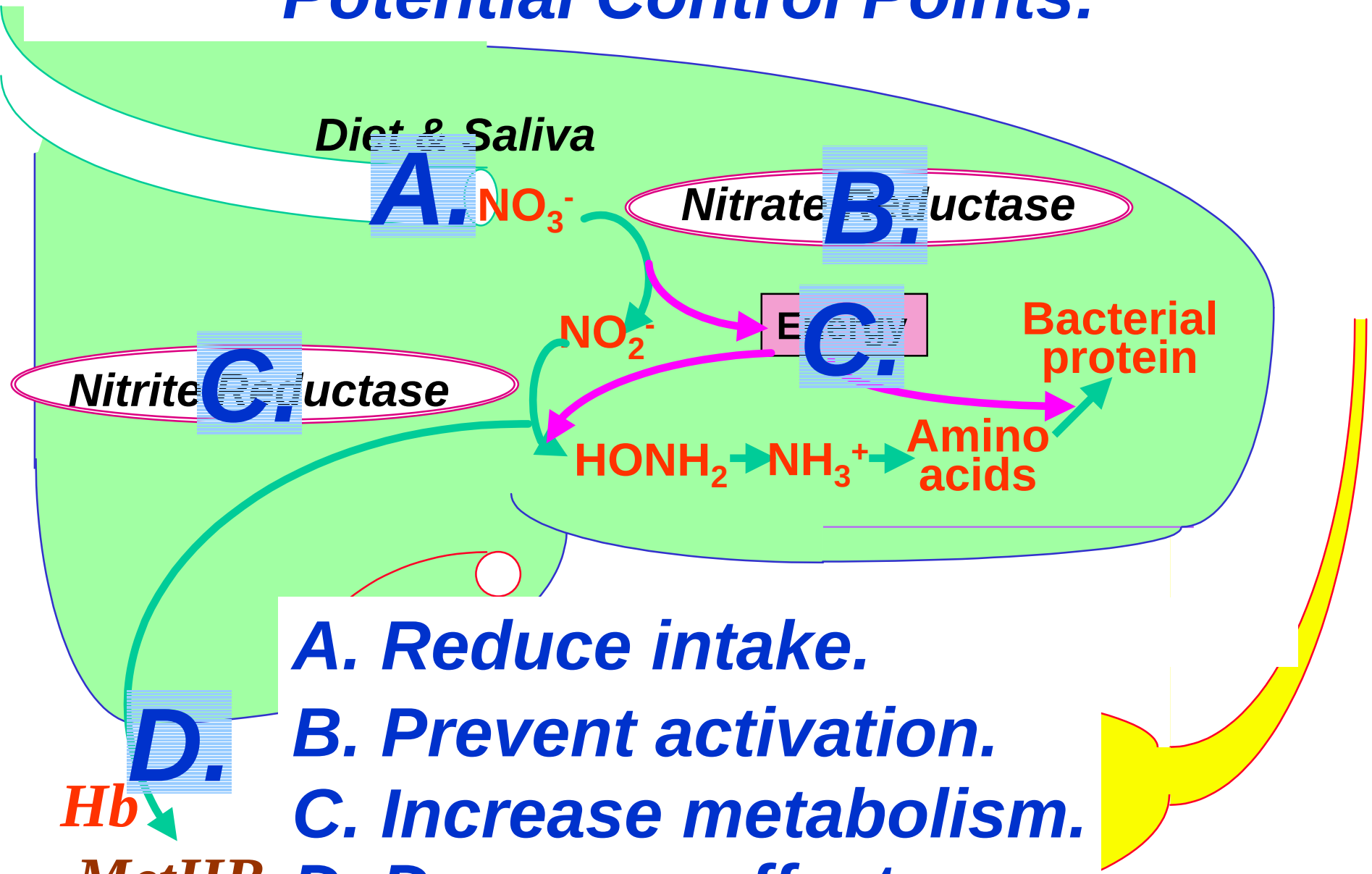
Nitrate-N in Diet - Safe & Hazardous Levels



What Happens to Nitrate-N in the Rumen?



Potential Control Points:



A. Reduce intake.

B. Prevent activation.

C. Increase metabolism.

D. Decrease effect.

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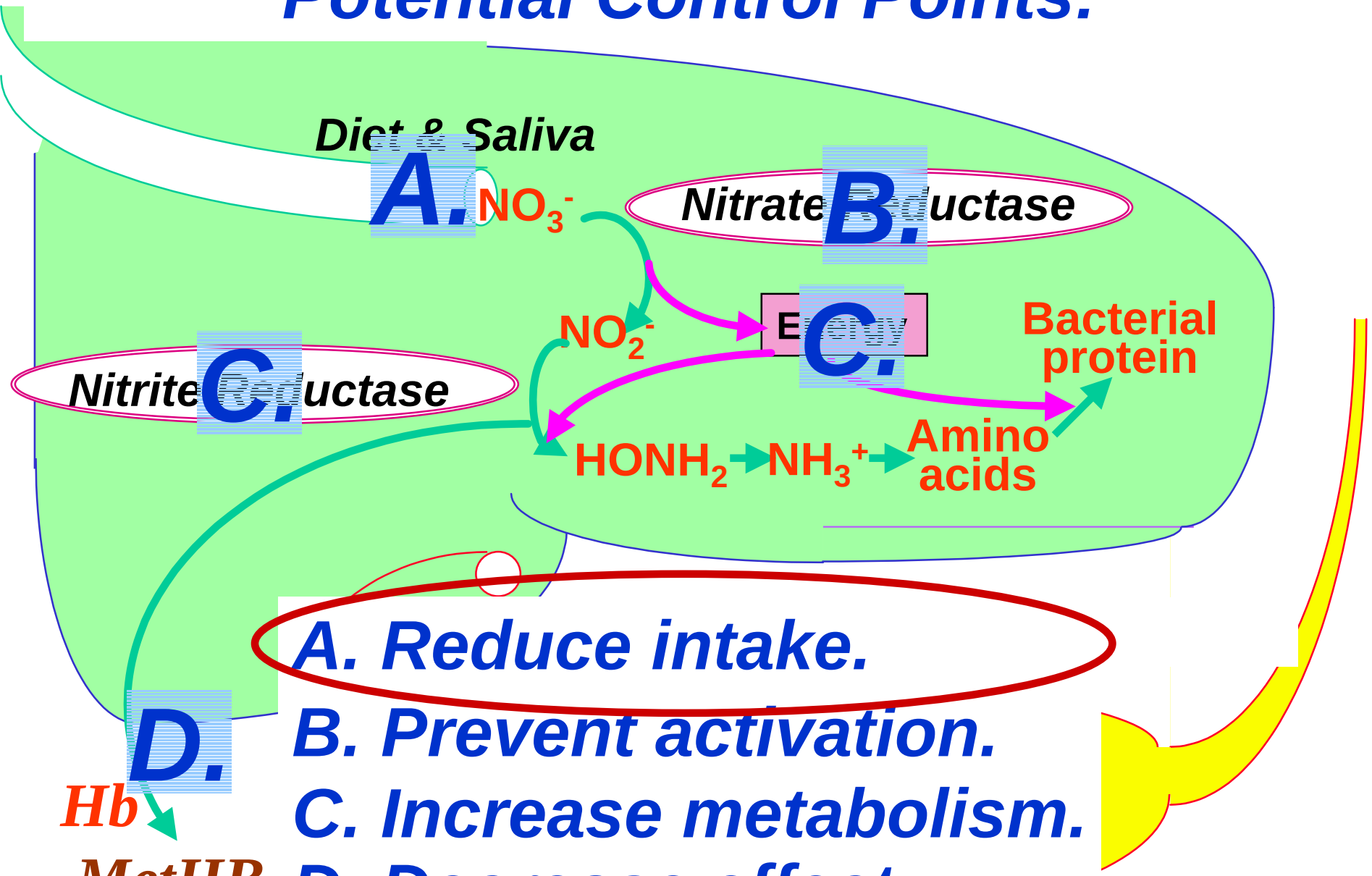

A. Reduce intake.

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A. Reducing Nitrate Intake

1. Forage concentration varies with:

Plant species

Crops: Sudans, sorghum-sudan, sorghum, corn, wheat, oats, rye, barley, millet

Grasses: fescue, johnsongrass

Weeds: pigweed, mustard, kocia, nightshade, lambsquarter, nettles, elderberry, burdock, thistle

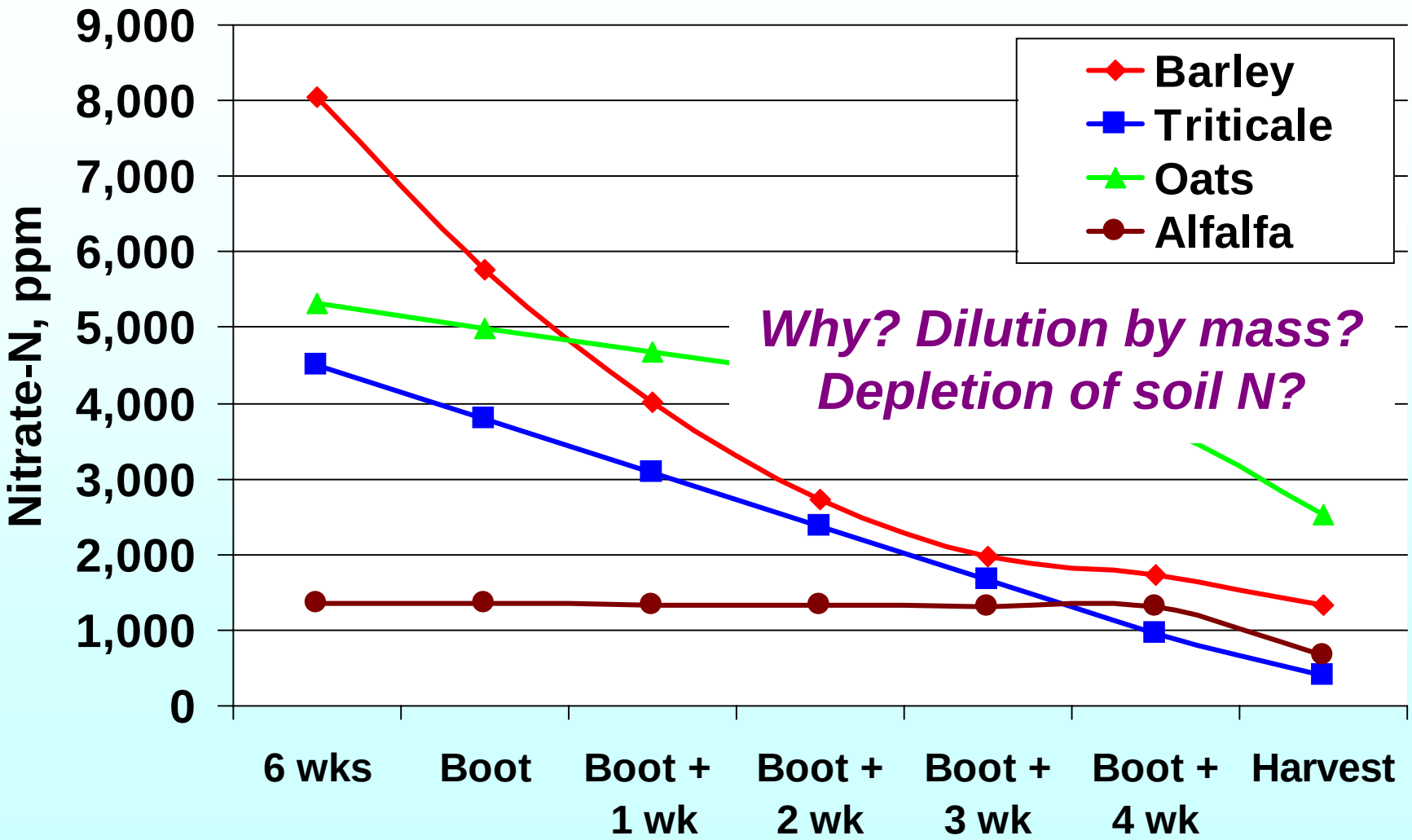
Legumes: Alfalfa?

Lower in perennials, woody plants.

