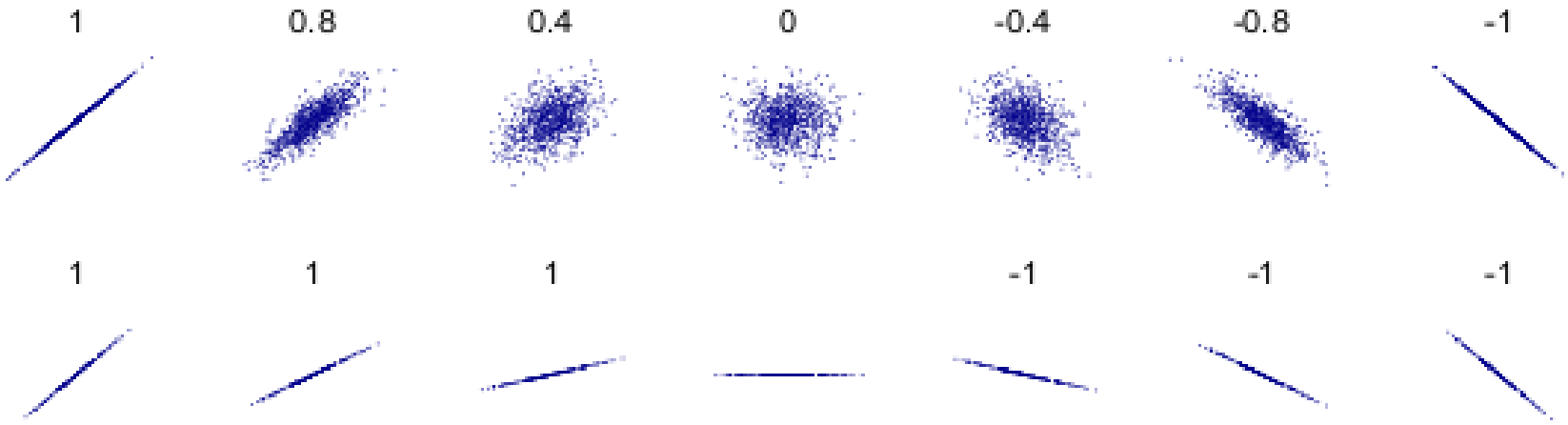
Practice Assignment

Tutorial 5

Tips: How to read correlations

The *r* number gives info on how related the two values are, while the plus or minus indicate direction. *P*-value determines significance.

Smaller the *r*, the greater the dispersion of the data. Generally only need to know direction and significance.



Study

Female Sprague-Dawley rats were treated with a colon-specific carciongen, DMH, or saline as a control group.

DMH has a stimulating effect on cell proliferation that lasts for a number of weeks.

Rats were subsequently fed either a high sucrose diet (46g/kg) or a high corn starch diet (46g/kg) for 30 days or 105 days.

At the end of the 30 or 105 days of feeding, a series of indices of cecal function were evaluated, as summarized in Table 1.

As well, the cecal and fecal content of total and individual SCFAs were evaluated (Figure 1,2, and Table 2).

