High tannin forages can improve sustainability of integrated crop-livestock production systems

Interim report to ARDI

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Background and objectives

Tannin containing legumes provide a good alternative to alfalfa for producers who want to use legume forages in pasture mixtures or as part of overwintering diets to provide an inexpensive source of protein, energy, minerals and vitamins for cattle. Further, we know that these forages can prevent bloat and can improve protein digestion and utilization in cattle. The value of these forages in reducing enteric methane emissions of cattle, reduced soil N losses as nitrous oxide or in water, and reduced pathogen and parasite loads in the animal gut has been identified in other subtropical countries, although plant species used in these countries differ from those used in the Western Canadian production environment.

This project seeks to better understand the role of tannin-containing plants in soil-crop-livestock systems. The research questions in the project are as follow:

1. Do tannin-containing plants within forage mixtures in fact increase total forage tannin production?
2. Does the tannin in the forage carry through to the manure in a beef cattle system?
3. How does tannin concentration in the manure affect greenhouse gas production?
4. Does the presence of tannin-bearing plant material affect pathogen colonization of beef cattle manure or soil?
5. Does manure differ in its greenhouse gas properties when the manure is derived from tannin-containing plants?
6. How does the application of tannin vs non-tannin plant-derived manure affect soil processes?

The research team has taken an integrated approach to evaluate the potential use of tannin containing legumes in beef cattle production systems. The questions posed have been addressed using an *integrated systems research* model which is described in Figure 1. The integrated systems model includes forage plants, beef cattle, manure and soils.

Project results

Do prairie-grown forages contain tannins?

Using data gathered from a previous ARDI-funded project, the research team identified forage species which have the highest tannin concentration. Plants were clipped to approximately 3.75 cm above the soil surface. Plants were either in the vegetative state (prior to flowering) or in the mature state (flower present). As indicated in Table 1, the species found to contain the greatest tannin concentration were purple prairie clover with an average plant condensed tannin concentration of 68.7 g/kg. There was considerable variation in this value, as the minimum concentration for this species was 37.9 g/kg while the maximum concentration was 92.9 g/kg. Other species which contain high levels of condensed tannins were sainfoin and Birdsfoot trefoil which contained average tannin concentrations of 46.0 and 15.1 g/kg, respectively.

Table 1. Condensed tannin concentrations of various fodder legumes						
Species	Site years	no. of varieties	Mean (g/kg)	Standard deviation	CV (%)	Min/Max
Alfalfa	7	9	0.0	0.0	453.3	0.0/0.1
Alsike clover	5	1	9.6	7.4	76.9	0.0/19.5
Cicer milkvetch	3	2	0.0	0.0	315.0	0.0/0.0
Kura clover	5	?	2.4	3.1	128.6	0.0/9.6
Purple prairie clover	5	1	68.7	22.6	32.9	37.9/92.9
Red clover	6	?	3.3	4.4	132.3	0.0/15.3
Sainfoin	3	6	46.0	19.3	42.0	16.3/94.4
Trefoil	8	2	15.1	6.3	41.6	0.0/25.7
White clover	5	?	2.3	4.6	201.5	0.0/11.9

As indicated in Table 2, tannin concentrations were found to be highest in mature plants in all species except sainfoin, where plant concentrations were highest in the vegetative forage.

Table 2. Average tannin concentration at different stages of maturity in various fodder legumes

Specie	n	vegetative (g/kg)	mature (g/kg)
Alsike clover	12	0 ± 4.4*a*	11.6 ± 2.0*b*
Kura clover	12	0.1 ± 1.1*a*	4.1 ± 0.9*b*
Purple prairie clover	6	N/A	68.7 ± 9.2
Red clover	20	0.2 ± 0.6*a*	8.1 ± 0.7*b*
Sainfoin	49	58.7 ± 3.0*a*	33.7 ± 3.0*b*
Trefoil	12	9.4 ±2.7*a*	18.6 ± 1.2*b*
White clover	7	0.0 ± 2.0	6.9 ± 2.3

LS mean ± standard error of the mean. Statistical significance ($P < 0.05$) is denoted by different letters within a specie.