A. Reducing Nitrate Intake

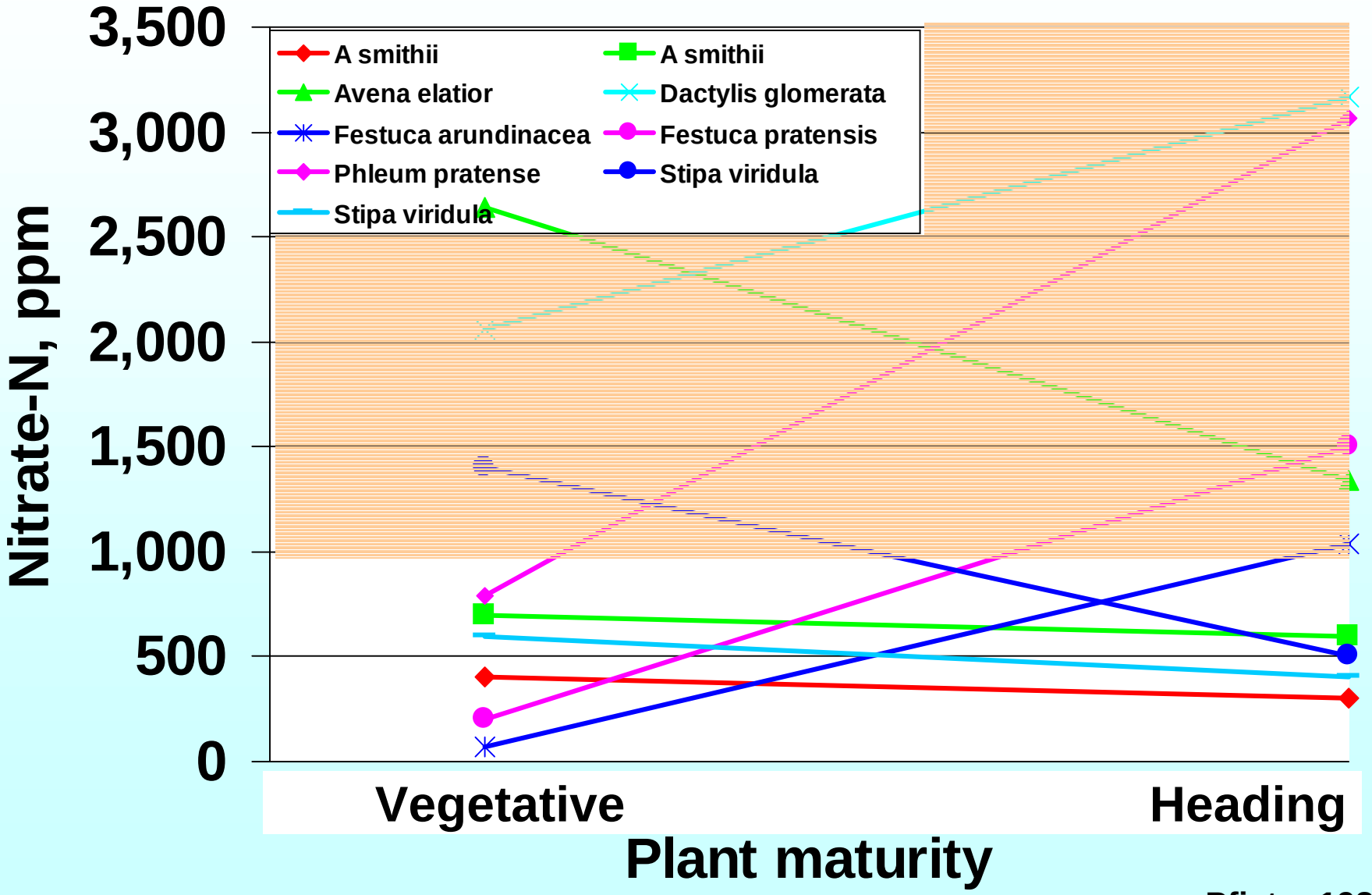
1. Forage concentration varies with:
   - Plant maturity

Plant Maturation and Nitrate



Khorasani et al. 1997

Plant Maturation and Nitrate

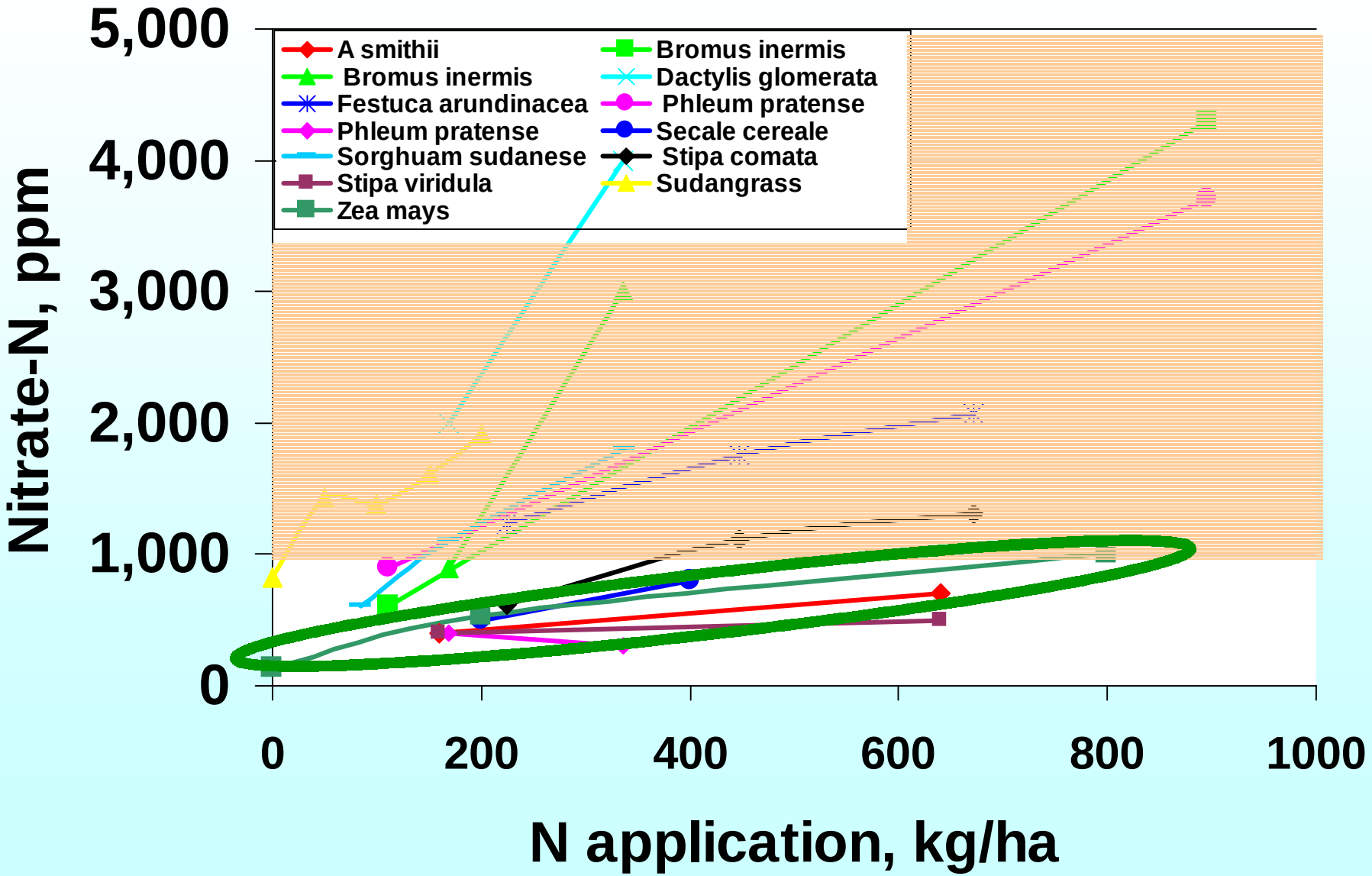


Pfister 1987

A. Reducing Nitrate Intake

1. Forage concentration varies with:
   - Plant species and maturity
   - Level of N fertility

Fertilization and Nitrate



Pfister 1987

A. Reducing Nitrate Intake

1. Forage concentration varies with:
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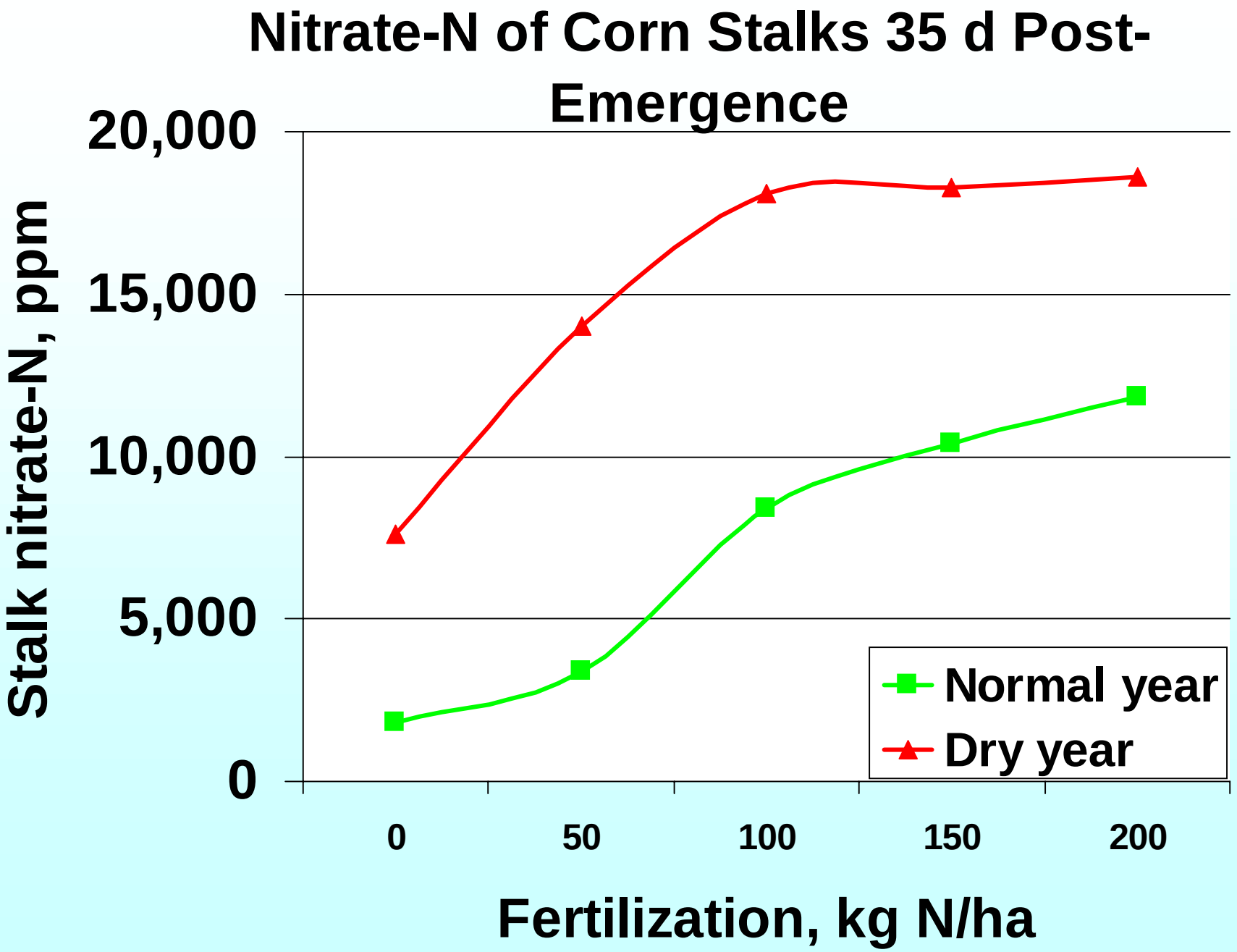
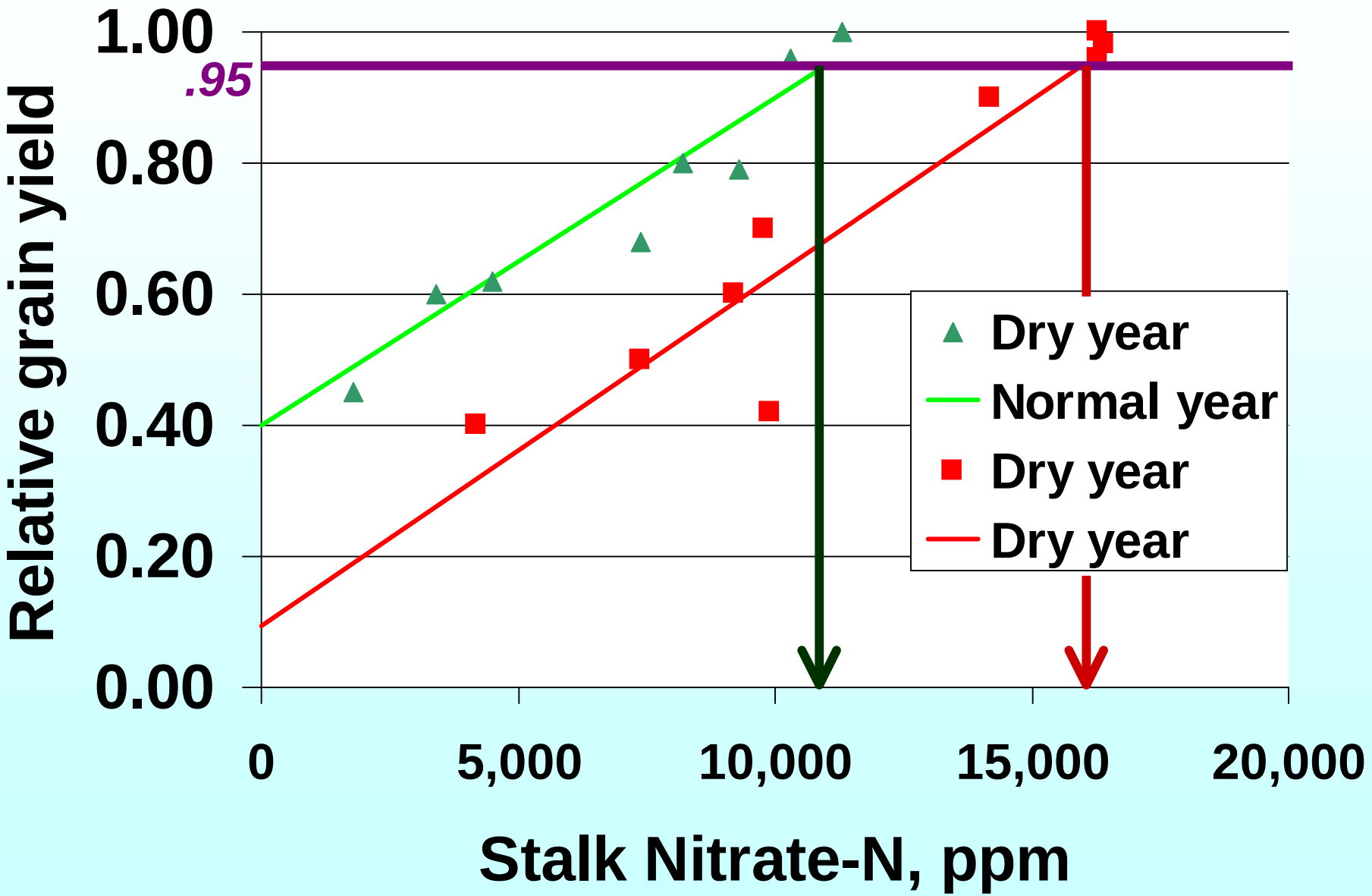
   - Level of N fertility
   - Plant stress
     - Frost, low temperatures.
     - Acid or deficient (P, S, Mb) soils.
     - Water shortage


Nitrate-N of Corn Stalks 35 d Post-Emergence
20,000
15,000
10,000
5,000
0
Stalk nitrate-N, ppm
0
50
100
150
200
Fertilization, kg N/ha
Normal year
Dry year

A. Reducing Nitrate Intake

- Forage nitrate-N concentration serves as an index of plant N status and grain yield.
- Thus, high levels are considered a desirable trait by grain growers and plant breeders!