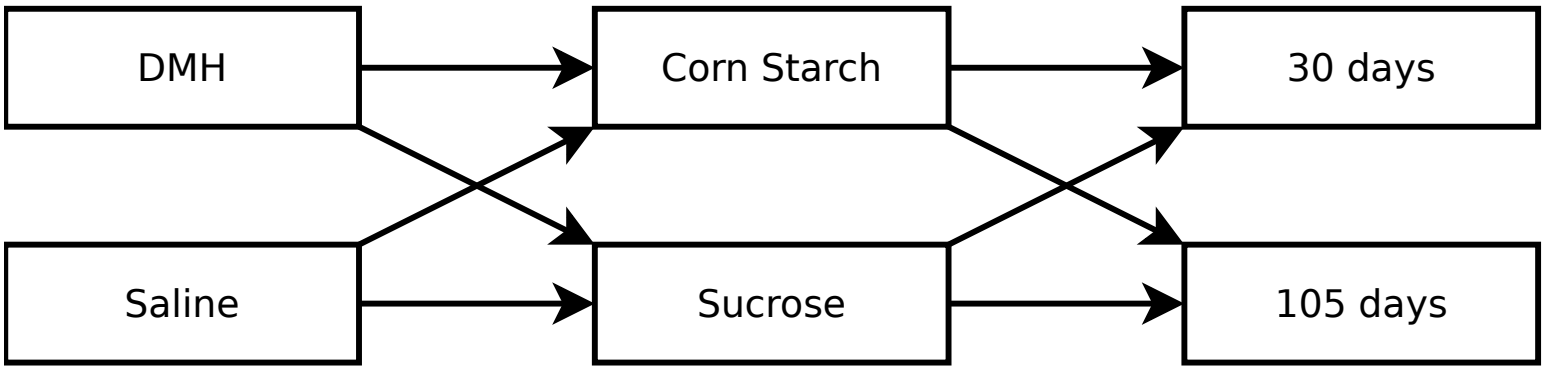
Study, con't

In these same rats, the proliferative activity of cells in the rectal mucosa was assessed by measuring the number of 3H-thymidine-labelled cells (Figure 3).

Proliferative activity in the colon was expressed as the number of labeled cells (LC)/crypt.

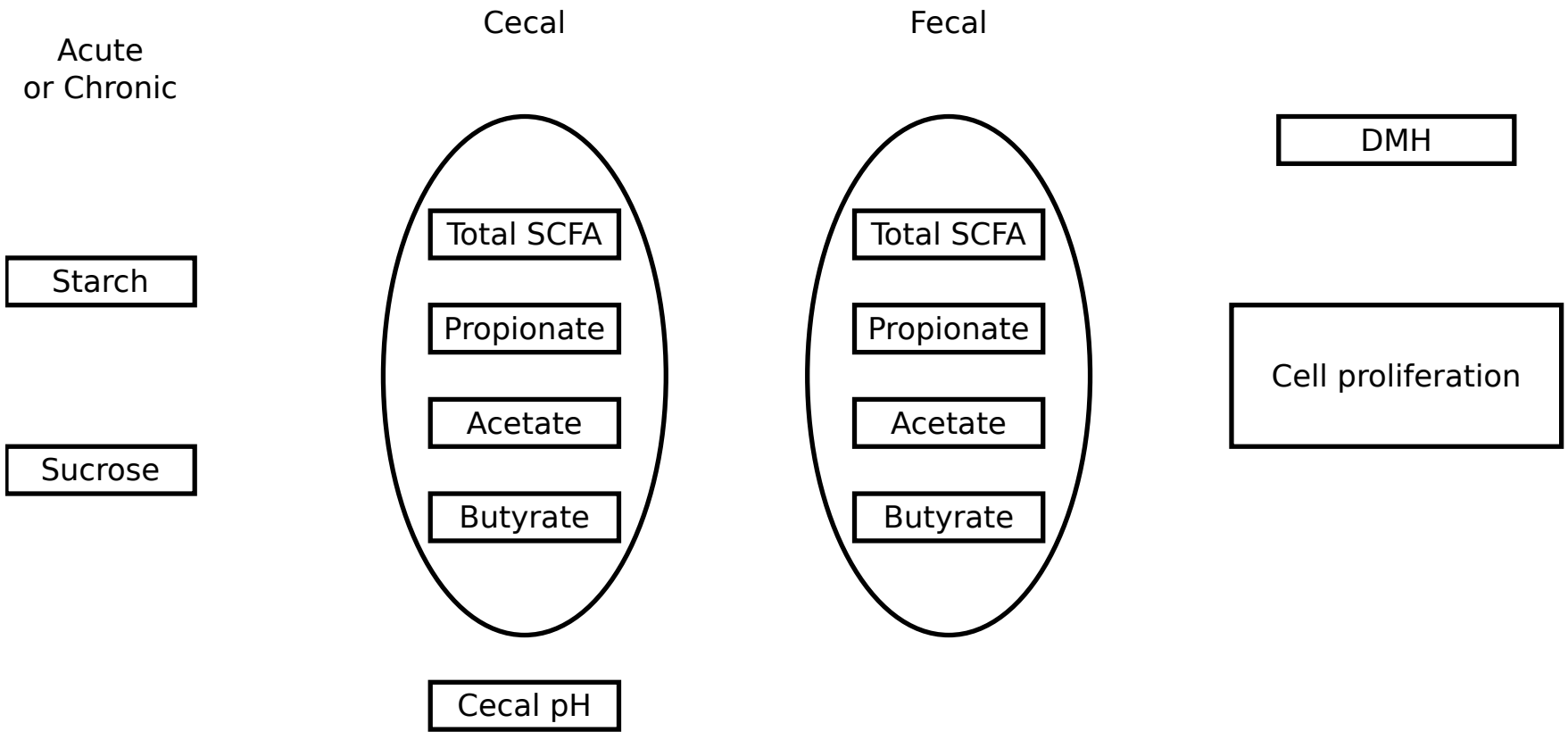
By linear regression analysis, rectal proliferation data was then compared to variations in cecal SCFAs at 105 days after feeding diets (Table 3).