This information was then used to determine which forage species to seed in the integrated systems approach described below.

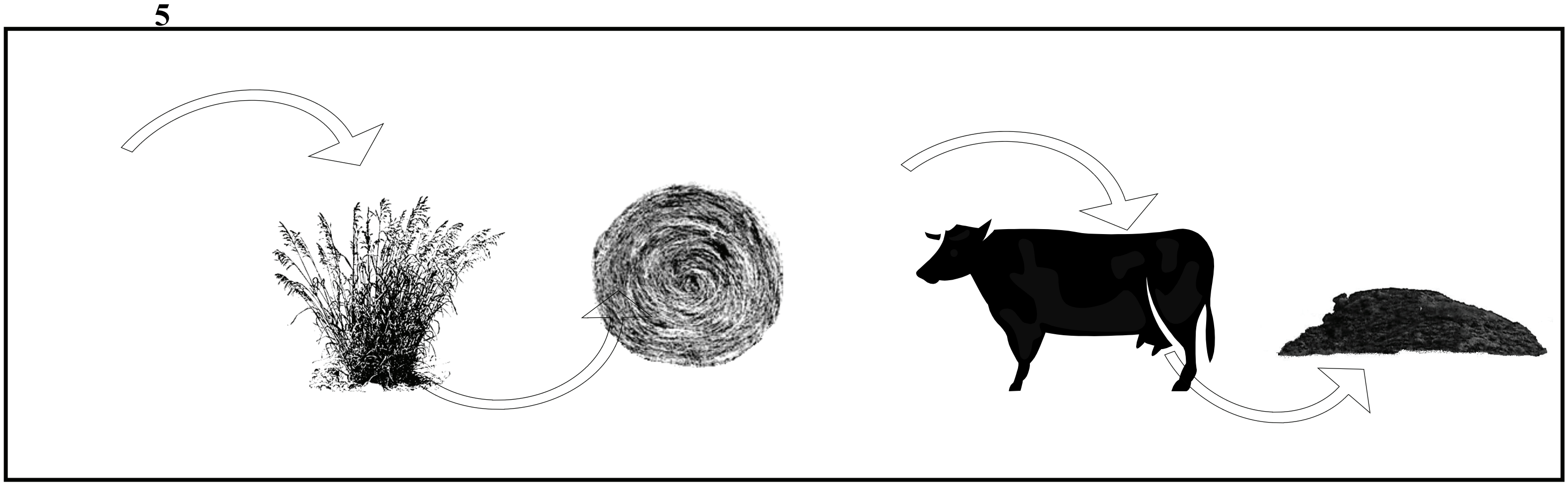
Results from the "integrated systems research"

The integrated forage-beef model is described in Figure 1. The results for each step within the model are explained separately.

Figure 1. Flowchart of activities on the "integrated tannin research".

Step 1: Growing forage at Brandon; Step 2: Preserving forage as dry hay or silage and transporting forage to Glenlea; Step 3: Feeding forage to beef cattle over winter – Glenlea; Step 4: collecting and evaluating manure - Glenlea; Step 5: spreading manure on long-term forage plots at Carman and Glenlea.

1 2 3 4 5



Step 1.

Two different forage species were established on a 30 acre field at the Agriculture and Agri-Food Canada Brandon Research Centre in 2005. Half of the field was seeded to alfalfa, a tannin-free forage species; the other half of the field was seeded to sainfoin, a tannin-containing forage species. Both species were successfully established. Sainfoin and alfalfa yields were in 2006 were 4741 kg ha^{-1} and 5064 kg ha^{-1}, respectively. In 2007, sainfoin and alfalfa yields were 6258 kg ha^{-1} and 8533 kg ha^{-1}, respectively. In both growing seasons, alfalfa yields were greater than that of sainfoin.

Step 2.