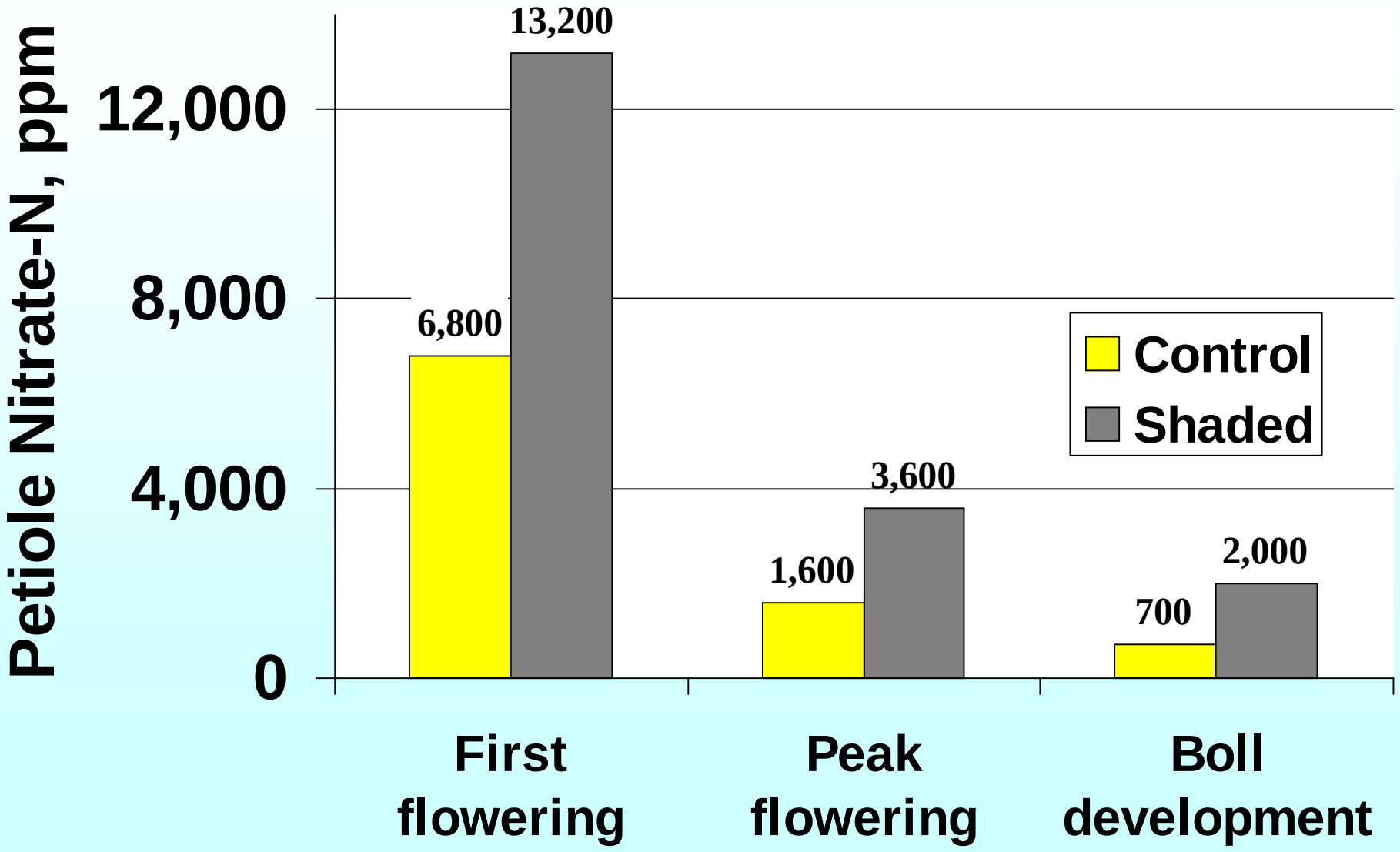
Corn Stalk Nitrate-N vs Grain Yield



A. Reducing Nitrate Intake

1. Forage concentration varies with:
   - Level of N fertility
   - Plant species and maturity
   - Plant stress
     - Frost, low temperatures.
     - Acid or deficient (P, S, Mb) soils.
     - Water shortage
     - Cool, cloudy weather, shade

Shading and Cotton Petiole Nitrate-N



Zhao, 1998

A. Reducing Nitrate Intake

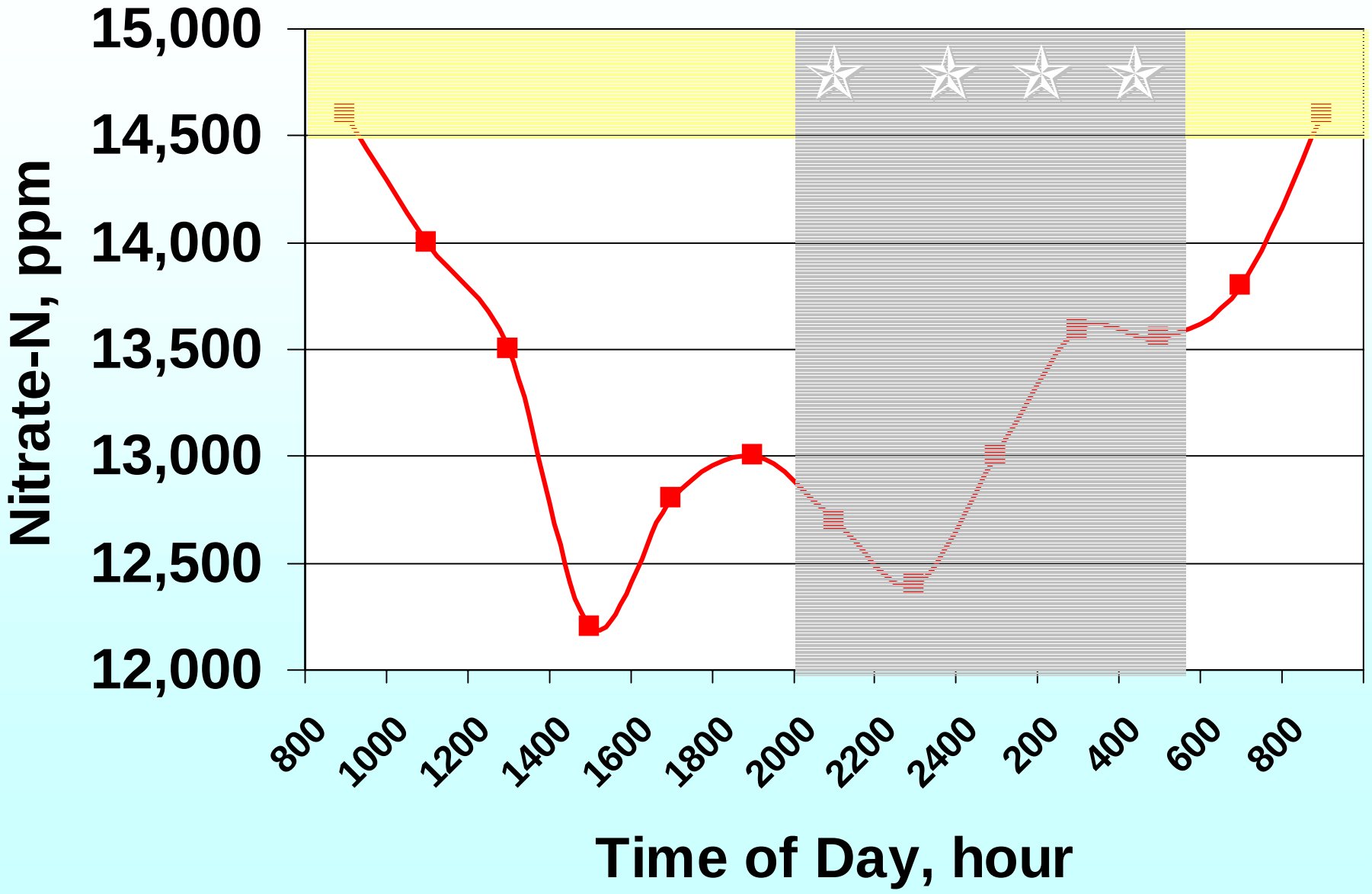
1. Forage concentration varies with:

- **Level of N fertility**
- **Plant species and maturity**
- **Plant stress**
  - **Water shortage**
  - **Weather & shading**
  - **Frost damage**
  - **Time of day**

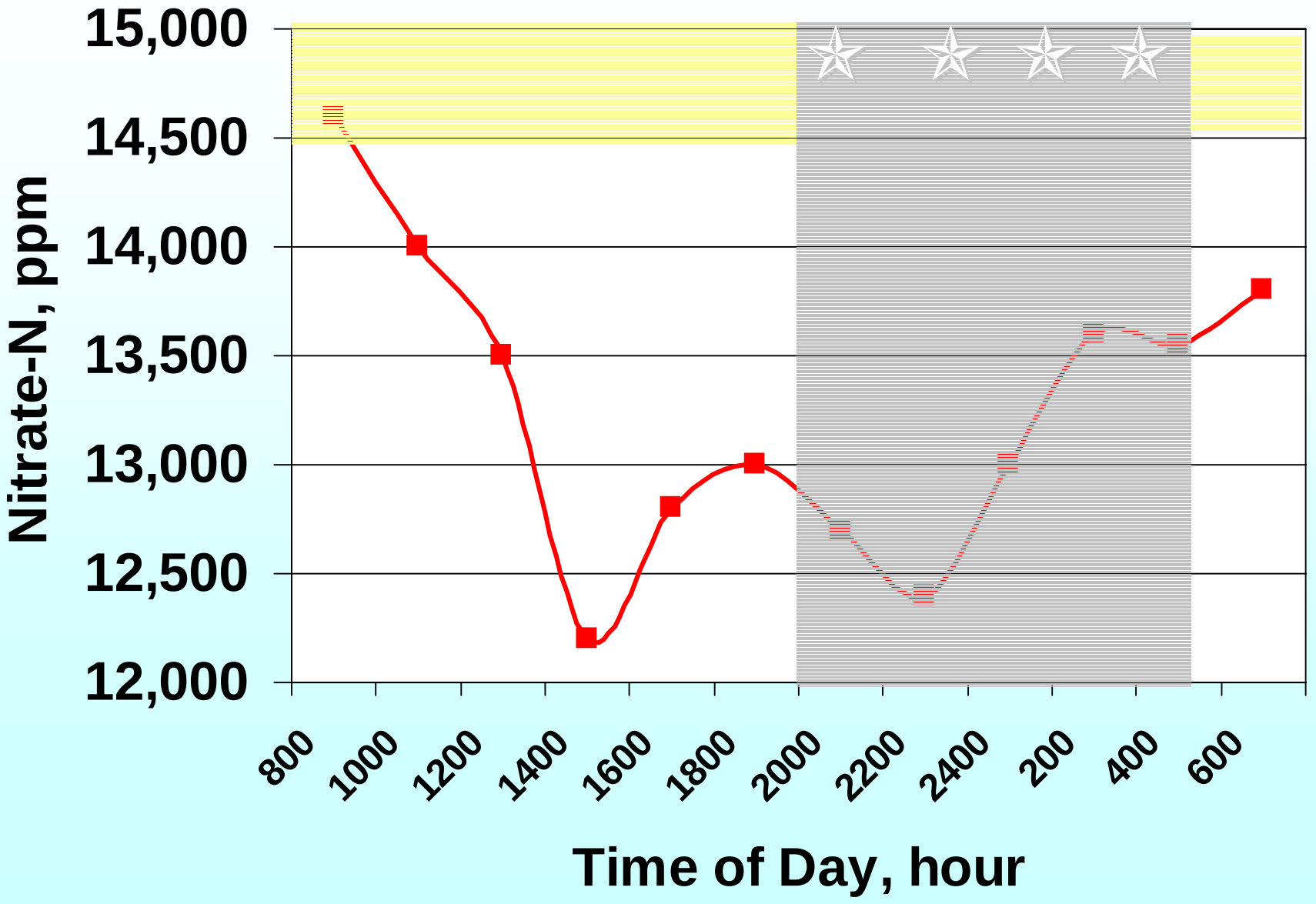
Nitrate uptake continues during the dark, but is depleted during photo-synthesis. Lowest late afternoon.

Avoid grazing or harvest in the morning.