Study Design



Cecal function
Total, Individual SCFA
# of labelled rectal cells

What is everything that is going on?



Question 1

Describe the effects of sucrose and starch-rich diets on cecal function (Table 1), cecal and fecal concentrations of SCFAs (Figures 1 & 2, and Table 2). (5)

Table 1 - Cecal variables from rats fed different diets for 30d or 105d[1]

	30d		105d	
	Sucrose	Starch	Sucrose	Starch
Wet cecal sac weight (g)	0.68+/-0.04	0.58+/-0.02	0.82+/-0.06	0.71+/-0.04
Wet cecal content weight (g)	1.31+/-0.09	1.17+/-0.08	1.61+/-0.14	1.47+/-0.11
Cecal pH	7.28+/-0.08	7.19+/-0.07	7.37+/-0.04	7.16+/-0.03[a]

[1] Values are means +/-SEM for 12 (30d) or 15 (105d) rats

[a] $p<0.01$ compared with the sucrose diet

Q1, con't

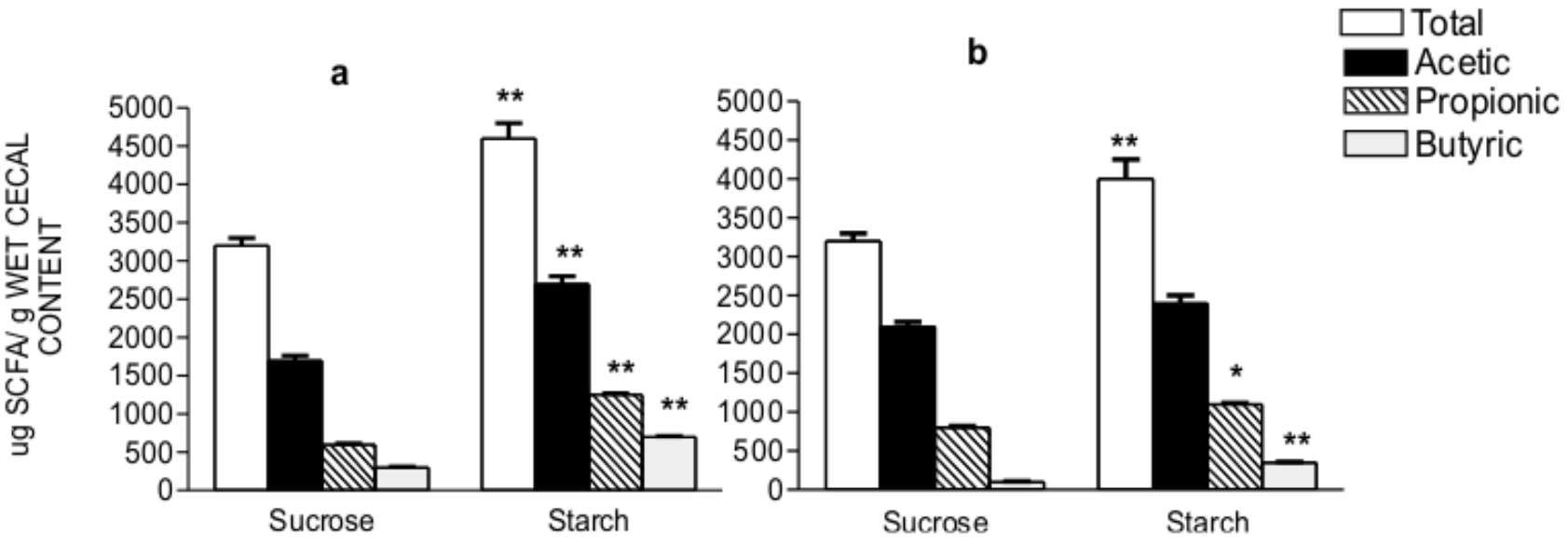


Figure 1- Concentration of SCFA (ug/g) in the wet cecal content of rats fed difference diets for 30d (panel a, n=12) and 105 d (panel b, n=15). Values are means ± SEM.

*P<0.05 as compared with the sucrose diet.

**P<0.01 as compared with the sucrose diet.

Q1, con't

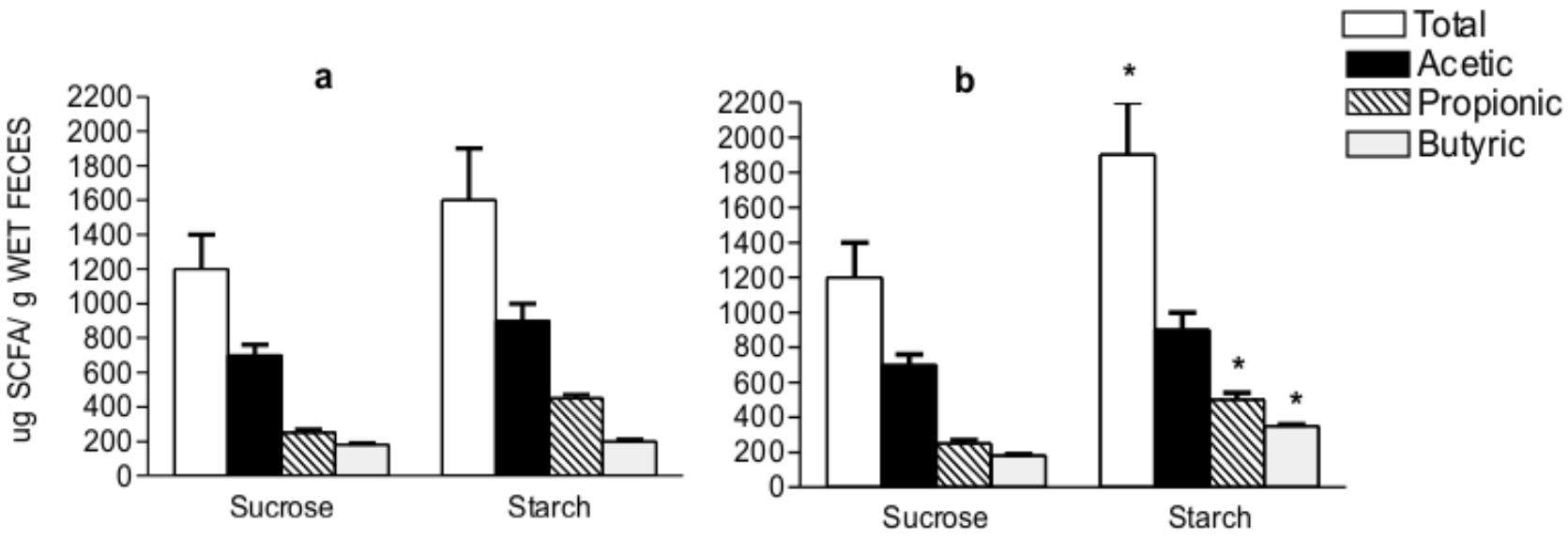


Figure 2- Concentration of SCFA (ug/g) in the wet feces of rats fed difference diets for 30d (panel a, n=12) and 105 d (panel b, n=15). Values are means ± SEM.
*P<0.05 as compared with the sucrose diet.