Sainfoin and alfalfa forage from the Brandon fields were preserved as round-bale silage or dry hay (also round bales). These bales were transported to the University of Manitoba's Glenlea Research Station. Crude protein concentrations were highest in sainfoin silage (18.3%) and alfalfa hay (17.16%), intermediate in alfalfa silage (15.47) and were lowest in sainfoin hay (12.4%). Gross energy ranged from 4.28 to 4.40 Kcal/g and was highest in the sainfoin silage diet. The phosphorus content of the feed was highest in sainfoin silage (0.31%) and lowest in alfalfa hay (0.16%).

Step 3.

An overwintering trial was conducted in which the forages described above were fed to backgrounded cattle. Intake (expressed as a percent of dry matter) and gross energy where greatest in alfalfa hay, followed by sainfoin hay. Intake and gross energy consumption were lowest in the silage diets. Average daily gain of the steers was not significantly different between diets with gains of 0.68. 0.62, 0.68 and 0.62 kg/d for cattle fed sainfoin silage, sainfoin hay, alfalfa silage and alfalfa hay respectively.

Step 4.

The animals created a manure pack during the winter feeding period. Greenhouse gases (CO_2 = carbon dioxide, N_2O = nitrous oxide and CH_4 = methane) were measured in the manure produced by the animals described above in a bucket type experiment. Manure produced by these animals was examined alone, mixed with straw, or mixed with sainfoin silage to assess the value of tannin in the bedding pack (Figure 2).