Nitrate-N of Corn Stalks



Nitrate-N of Corn Stalks

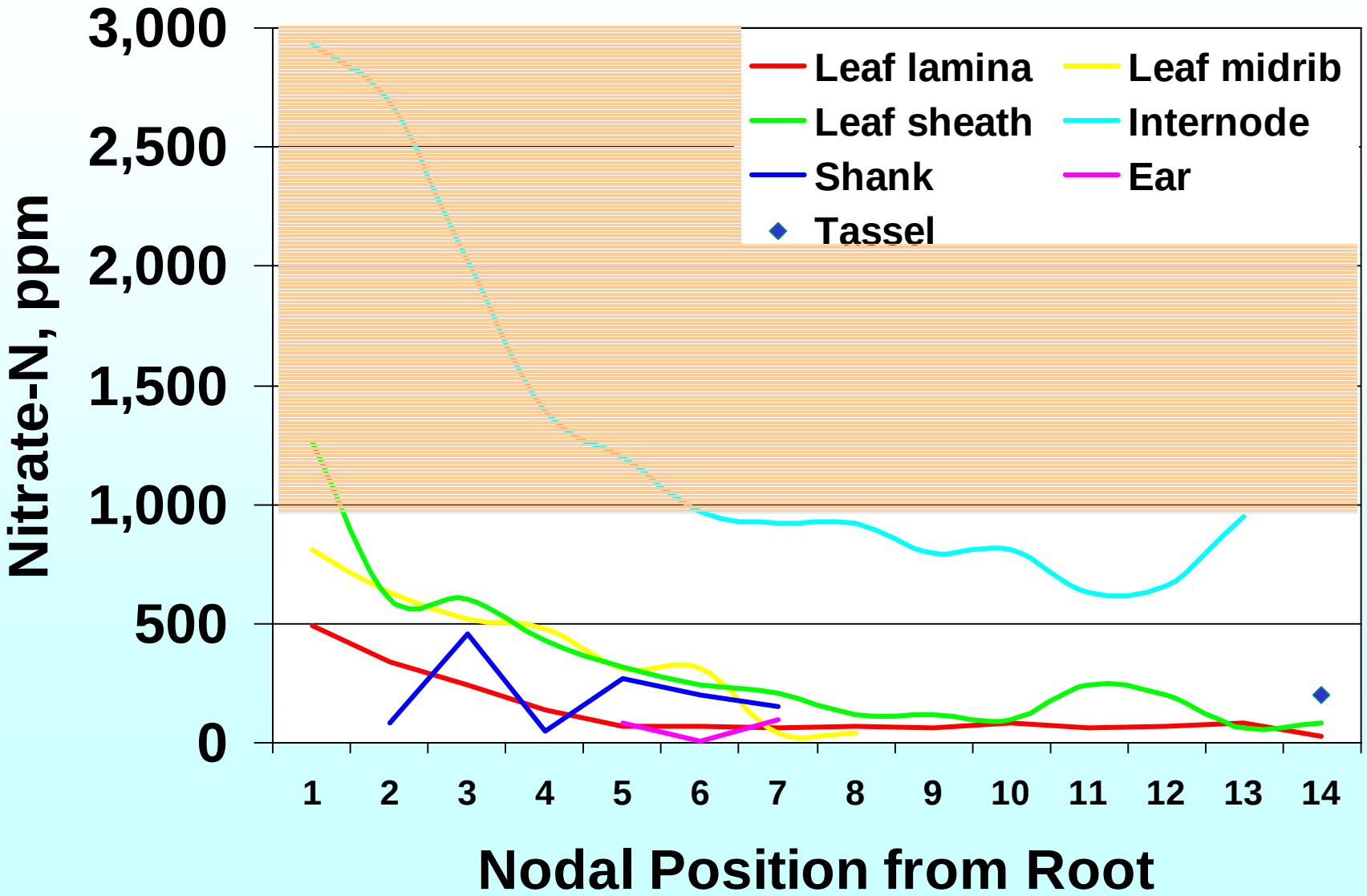


A. Reducing Nitrate Intake

1. Forage concentration varies with:
    - Level of N fertility
    - Plant species and maturity
    - Plant stress
        - Water shortage
        - Weather & shading
        - Frost damage
    - Time of day
    - Plant part

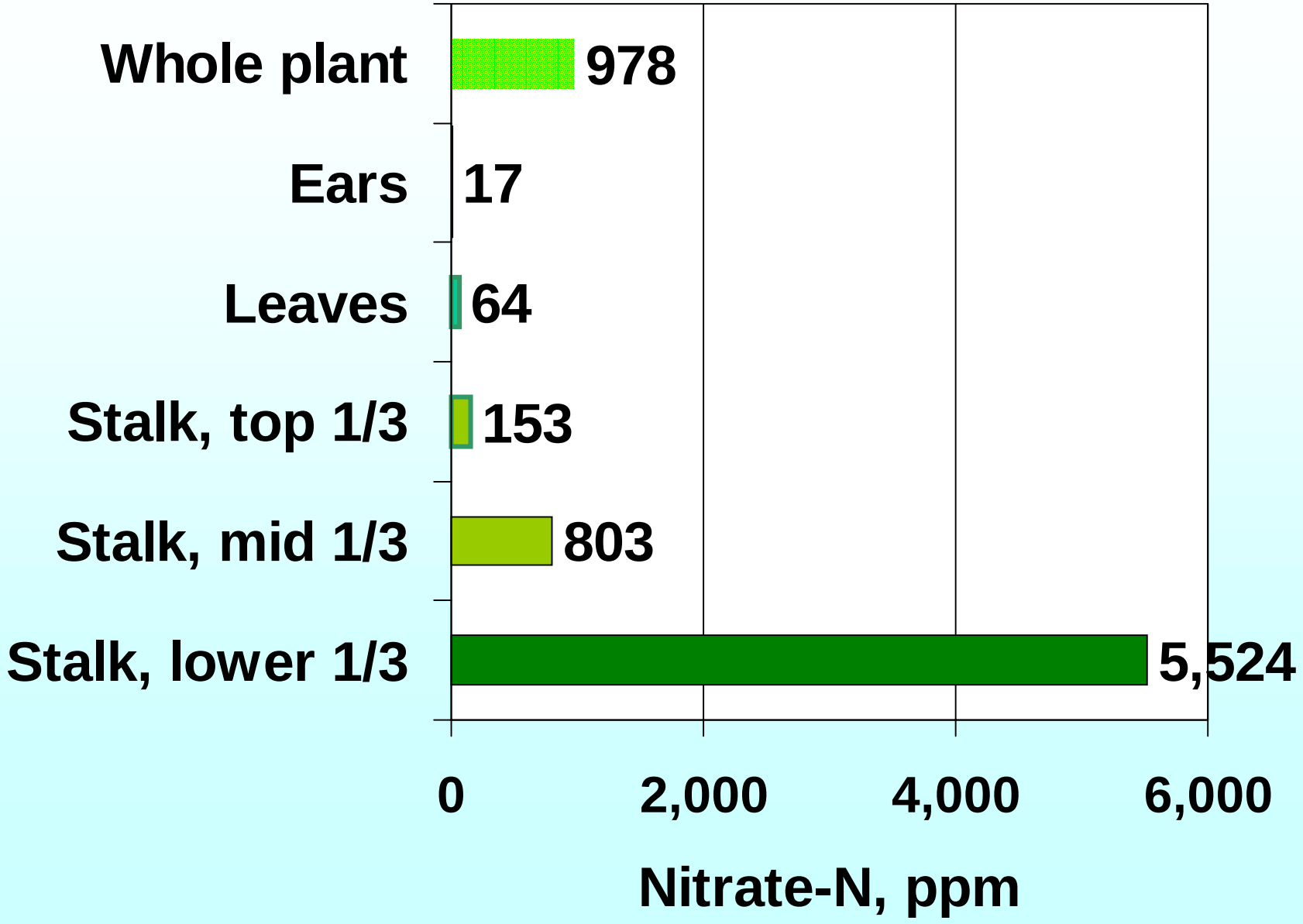
Uptake by plant roots; transported to leaves for reduction to nitrite.

Nitrate-N of Corn Plants

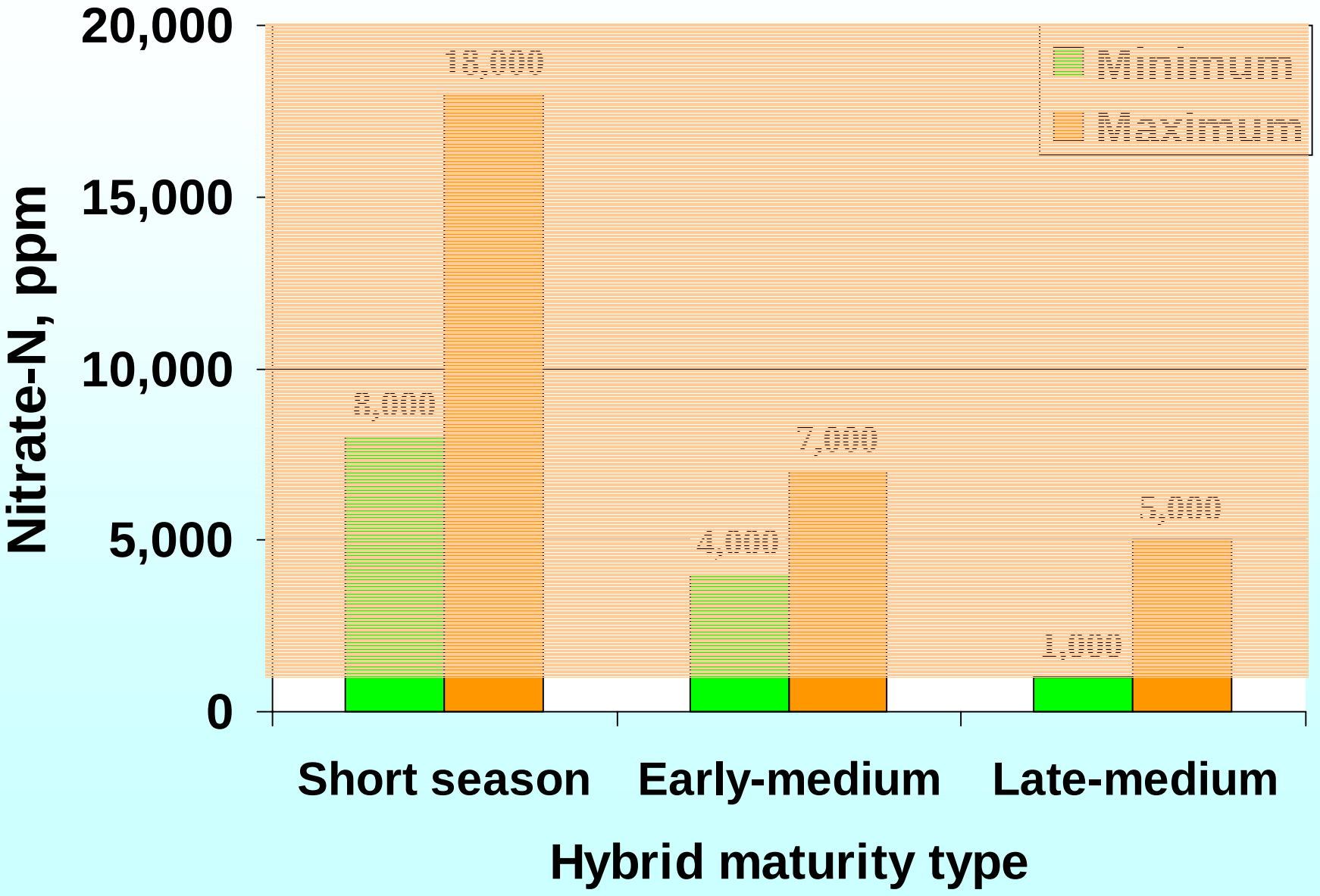


Whitehead 1952

Nitrate in Corn Plant Parts



Nitrate-N in Stalk



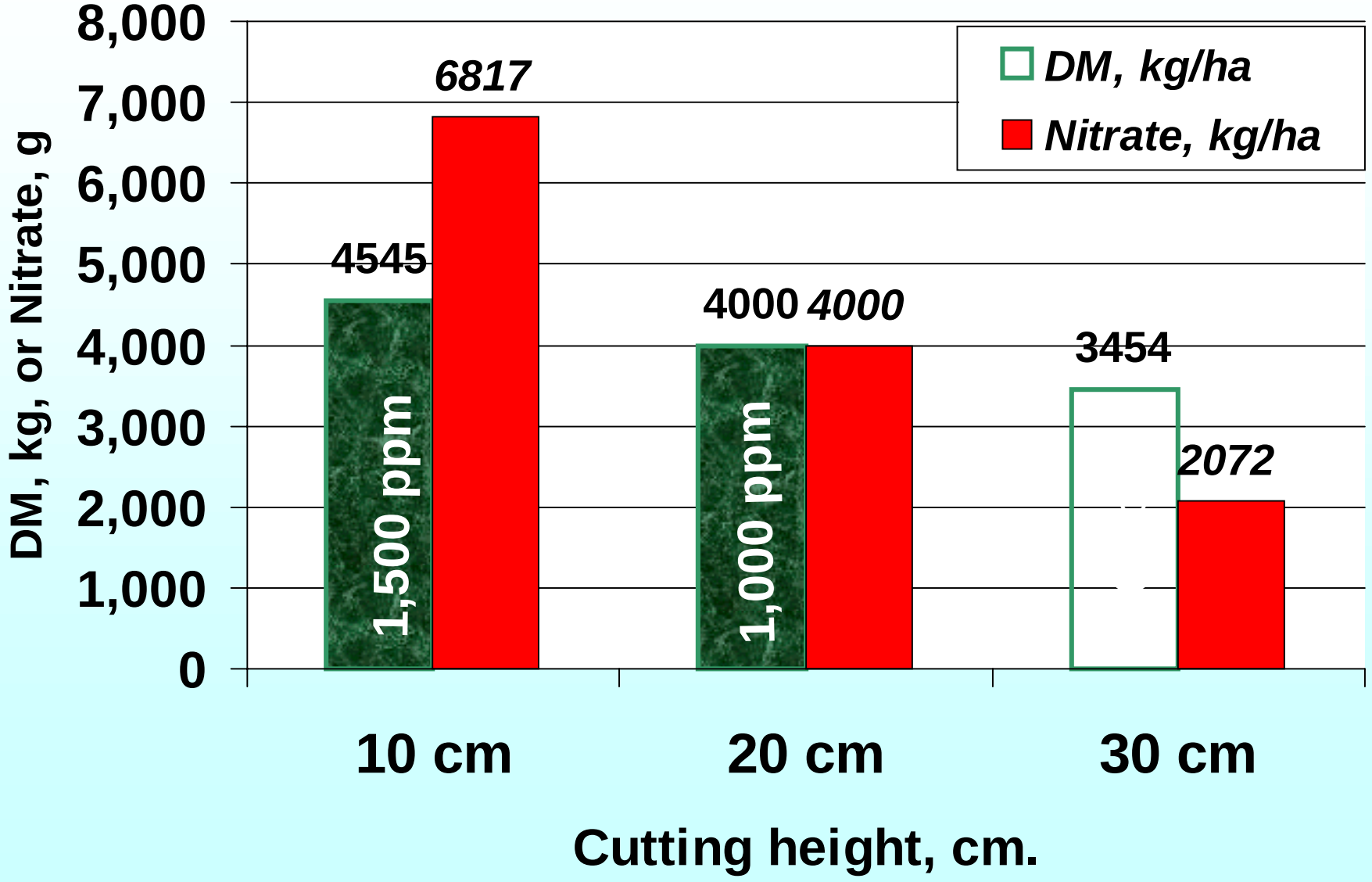
A. Reducing Nitrate Intake

1. Forage concentration varies with:
    - Level of N fertility
    - Plant species and maturity
    - Plant stress
        - Water shortage
        - Weather & shading
        - Frost damage
    - Time of day
    - Plant part

Low stocking rate - skip lower leaves.