Q1, con't

Table 2 - Percentage of individual SCFAs in the cecal content of rats fed different diets for 30 or 105 d[1]

	30d		%	105d	
	Sucrose	Starch		Sucrose	Starch
Acetic	58.1+/-1.1	58.3+/-1.3		62.3+/-1.0	58.4+/-0.8[a]
Propionic	22.3+/-1.1	23.3+/-0.9		25.2+/-0.4	24.7+/-0.5
Butyric	12.3+/-0.9	13.3+/-0.9		5.1+/-0.8	9.0+/-0.4[a]
Acetic/Butyric	5.0+/-0.4	4.1+/-0.4		14.7+/-1.7	8.3+/-0.4[a]

[1] Values are means +/-SEM for 12 (30d) or 15 (105d) rats

[a] $p<0.01$ compared with the sucrose diet

Q2, con't

Discuss a mechanism to explain why the cecal and fecal content of total and individual SCFAs may differ in rats fed sucrose and starch-rich diets for 105 days? (10)

Q2, con't

Possible A:

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Digestion/Absorption differences:

Sucrose is a simple sugar and would have very efficient digestion & absorption.

Q2, con't

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Sucrose is a simple sugar and would have very efficient digestion & absorption.

Starch is a complex polysaccharide, has a slower digestion process and would reach the colon allowing for greater bacterial fermentation and greater production of SCFA (Fig 1: ↑ total production of SCFAs at 30 & 105d, Table 1: ↓ pH on starch at 105 d)

Q2, con't

Differences in Colonic bacterial populations:

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Starch feeding results in significant differences in proportion of individual SCFAs produced in cecum at 105 days suggesting the stimulation of specific bacterial populations at this time. (Table 2)

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By 105 days, bacteria are very efficient at producing SCFA (see sig. ↓ pH at this time) and the absorptive capacitiy of the cecum has been exceeded, resulting in significant excretion of SCFAs in feces (Fig 2).

Q3a

Describe the effects of sucrose and starch-rich diets on proliferative activity in the rectal mucosa of control and DMH-treated rats fed the diets for 30 and 105 days (Figure 3) (5)