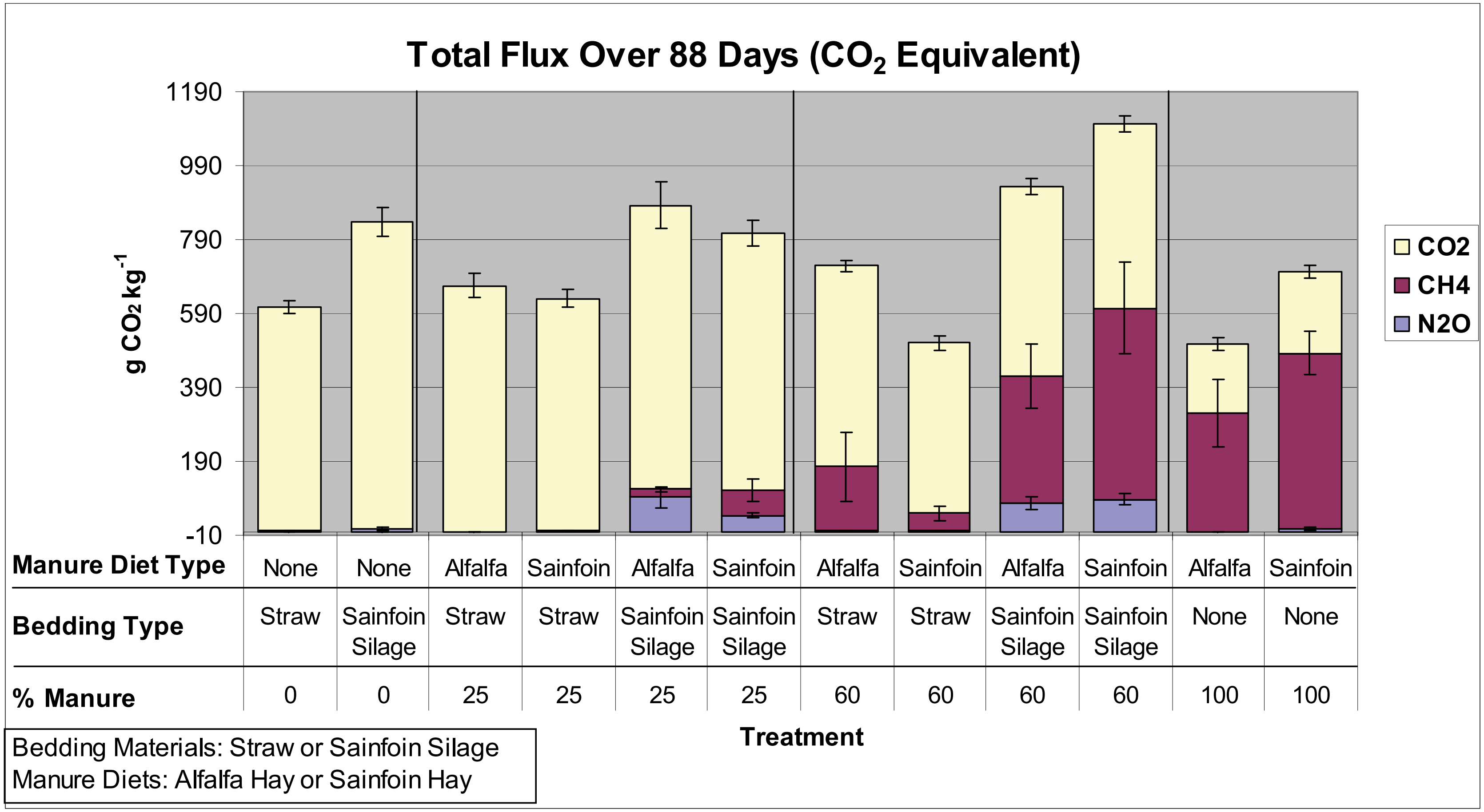


Figure 2. Cumulative carbon dioxide equivalents of the greenhouse gases, CO_2, CH_4 and N_2O produce during incubation of various combinations of bedding (straw and sainfoin), and manure from cattle feed diet of alfalfa or sainfoin silage. Note greenhouse gas emissions were lower with straw bedding, sainfoin bedding promoted N_2O and CH_4 emission and CH_4 increased with manure content

The bedding pack incubation experiment showed an effect of manure addition rate and bedding type on the total amount and types of greenhouse gas emitted. Total emission of greenhouse gases was highest when manure of either diet was added to sainfoin silage bedding. The total level of greenhouse gas emitted increased with sainfoin bedding when manure was a greater proportion of the bedding pack (at 60% manure content). The increase in greenhouse gas emissions with sainfoin bedding was partly attributable to the production of nitrous oxide that was absent with straw bedding treatment. Further methane production was enhanced with sainfoin bedding compared to Straw bedding.

The feeding trial described in Step 3 above utilized straw and sainfoin as the bedding sources. Greenhouse gases, as well as odour were measured from the pack and are presently being analysed. Upon completion of the trial in the spring, the bedding pack was turned several times in an effort to mix the manure and to reduce the manure pile volume. Subsequently, the manure was removed from the pen, sampled for nutrient and tannin concentrations, and spread on the crops described in Step 5 below. This is the focus of Robert Kazuk's MSc thesis.

Table 3. Analysis of manure from alfalfa and sainfoin crops grown at Brandon and fed to beef cattle at Glenlea. August, 2007.

	As recieved per Tonne (*t*) in kg.			
Sample	**Available N***	**Total S**	**P_2O_5**	**K_2O**
Sainfoin silage	67.000	14.400	0.403	0.447
Sainfoin hay	94.000	8.200	0.364	0.542
Alfalfa silage	96.000	6.400	0.094	0.530
Alfalfa hay	99.000	7.900	0.161	0.406

*Available N calculated as per *Characterization of Solid Beef Manure* protocol on MAFRI website, prepared by P. Loro, where Available Nitrogen assumes 25% of organic N is labile at application, and 25% of Ammonium N is volatilized (this parameter may be significantly higher given the manure will not be incorporated post-application).

Step 5.

The final part of the integrated systems research involves the field experiments. Two field experiments were established – one at the Carman research station and a second at the Glenlea research station. Field plot maps of these two locations are given in Appendix 1.

The Carman experiment includes both tannin-containing (birdsfoot trefoil; sainfoin; purple prairie clover) and tannin-free (alfalfa) legumes. These legumes are grown both as a monoculture and in combination with grass forage (meadow bromegrass).

The experiment was seeded in spring of 2006. Because of drought conditions that year, two of the forage species (birdsfoot trefoil and purple prairie clover) did not establish well. Therefore, these plots were reseeded in spring 2007. These species did establish well in 2007.