Avoid harvesting the bottom stems of the corn plant. Cuts yield & nitrate.

Cutting Height and Nitrate in Corn Silage



A. Reducing Nitrate Intake

1. Forage concentration varies with:
    - Level of N fertility
    - Plant species and maturity
    - Plant stress
        - Water shortage
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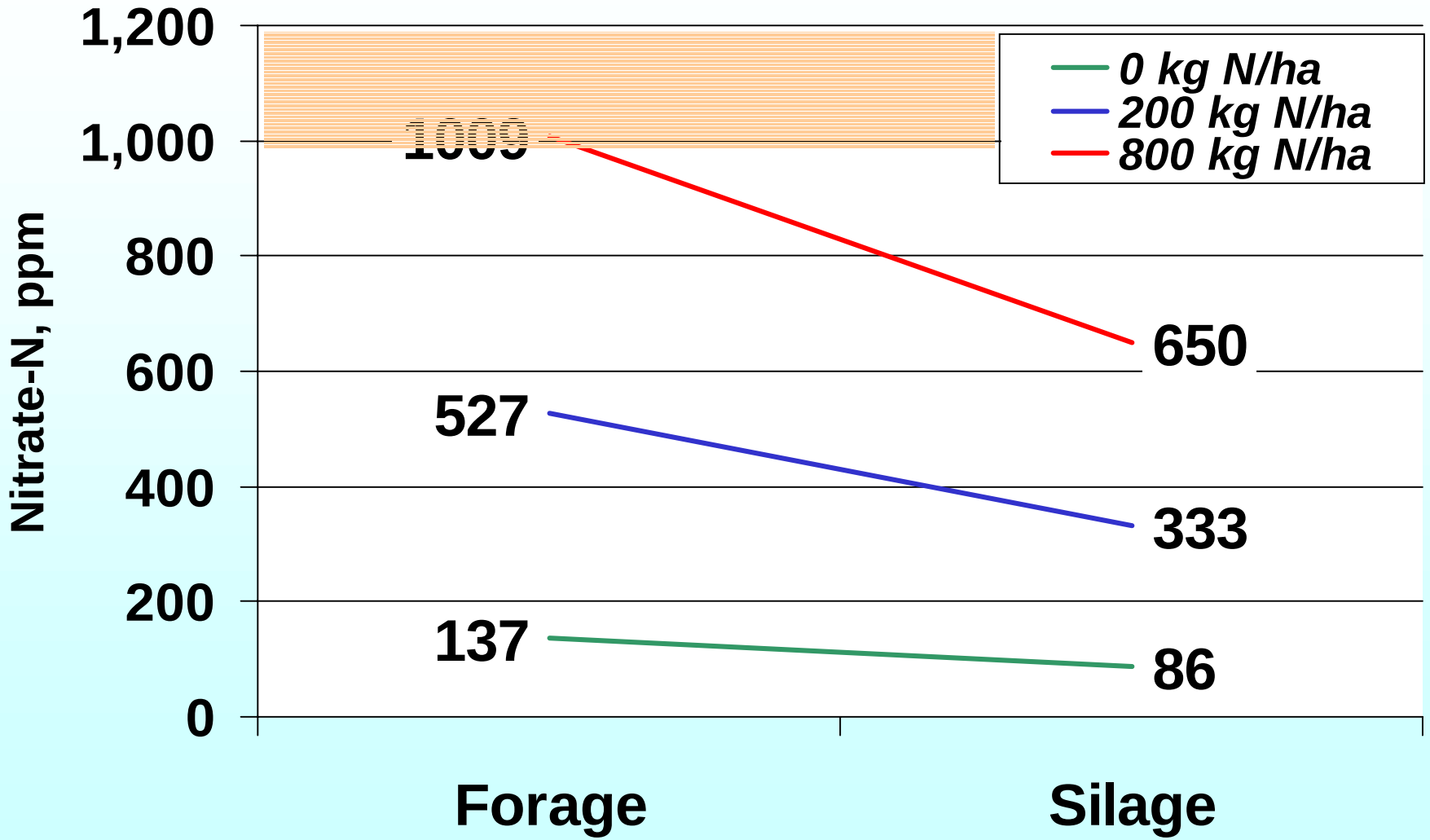
        - Frost damage
    - Time of day
    - Plant part
2. Reduce by fermentation/ensiling.

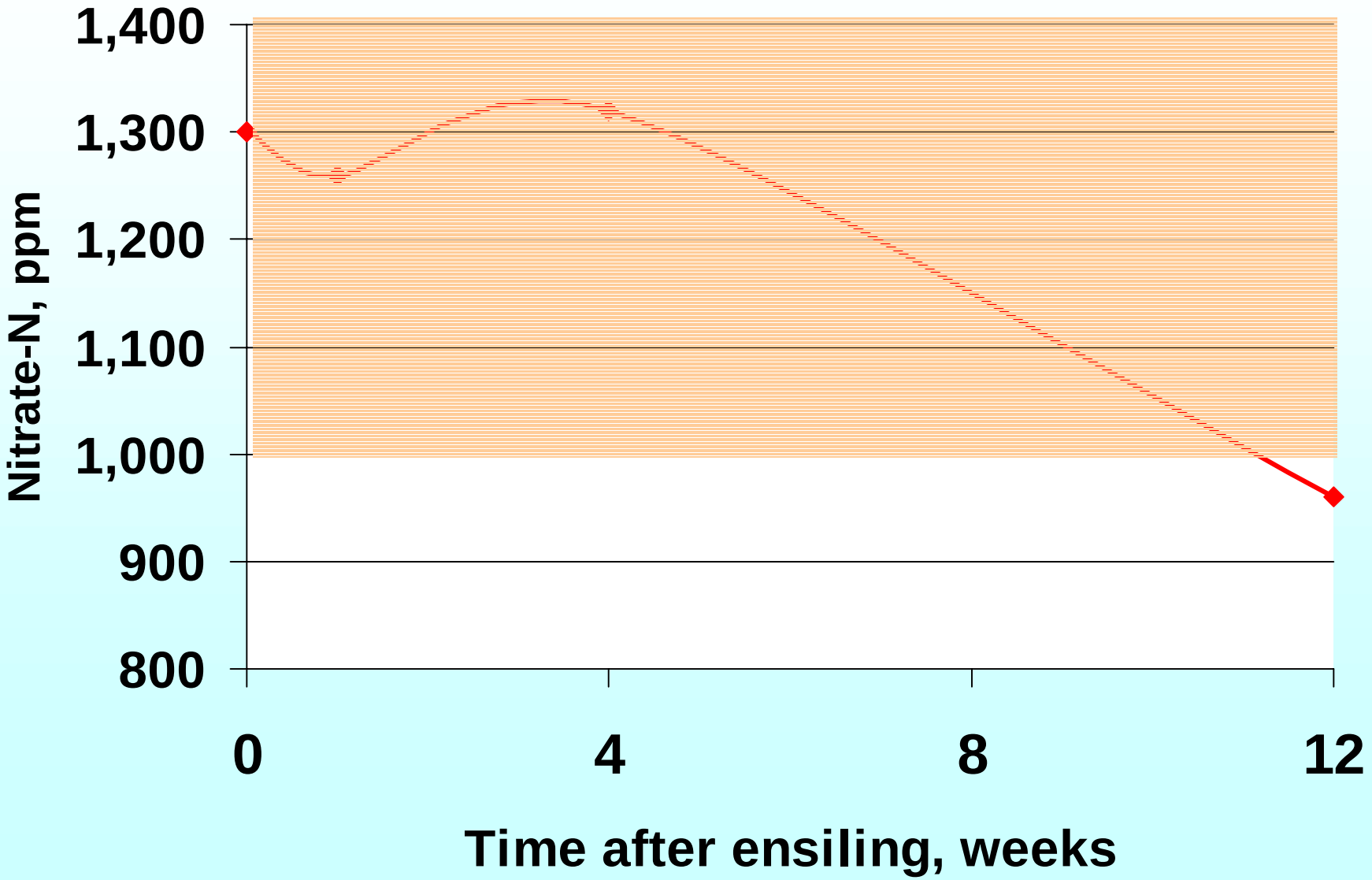
Nitrate-N converted to gaseous form.

Corn Plant Ensiling and Nitrate

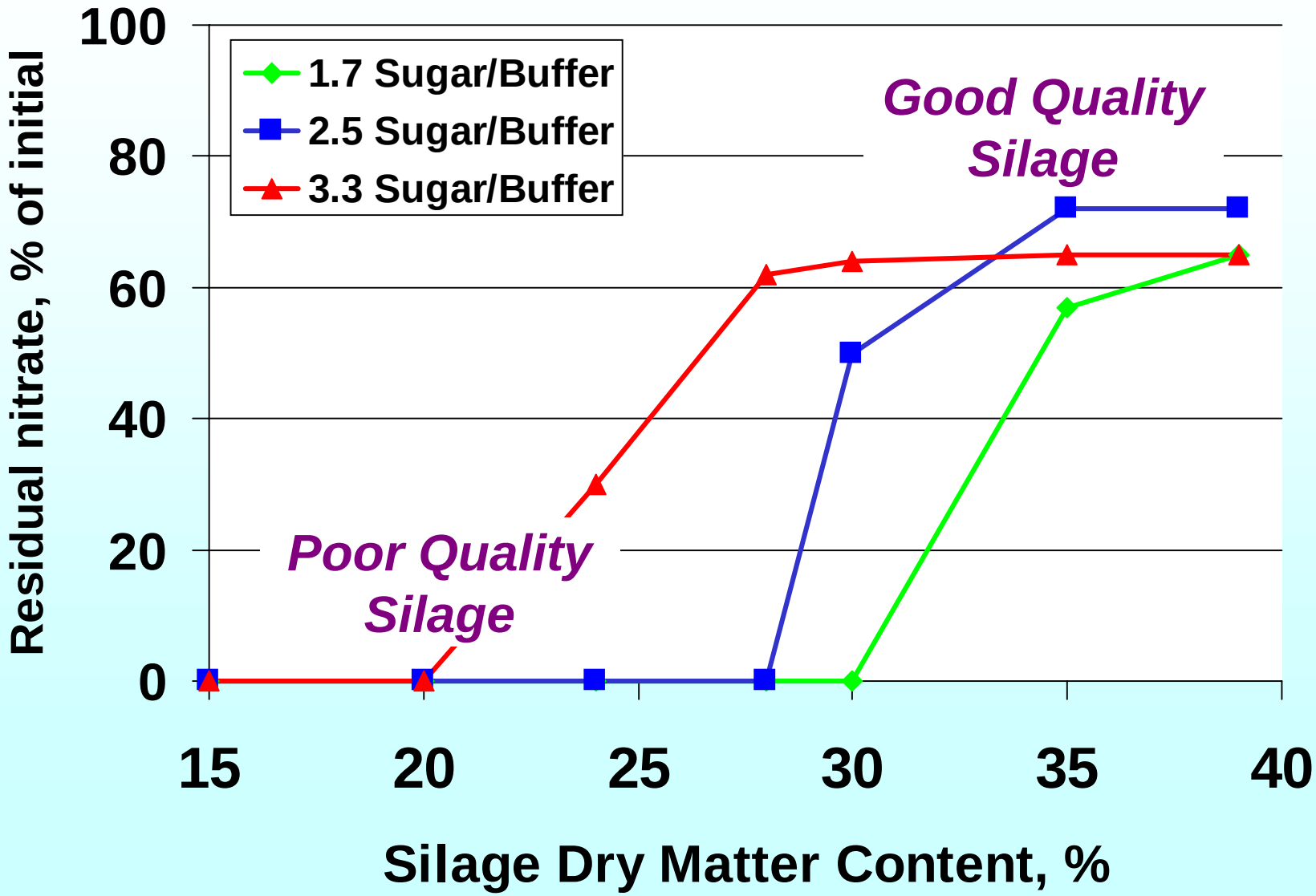


Rhykerd 1988

Small Grain Ensiling and Nitrate



Nitrate Remaining after Grass Silage Fermentation



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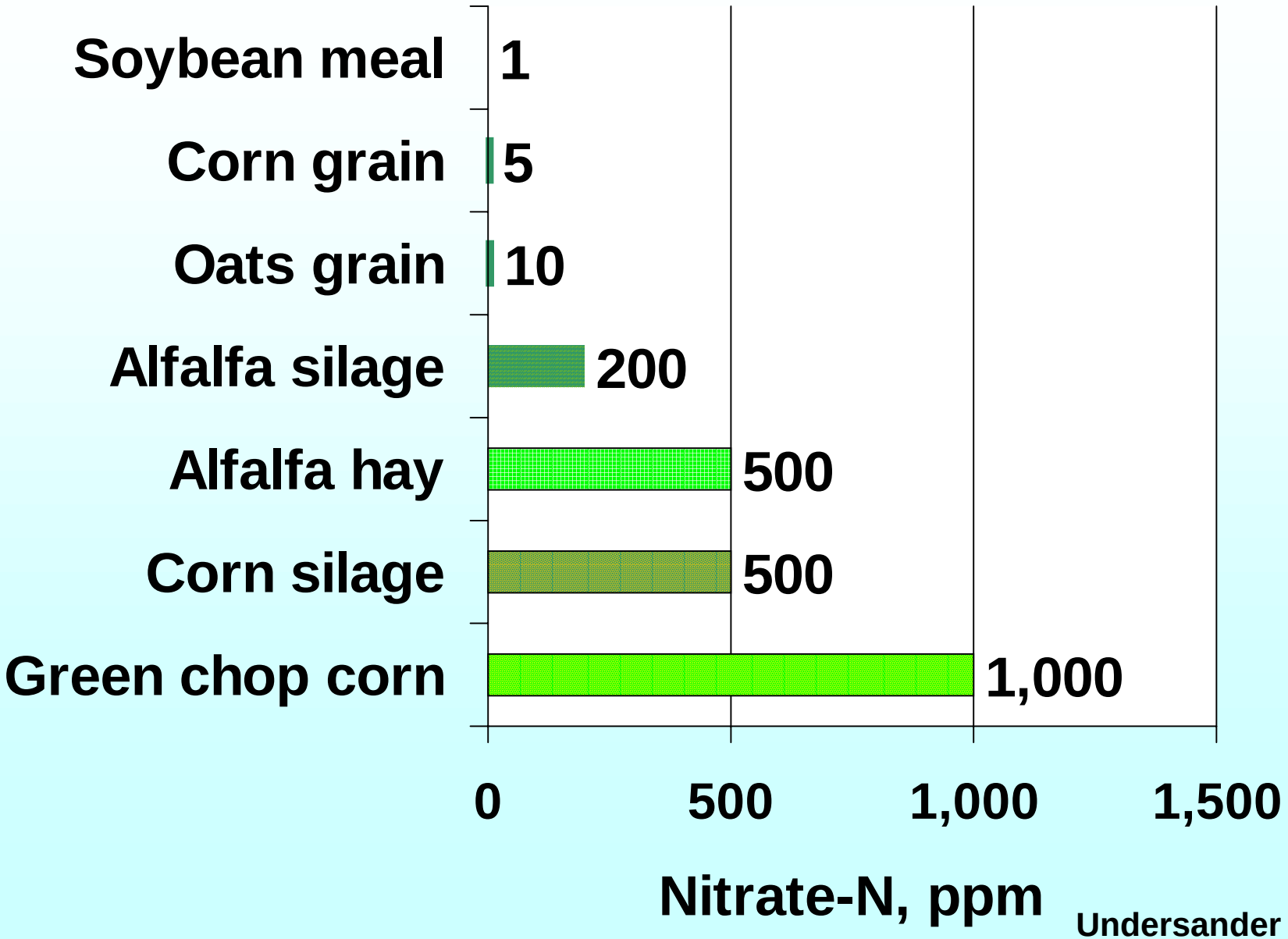
    - Plant part
2. Reduce by fermentation/ensiling.
3. Dilute diet with low-nitrate forage/grain.