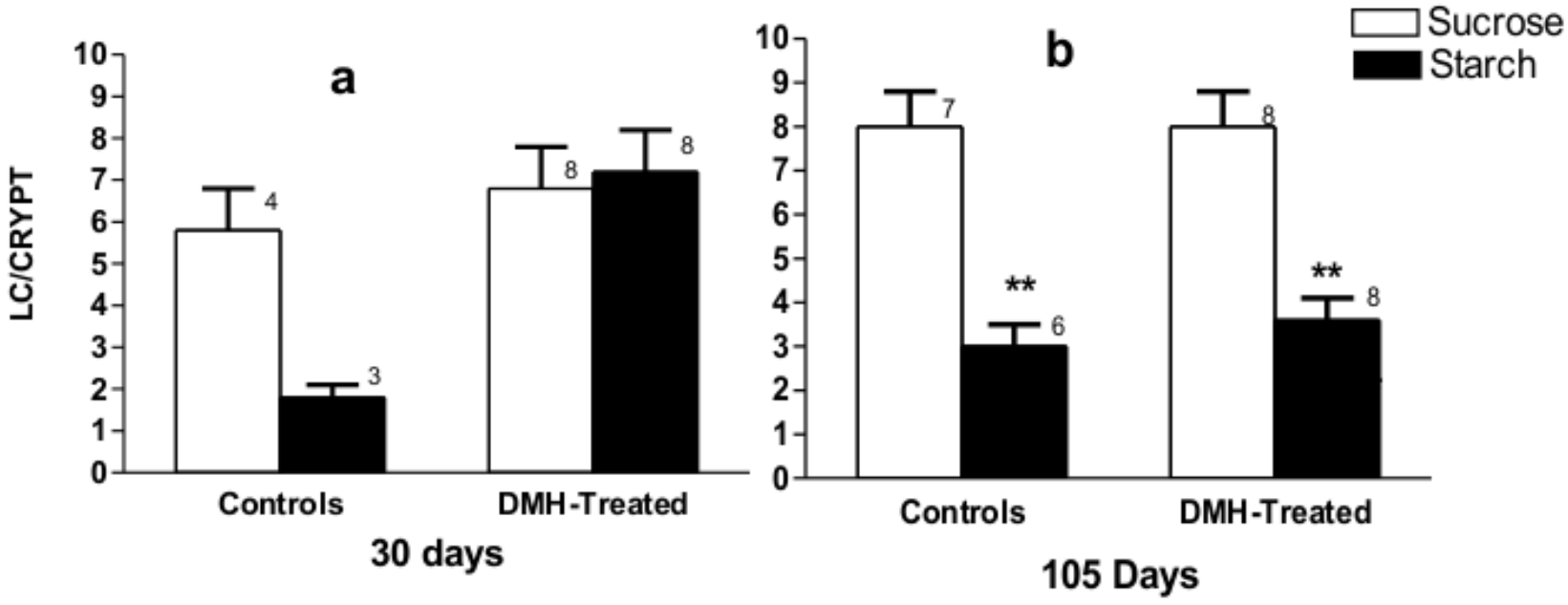

Figure 3- Proliferative Activity [labelled cells (LC)/crypt] in rectal mucosa of rats fed different diets for 30d (panel a) and 105 d (panel b). Numbers above bars represent # of animals in each treatment group.
**P<0.01 as compared with the sucrose diet.

Q3b

Describe the correlations between colonic proliferative activity in rats and SCFA concentrations in rats fed sucrose and starch-rich diets for 105d (Table 3). (5)

Table 3 - Parameters of Linear Correlation between Proliferative Activity (LC/crypt) in the rectum and variations in Cecal SCFAs 105d after feeding starch or sucrose diets[1]

	Labelled cells/crypt	
	r	P
Total SCFA concentraton	-0.14	0.47
Acetate Concentration	-0.08	0.69
Butyrate Concentration	-0.65	0.00011
Acetate/total SCFA, %	0.64	0.00018
Butyrate/total SCFA, %	-0.71	0.00001
Acetate/Butyrate	0.63	0.00024
Cecal pH	0.46	0.011

1 Correlation Coefficient was calculated by Pearson Correlation

r = correlation coefficient and P = probability values

Q4

Based on all of the data presented, discuss a mechanism for the effect of dietary carbohydrates on colonic proliferative activity? (10).