Forage plant density and forage dry matter yield have been assessed. Forage plant density data is given in Table 4. The results indicate adequate plant population densities for all monoculture forages. Even purple prairie clover, which is considered a difficult to establish plant, established well in the monoculture treatment.

In the mixture plots (i.e., grass and legume), relatively equal proportions of grasses and legumes were achieved for meadow brome-alfalfa and meadow brome-sainfoin treatments. However, for birdsfoot trefoil and purple prairie clover, populations of these legumes were only 10% of the meadow bromegrass (Table 4).

Dry matter samples were harvested from these plots twice during the growing season. These samples are presently undergoing tannin analysis.

Table 4. Forage species plant density and plant height of tannin-containing species.

Treatment	Plant Species in mix	Plant density		Plant height – Oct 23
		Sept. 12	Oct. 23	
		plants m^{-2}		cm
Alfalfa		45	48	11
Birdsfoot trefoil		56	55	13
Sainfoin		56	70	10
Purple prairie clover		25	31	8
Meadow brome		78	99	26
Meadow brome / alfalfa mix	Meadow brome	29	44	19
	Alfalfa	40	40	9
Meadow brome / birdsfoot trefoil mix	Meadow brome	83	87	21
	Birdsfoot trefoil	8	7	10
Meadow brome / sainfoin mix	Meadow brome	49	51	20
	Sainfoin	27	34	11
Meadow brome / purple prairie clover mix	Meadow brome	49	57	21
	Purple prairie clover	4	7	5

Manure was also analysed for pathogens, as indicated in Table 5.

Table 5: Preliminary synopsis of pathogen identification in tannin and non-tannin containing forage plots with three manure treatments.

Forage Type	Block Number	Manure Treatment	Species Cultured from Soil + Manure Solution	Species Identified Through PCR Product Sequencing (Isolated from Soil + Manure Solution)
Sainfoin	1	Alfalfa	7	*Acinetobacter calcoaceticus*
		Sainfoin	8	*Rahnella sp.*
		Control	5	
	2	Alfalfa	7	
		Sainfoin	6	*Serratia plymuthica*
		Control	7	
	3	Alfalfa	8	
		Sainfoin	7	*Rahnella sp.*
		Control	6	*Serratia plymuthica*
	4	Alfalfa	9	*Shewanella putrefaciens*
		Sainfoin	7	*Serratia fonticola*
		Control	6	
Meadow Brome	1	Alfalfa	5	*Bacillus pumilus*
		Sainfoin	4	*Serratia sp.*
		Control	2	
	2	Alfalfa	7	
		Sainfoin	6	*Rahnella aquatilis*
		Control	4	*Serratia fonticola*
	3	Alfalfa	7	*Serratia sp.*
		Sainfoin	8	
		Control	5	
	4	Alfalfa	9	
		Sainfoin	7	*Serratia plymuthica*
		Control	3	

As indicated in Table 5 a range of proteobacteria were observed in the manure. We were expecting to see some of the classical gram negative pathogens typically associated with manure such as Escherichia coli and Salmonella spp. This did not occur but may be due to the simple medium we used for cultivation. The following bacteria were isolated:

Acinetobacter calcoaceticus: Acinetobacter spp are widely distributed in nature and is a common soil microorganisms. Acinetobacter species are generally considered nonpathogenic to healthy individuals. However, several species persist in hospital environments and cause severe, life-threatening infections in compromised patients. A. baumannii is the second most commonly isolated nonfermenting bacteria in human specimens

Rahnella spp: This is a common species of bacteria frequently isolated from soil. In some circumstance certain species can be pathogenic to humans and animals.

Serratia spp: Serratia are opportunistic gram-negative bacteria classified in the Enterobacteriaceae. Serratia marcescens is the only pathogenic species of Serratia, except for rare reports of disease resulting from infection with Serratia plymuthica, Serratia liquefaciens, Serratia rubidaea, and Serratia odorifera.

Shewanella spp: These are common species in nature and are not typically associated with pathogenic processes. They belong to the gram-negatives, and have the unique ability to grow anaerobically as well as aerobically and can utilize different minerals at various oxidation states to generate ATP.