Typical Feed Nitrate Levels



Undersander 1997

A. Reducing Nitrate Intake

1. Forage concentration varies with:
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    - Plant species and maturity
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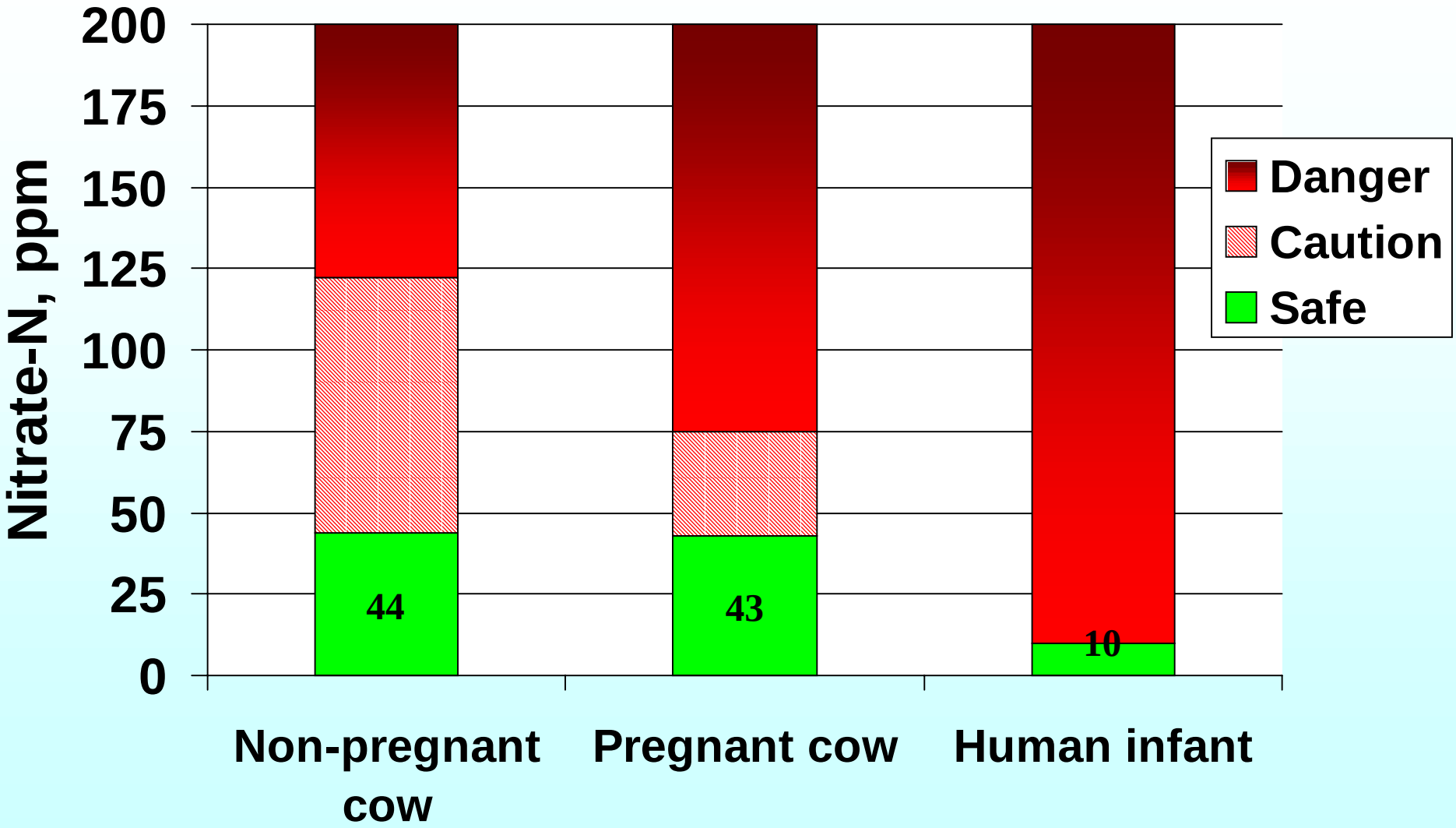
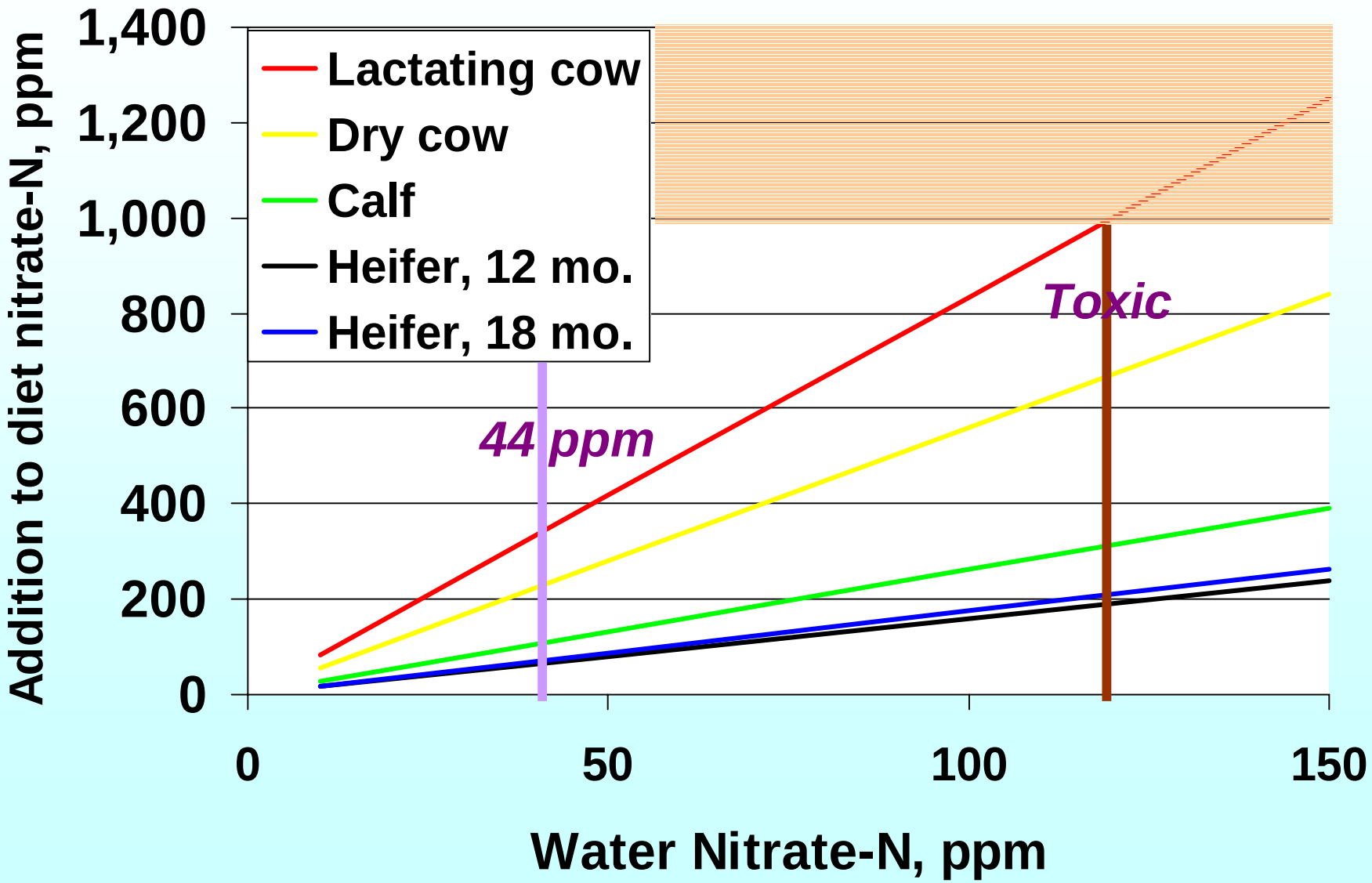
        - Water shortage
        - Weather & shading
        - Frost damage
    - Time of day
    - Plant part
2. Reduce by fermentation/ensiling.
3. Dilute diet with low-nitrate forage/grain.
4. Avoid water sources high in nitrate.

Nitrate-N in Water - Safe & Hazardous Levels



Water's Contribution to Nitrate-N Intake



Nitrate in Drinking Water

Nitrate Removal from Water

1. Distillation - costly to install.
2. Reverse osmosis - costly to install/run.
3. Anion exchange columns - short life.
4. Bottled water.

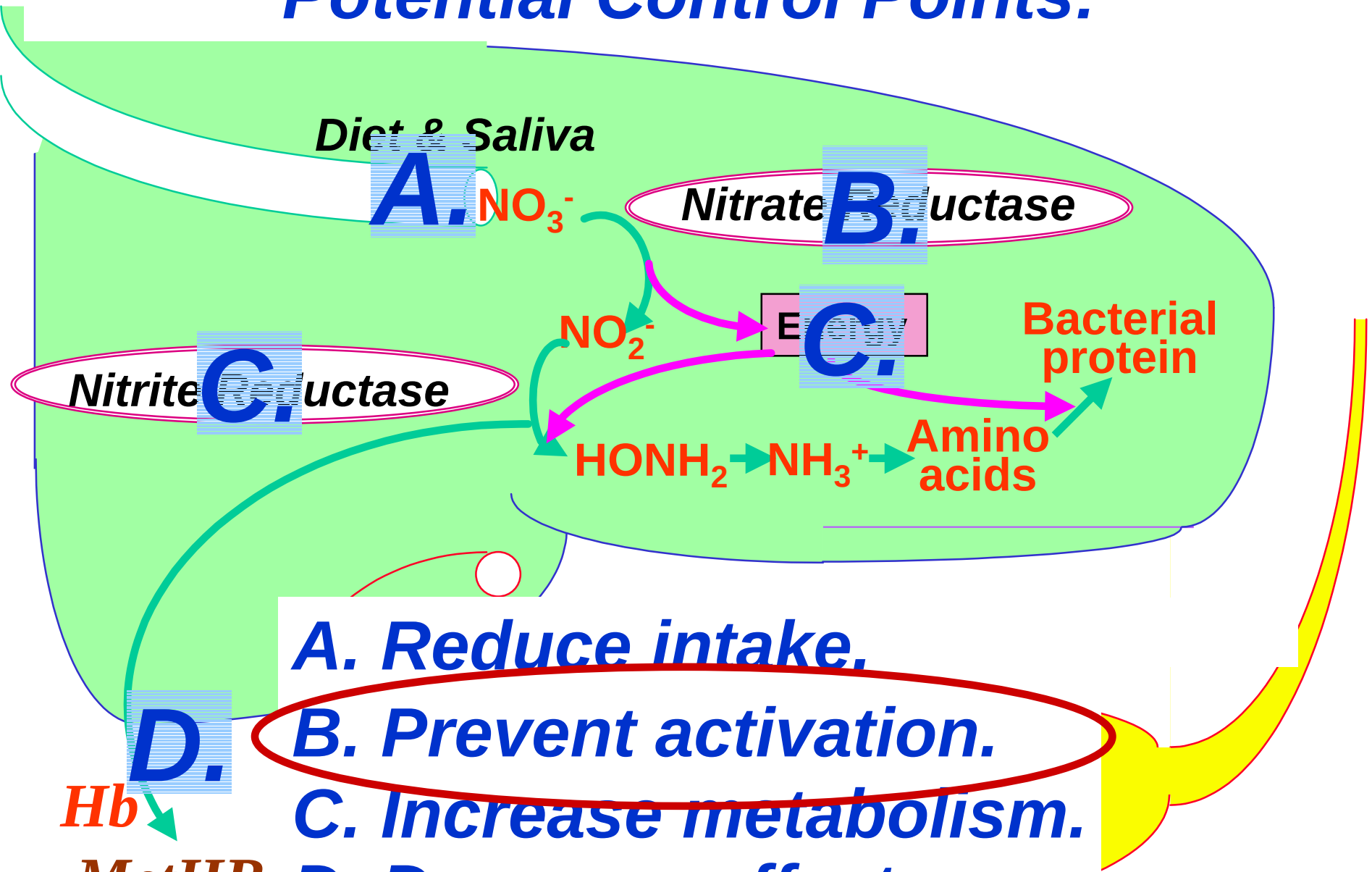
Boiling useless - Concentrates nitrate.
Aluminum pan - converts nitrate to nitrite

Collect rainwater - Possibility (Pb, asbestos concerns)

Nitrate Assay Methods

1. Field test:
   Diphenylamine spot test.
   Diphenylamine + H_2SO_4 + NO_3^-
   yields blue/black spot on forage.
   Test inside of stems.
2. Laboratory tests:
   Colorimetric.
   Nitrate electrode.
   Nitrate reductase (from corn plants).