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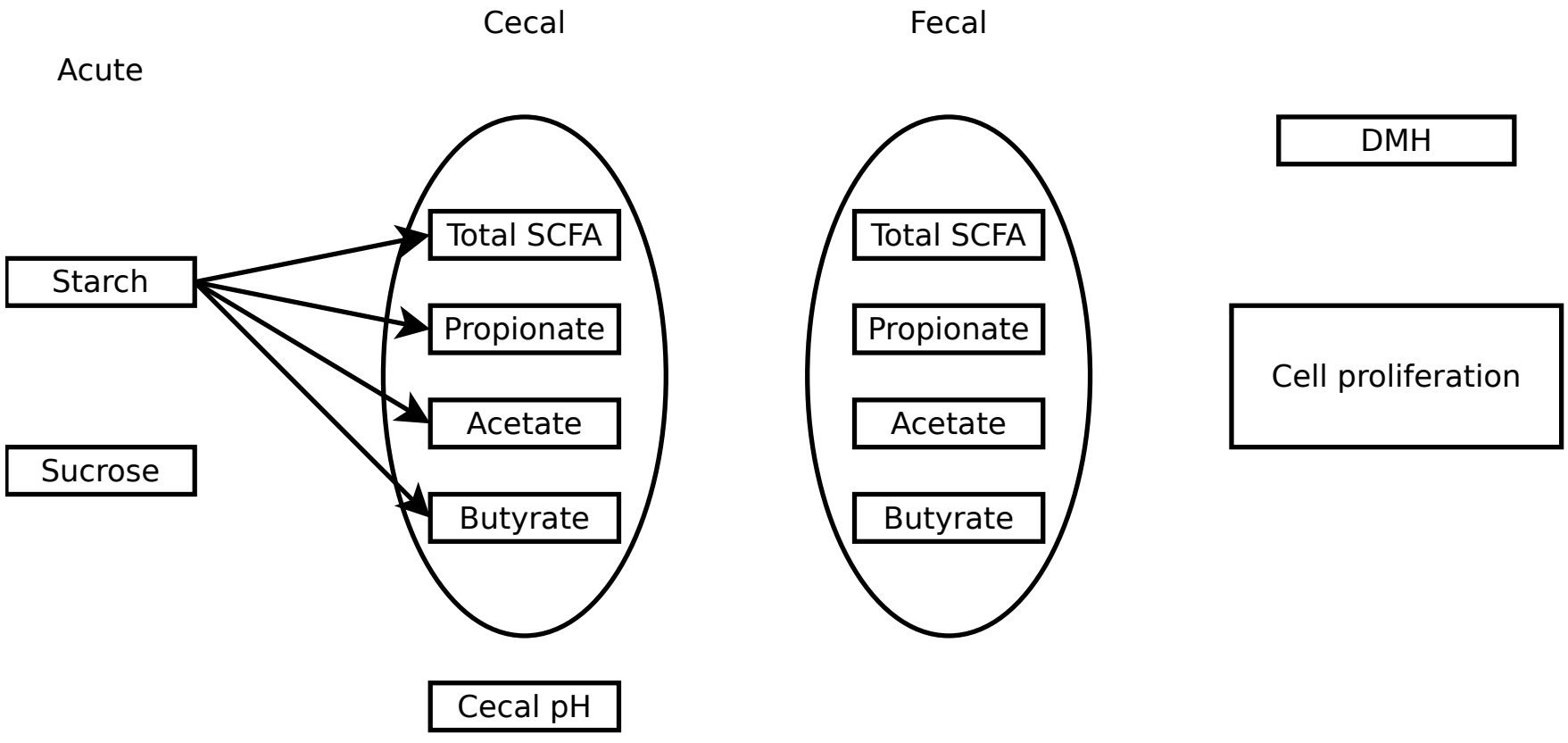
First, use the diagram to work through what is going on.