Extension Activities:

Numerous extension activities have been conducted to highlight the results of the tannin research program at the University of Manitoba

Crop Diagnostic School - Carman, MB, 2 weeks in July, 2007. 380 professional agronomists.

Glenlea long-term rotation field day. July, 2007. 70 farmer and industry/extension participants

MP Tour – July 2007

Transition to organic workshop - Carman, MB Feb 21, 2008. 25 transitioning farmers

Natural systems agriculture - Invited presentation at Saskatchewan Soil Conservation Meeting, Regina, Feb 12, 2008. 200 farmers

Go Organic Conference. Invited set of presentations on sustainable organic grain and forage-beef systems. Camrose, AB March 4, 5, 2008

NCLE Field Tour – September 25, 2008.

Appendix 1

This appendix contains the field plan layout for the Carman and Glenlea field experiments. Both experiments are designed as randomized complete block studies. At Carman, the experiment is a factorial arrangement with growing forage species as the mainplot and manure type (alfalfa vs sainfoin) as the subplot. Manure was applied to the plots at a rate of 20 tons/acre in October, 2007.

The Glenlea site involves three manure rates. Both the alfalfa and sainfoin manure types were applied at 10, 20 and 30 tons/acre. Manure application at Glenlea took place in October, 2007. The plant species at Glenlea is forage rye.

==> North

Block 4

Sainfoin	Alfalfa	Alfalfa	Alfalfa		Alfalfa		Alfalfa	Alfalfa
Alfalfa		Sainfoin		Alfalfa		Sainfoin	Sainfoin	Sainfoin
	Sainfoin		Sainfoin	Sainfoin	Sainfoin	Alfalfa		
6	**4**	**2**	**9**	**5**	**1**	**3**	**7**	**8**

Block 3

	Alfalfa	Sainfoin		Alfalfa	Sainfoin			Sainfoin
Sainfoin			Alfalfa	Sainfoin	Alfalfa	Alfalfa	Sainfoin	Alfalfa
Alfalfa	Sainfoin	Alfalfa	Sainfoin			Sainfoin	Alfalfa	
2	**3**	**8**	**4**	**7**	**6**	**5**	**1**	**9**

Block 2

Sainfoin		Alfalfa	Sainfoin	Alfalfa	Alfalfa	Alfalfa	Sainfoin	Sainfoin
Alfalfa	Sainfoin	Sainfoin	Alfalfa	Sainfoin		Sainfoin		Alfalfa
	Alfalfa				Sainfoin		Alfalfa	
5	**9**	**6**	**1**	**3**	**8**	**7**	**2**	**4**

Block 1

Alfalfa	Sainfoin	Sainfoin	Alfalfa			Sainfoin	Alfalfa	Sainfoin
	Alfalfa	Alfalfa	Sainfoin	Sainfoin	Alfalfa	Alfalfa	Sainfoin	Alfalfa
Sainfoin				Alfalfa	Sainfoin			
1	**7**	**4**	**8**	**9**	**2**	**5**	**6**	**3**

Key

1	Alfalfa	**6**	Alfalfa + Meadow Brome
2	Birdsfoot Trefoil	**7**	Birdsfoot Trefoil + Meadow Brome
3	Sainfoin	**8**	Sainfoin + Meadow Brome
4	Purple Prairie Clover	**9**	Purple Prairie Clover + Meadow Brome
5	Meadow Brome		