Potential Control Points:



A. Reduce intake.

B. Prevent activation.

C. Increase metabolism.

D. Decrease effect.

B. Prevent Nitrite Formation

Nitrate **itself is NOT toxic.** ***Nitrite*** **is.**

Nitrate (NO_3^-)---------->*Nitrite (NO_2^-)*

Nitrate reductase

Bacteria, Fungi, Algae, Plants, Animals?

A. Inducible, particulate form

B. Soluble, constitutive form.

NADH or NADPH are electron donors.

Activity varies diurnally in plants.

1 mole Molybdenum / mole enzyme.

B. Prevent Nitrite Formation

Nitrate-Reducing Bacteria

E. coli, Shigella, Salmonella, Klebsiella, Enterobacter, Proteus (Energy source for anaerobic bacteria)

Nitrate-Sensitive Bacteria

Clostridia. Prevents C. botulinum growth in foods, clostridial degradation of silage.

Nitrate/Nitrite added to processed meats: bright red color = nitrosohemoglobin.

Nitrate or vitamin C-Botulism prevention.

Nitrate Reductase contains Molybdenum (Mo)

Tungsten (W) is competitive Inhibitor for Mo.

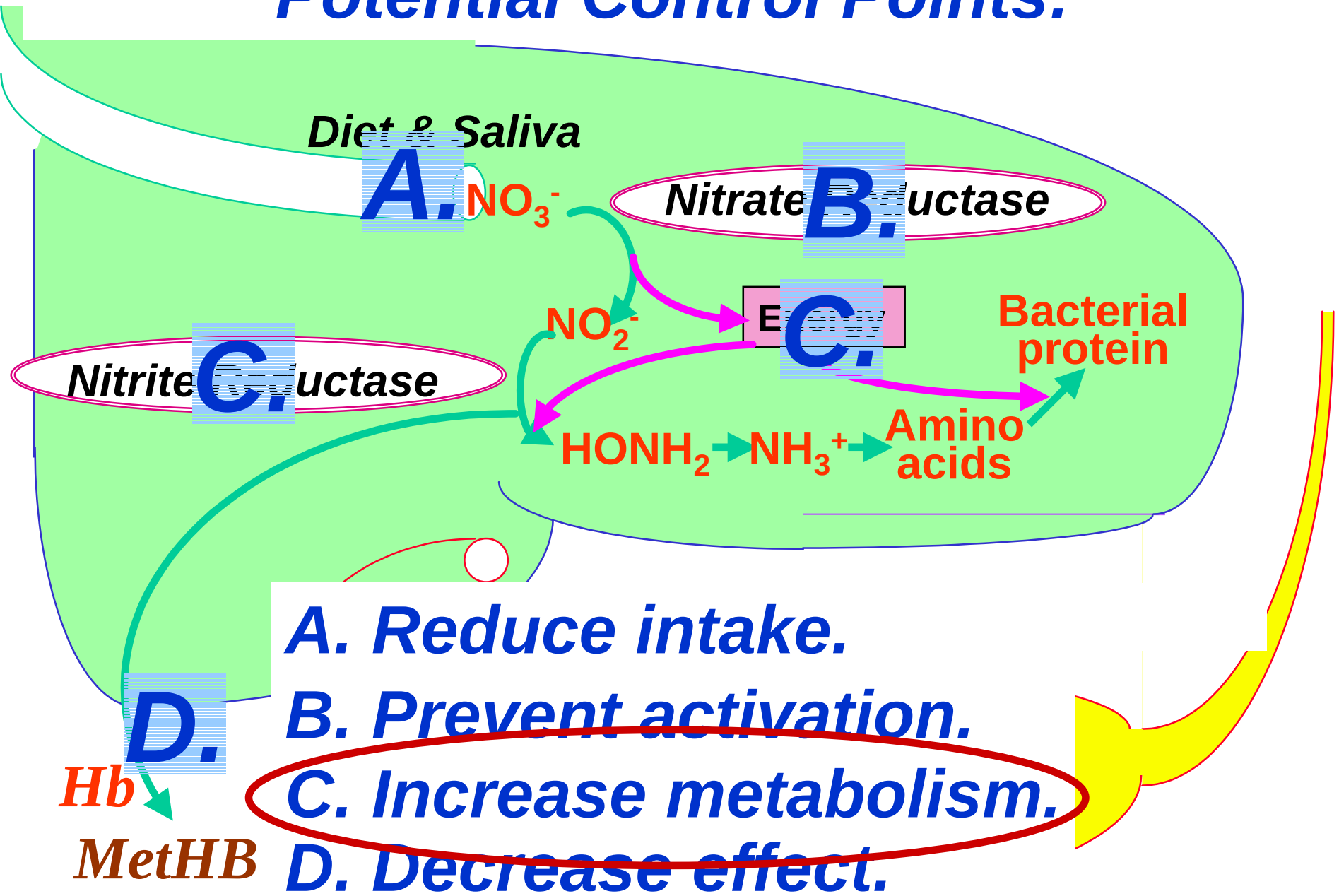
B. Prevent Nitrite Formation

Tungsten (*W*) inactivates nitrate reductase
Lactating cows research: Netherlands.

Rumen cultures: 100 to 500 uM of W prevented nitrate reduction.
Feeding 6 - 12 mg W/kg body weight prevented toxicity from 10,000 ppm.
Higher levels needed with Mb rich diets.

Concerns about Tungsten:
W in milk (.4% of dose).
Soil/plant effects?

Potential Control Points:



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C. Increase Nitrite Removal

Nitrite (NO_3^-) Hydroxylamine Ammonia (NH_4)

Nitrite reductase

Plants form - Not use NADH or NADPH.
Bacteria - Defense mechanism. NADH dependent & NADPH dependent forms.
Flavin dependent.
pH optimum 5.6.
Contains Fe.
Ammonia-useful NPN source.
Requires energy - Concentrate feeds.

C. Increase Nitrite Metabolism

Adaptation:

1. Ruminal bacteria:

- **Short term: Microbes adapt in 2 - 4 h.**
- **Long term: More nitrite reducers.**
  - **Propionibacterium inoculants.**
    - **Heat-resistant, cheese fermenters**
    - **Become permanent residents.**
    - **Ionophore sensitive?**

2. Pre-adapt to nitrate?

- **May prime nitrate reduction.**

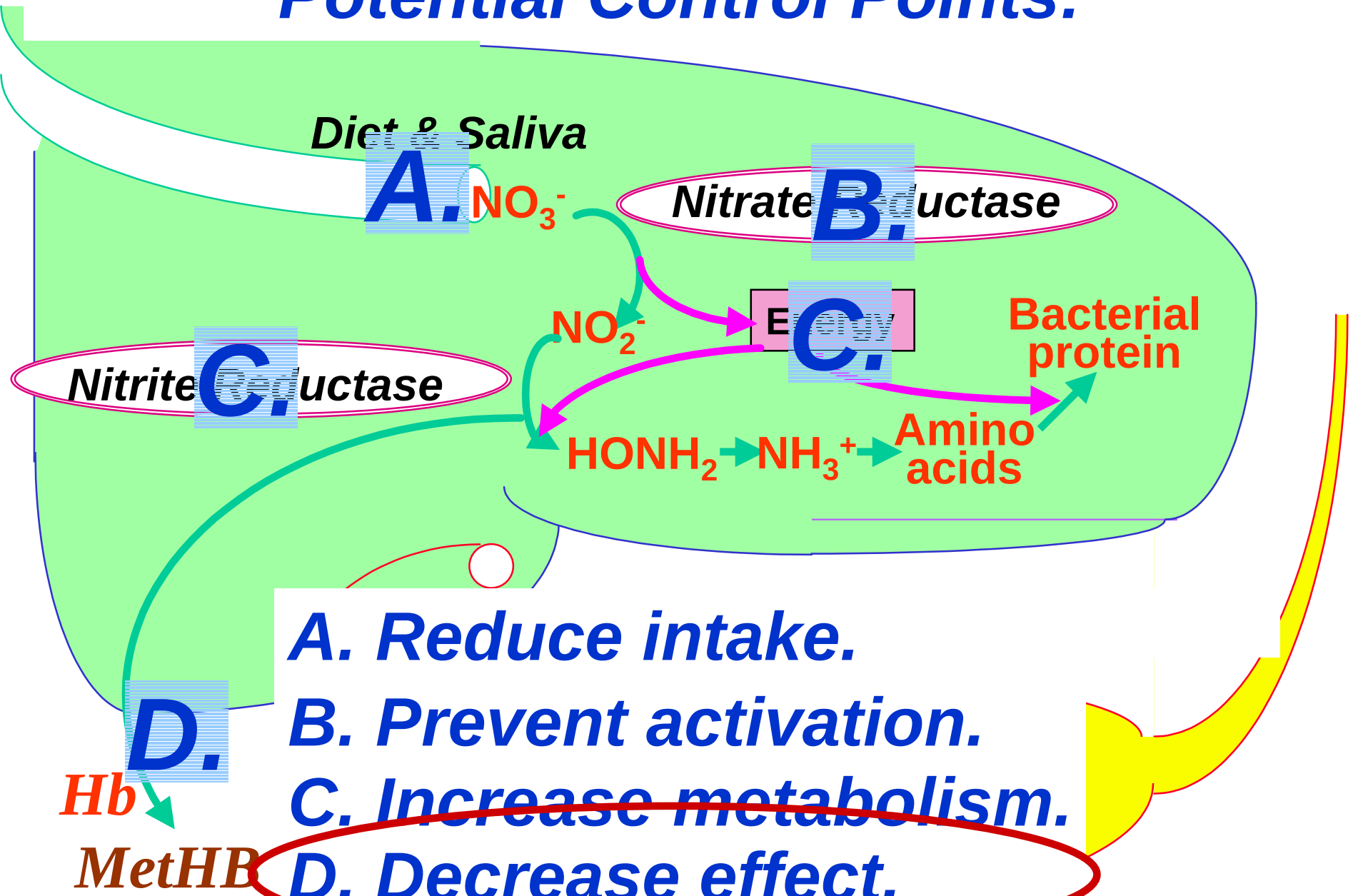
3. Pre-adapt to nitrite?

- **Not tried to date.**

C. Increase Nitrite Metabolism

1. Small frequent meals.
   - Feed hay before pasturing cattle.
2. Totally mixed dry rations.
3. Avoid damp forages (hay, fodder).
4. Gradually adapt to high nitrate feeds.
   - Bacterial adaptation/inoculation.
5. Increased dietary energy supply:
   - Carbohydrate supplements.
6. Other diet components:
   - Extra energy (concentrate).
   - Vitamin A?
   - Replace soybean meal with urea?

Potential Control Points:



A. Reduce intake.

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D. Decrease Nitrite Effects

Acute toxicosis:

Nitrite reacts with hemoglobin to stop oxygen transport by blood. Short term drastic effects.

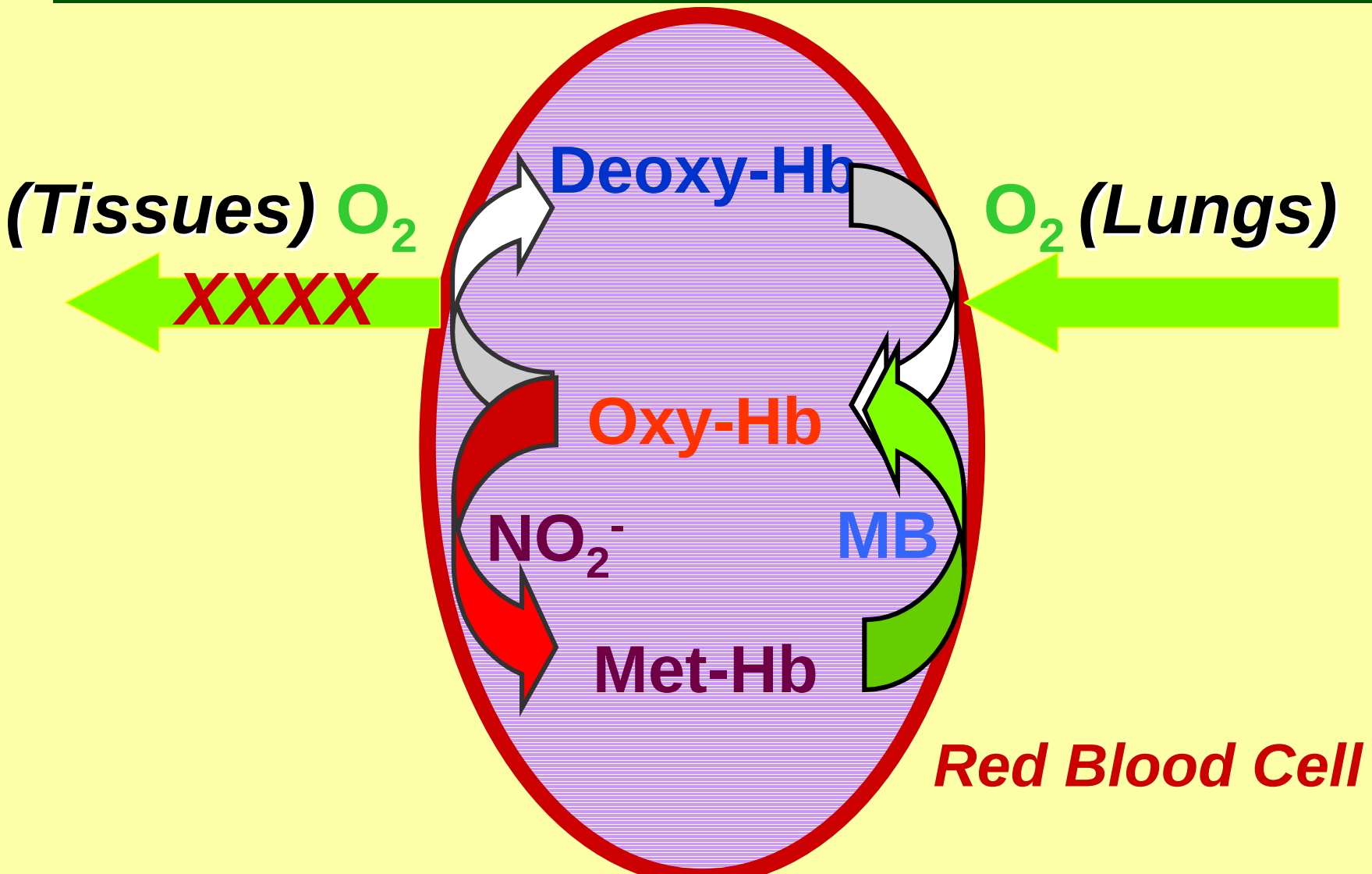
Chronic toxicosis:

Depressed feed intake.