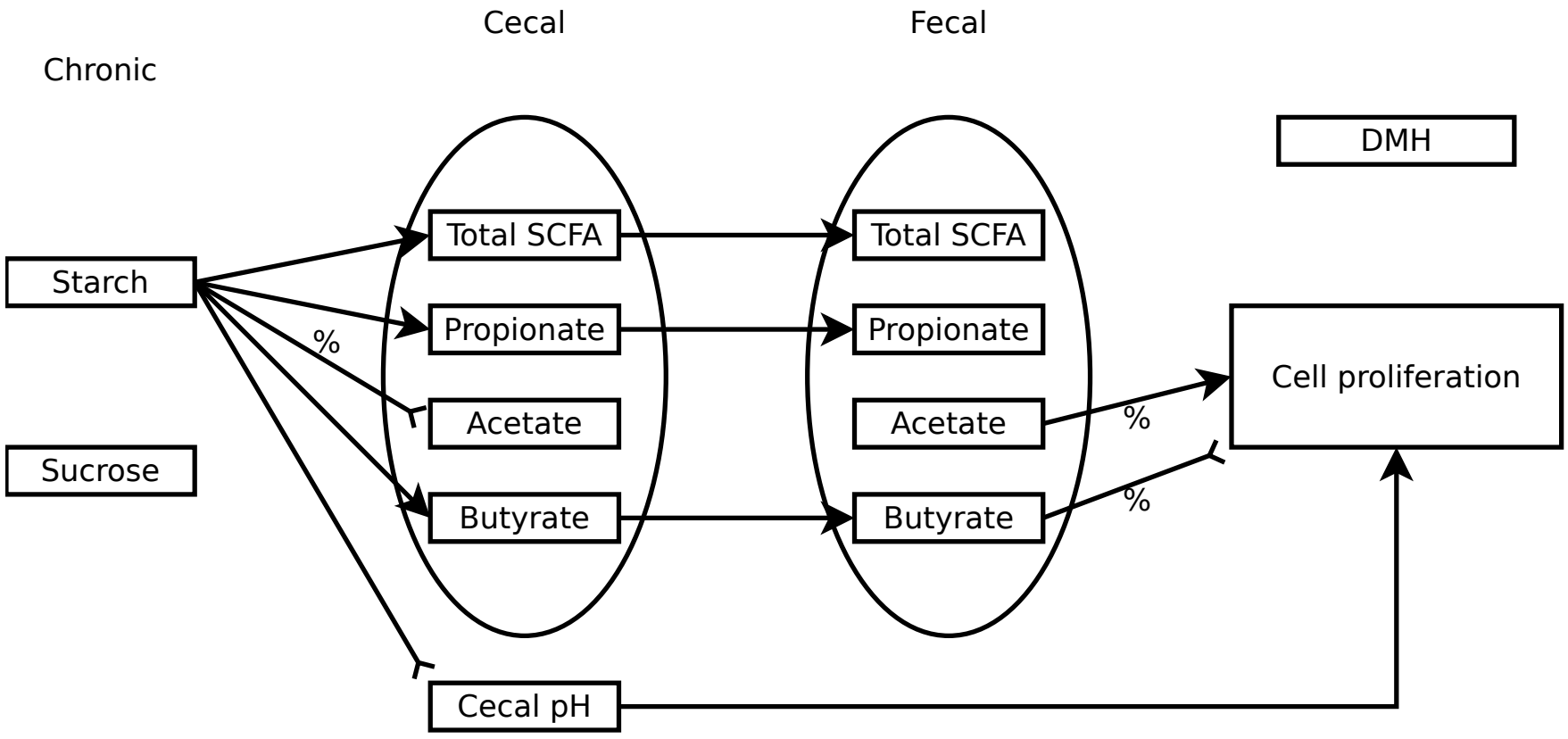
Q4, Short-term (Acute) feeding

Arrow = increase



Q4, Long-term (Chronic) feeding

Arrow = increase, inverse arrow = decrease



Q4, con't

Possible A:

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High starch diet vs high sucrose diet $\rightarrow \uparrow$ SCFA production in the gut (Fig. 1)

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SCFA's $\downarrow$ the pH of the gut (Table 1) and may in turn affect microfloral balance and $\uparrow$ in beneficial, protective bacteria (Table 2 shows different proportions of SCFA)

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High starch diet vs high sucrose diet $\rightarrow \uparrow$ SCFA production in the gut (Fig. 1)

SCFA's $\downarrow$ the pH of the gut (Table 1) and may in turn affect microfloral balance and $\uparrow$ in beneficial, protective bacteria (Table 2 shows different proportions of SCFA)

Supported in table 3 showing a significant positive correlation between cecal pH and proliferative activity.

Q4, con’t

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High starch diet = different proportions of SCFAs in cecum ↑ Butyrate concentration & proportion and ↓ proportion of acetate & acetate:butyrate ratio (Table 2)

High starch diet = different proportions of SCFAs in cecum Butyrate concentration and percentage are both significantly negatively correlated with proliferative activity in the rectum (Table 3).

Q4, con't

Butyrate is preferred energy source for colon; helps repair cells and improve colonocyte maintenance. *(Note: this information wasn't given so we wouldn't expect it in the answer)*

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Butyrate is preferred energy source for colon; helps repair cells and improve colonocyte maintenance. *(Note: this information wasn't given so we wouldn't expect it in the answer)*

High starch diet = different proportions of SCFAs in cecum SCFA's (esp. butyrate) may inhibit colon cancer by inhibiting cell proliferation (Figure 3) *(Can't say this conclusively as no difference in rectal proliferation between saline and DMH-treated groups)*

Q5

Discuss the purpose of 2 experimental periods in the study (i.e. 30d & 105d)

Possible A:

Adaptation (how long does it take to see changes in bacterial populations?)

Acute vs chronic effects

A number of answers may be given as long as they are logical and can be substantiated with data.