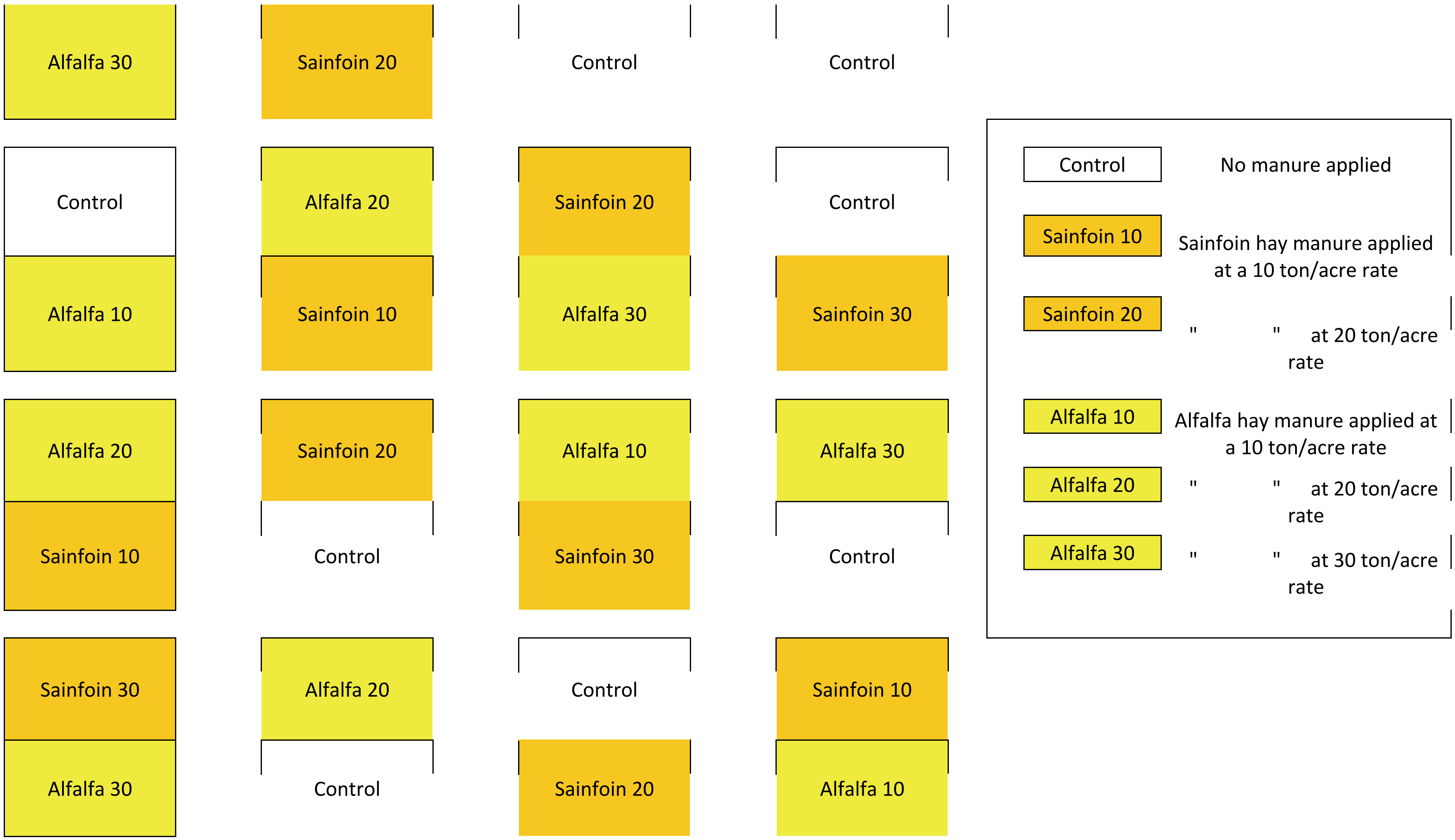
Sainfoin	- Sanfoin Hay Manure App. 20 tons/ac.
Alfalfa	- Alfalfa Hay Manure App. 20 tons/ac.
	- No Manure (Control)

Sainfoin 10

Sainfoin 30

Alfalfa 20

Alfalfa 10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Sainfoin 20
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Alfalfa 10
Control
No manure applied
Sainfoin 10
Sainfoin hay manure applied at a 10 ton/acre rate
Sainfoin 20
" " at 20 ton/acre rate
Alfalfa 10
Alfalfa hay manure applied at a 10 ton/acre rate
Alfalfa 20
" " at 20 ton/acre rate
Alfalfa 30
" " at 30 ton/acre rate