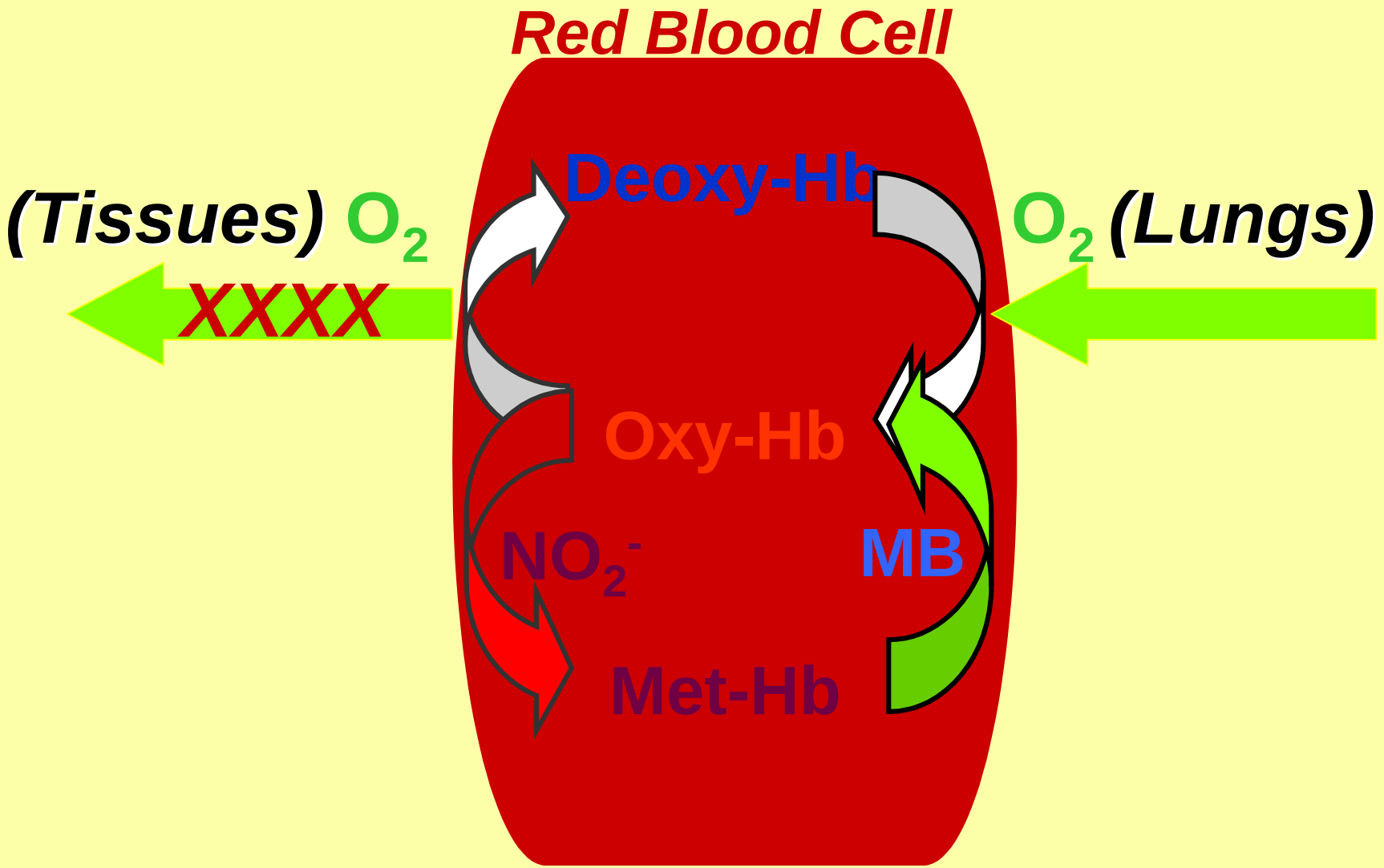
Oxygen depletion of uterus or fetus.

Increased blood ammonia may reduce implantation and fertility?

Hemoglobin (Hb) Metabolism



Hemoglobin (Hb) Metabolism



D. Decrease Nitrite Effects

Animal adaptation:

Nitrate is vasodilatory agent.

With gradual adaptation:

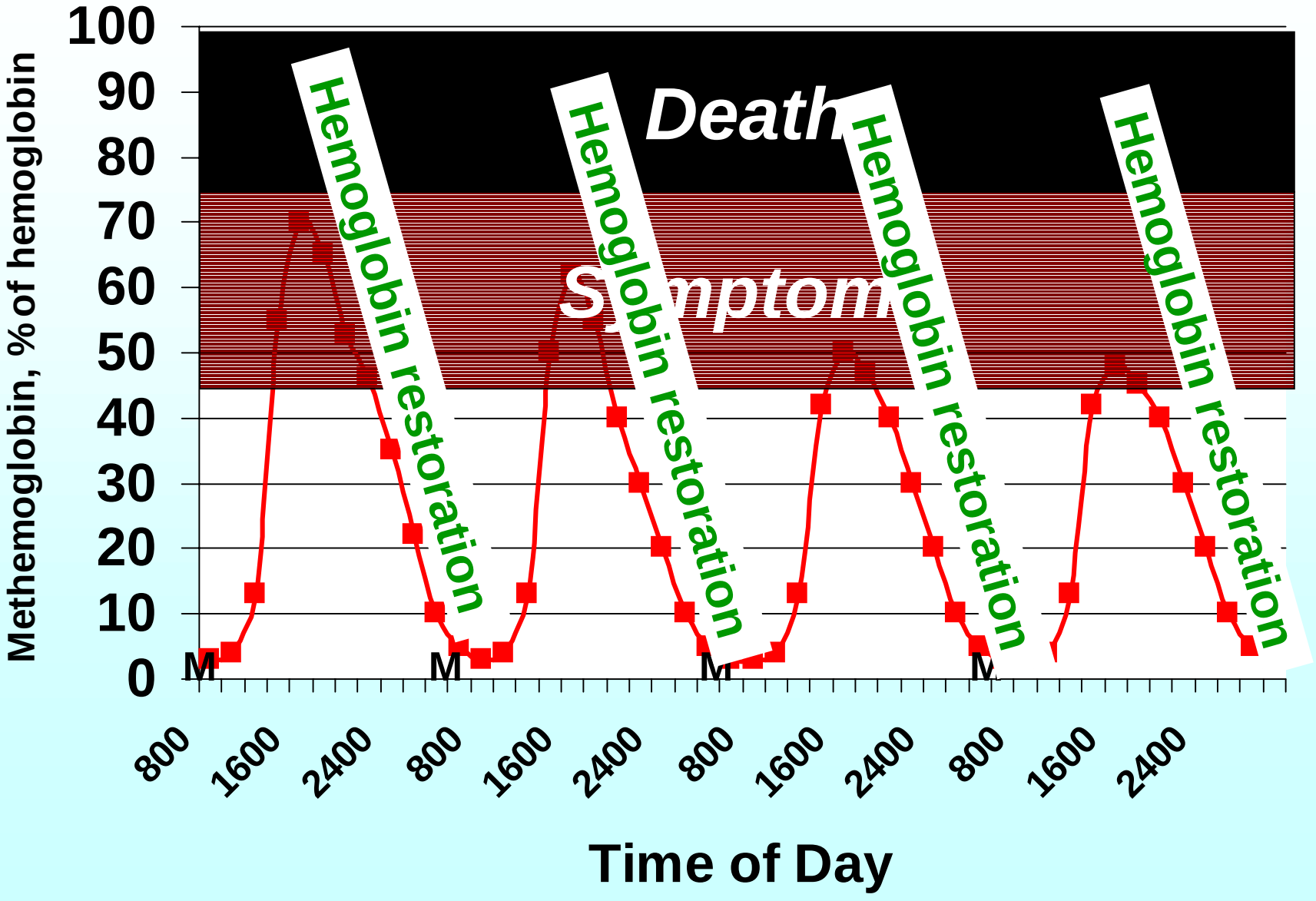
- **Increased hemopoiesis (More Hb).**
- **Increased blood volume.**
- **Increased hemoglobin restoration?**

Methemoglobinemia - Transitory.

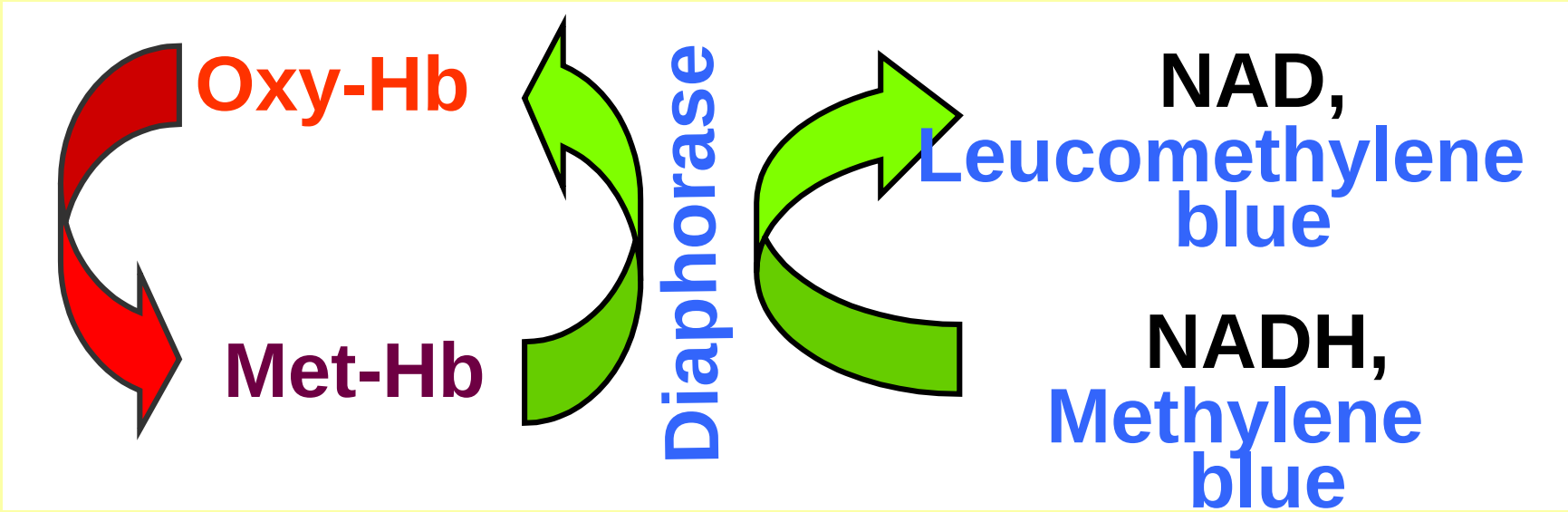
- **NADH dependent diaphorase in red blood cells. Higher blood glucose speeds hemoglobin restoration.**

Adapted grazers tolerate 3400-4600 ppm forage (>3 times toxic dry dose)

Methemoglobin Concentrations



D. Hemoglobin Restoration



Diaphorase: Present in red blood cells.

NADH generation from glucose.

Ruminants: Low blood glucose (NADH), esp. with roughage diets. Benefit from added concentrate. Ionophores?

D. Nitrite Toxicity Treatments

1. Acute toxicosis treatment:
   Intravenous infusion - 100 ml/500 kg body weight of a 4% methylene blue solution (Stanton, 1999).
   2 to 4 (or up to 15) mg methylene blue per kg body weight.
2. Sodium tungstate - preventative.
3. Ascorbate, menadione, glutathione, vitamin E?
4. Chlortetracyclines.

D. Gas Toxicity Concern

Silage Fermentation Gases:

NO, NO_2, N_2O_4, CO_2,

Released first 1 - 10 d of fermentation.

5 ppm NO_x odor threshold (8 h max).

75 ppm visual (brown) detection.

100 ppm danger.

700 ppm fatal in 30 min - Man & Animal!

Reacts with lung fluids to form nitric acid!

Permanent lung damage.

Ventilate silo. Heavy gases - sink.

Conclusions

Forage/Silage Control Points

1. Avoid high nitrate forage species.
2. Avoid excess N fertilization/drought.
3. Test suspicious forage & water for nitrate.
4. Avoid harvesting or grazing forages in wilting stage.
5. Harvest crops late in the day.
6. Leave lower stems in the field.
7. Ensile high nitrate forages.

Conclusions

Feeding/Management Control Points

1. **Feed high nitrate forages only to non-pregnant heifers and dry cows.**
2. **Dilute high nitrate forages with grain or low nitrate feeds. Totally mixed rations.**
3. **Adapt animals gradually to high nitrate forages.**
4. **Avoid high meal size of suspect forage.**
5. **Be alert for symptoms and have antidote (methylene blue) on hand.**
6. **Avoid silage gases for 10 days after ensiling crop.**
7. **Consider propionibacteria inoculation.**

Thanks for your Attention

Shalom Aleichem