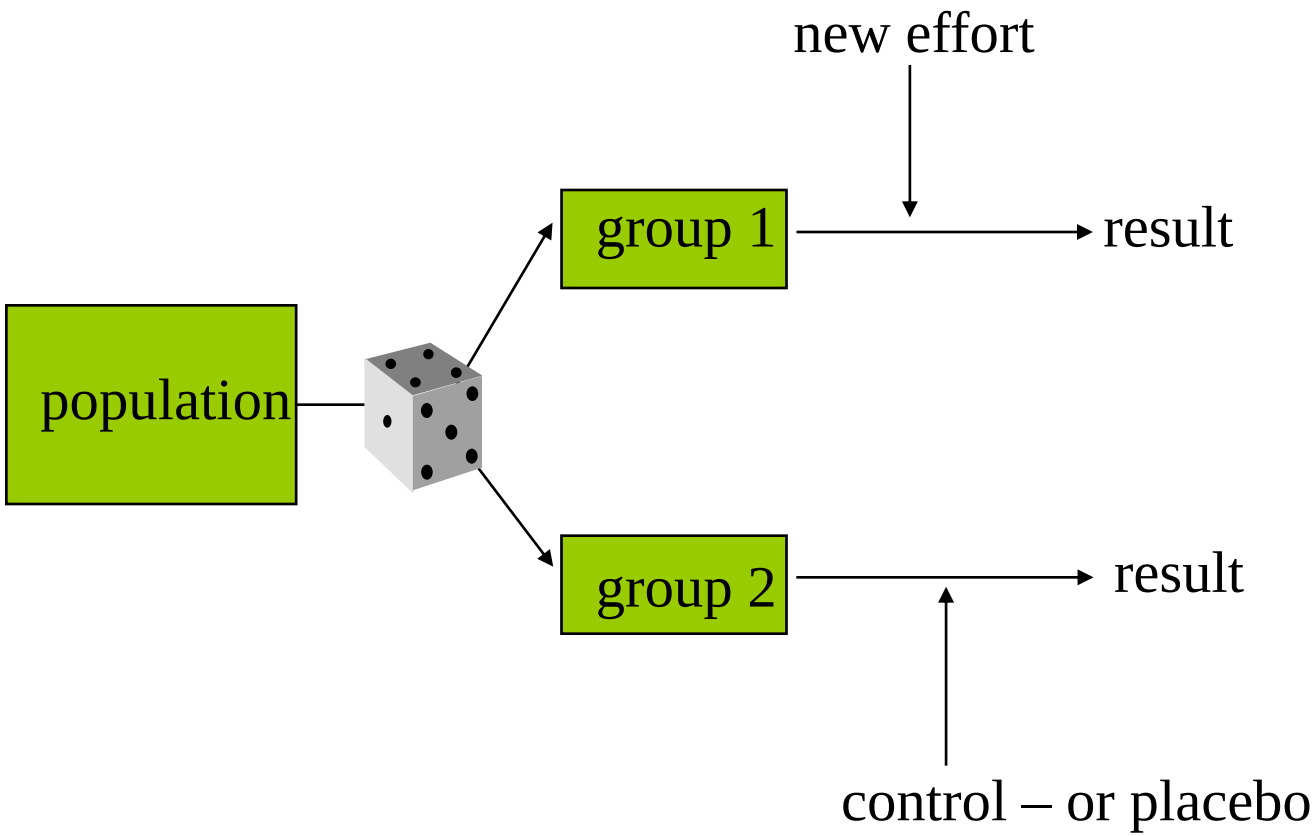
FROM VISION TO DECISION

Design of experiments

ragnar.nortvedt@helse-bergen.no

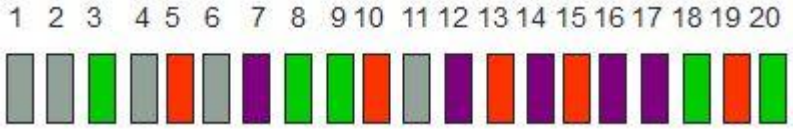
Randomized controlled trial (RCT)



Blinded – the patients do not know which group they belong to

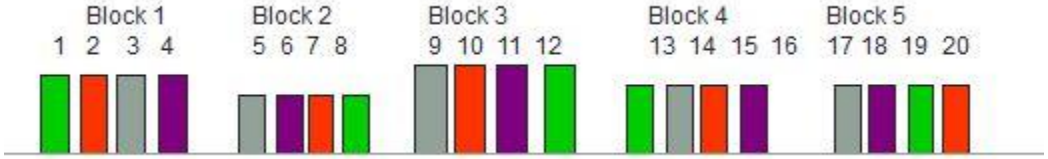
Double blinded – neither the patients nor the researchers know which group the patients belong to

The completely randomised design:



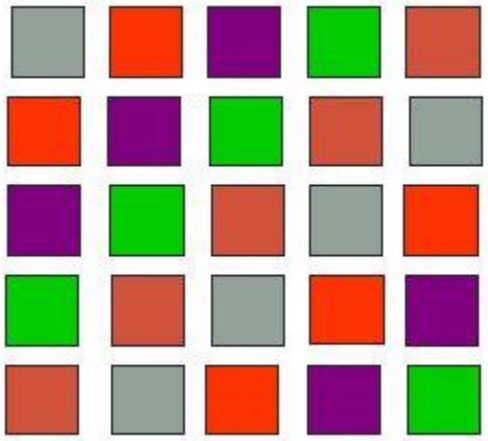
-each experimental unit is assigned to a treatment strictly at random without taking account of any individual characteristics
-demands relatively homogeneous experimental units

The randomised block design:



-used to control a source of random variation which might otherwise obscure the effect of a treatment
-it is assumed that differences between treatments *are* of interest while differences between blocks, which are random effects are of *no* interest

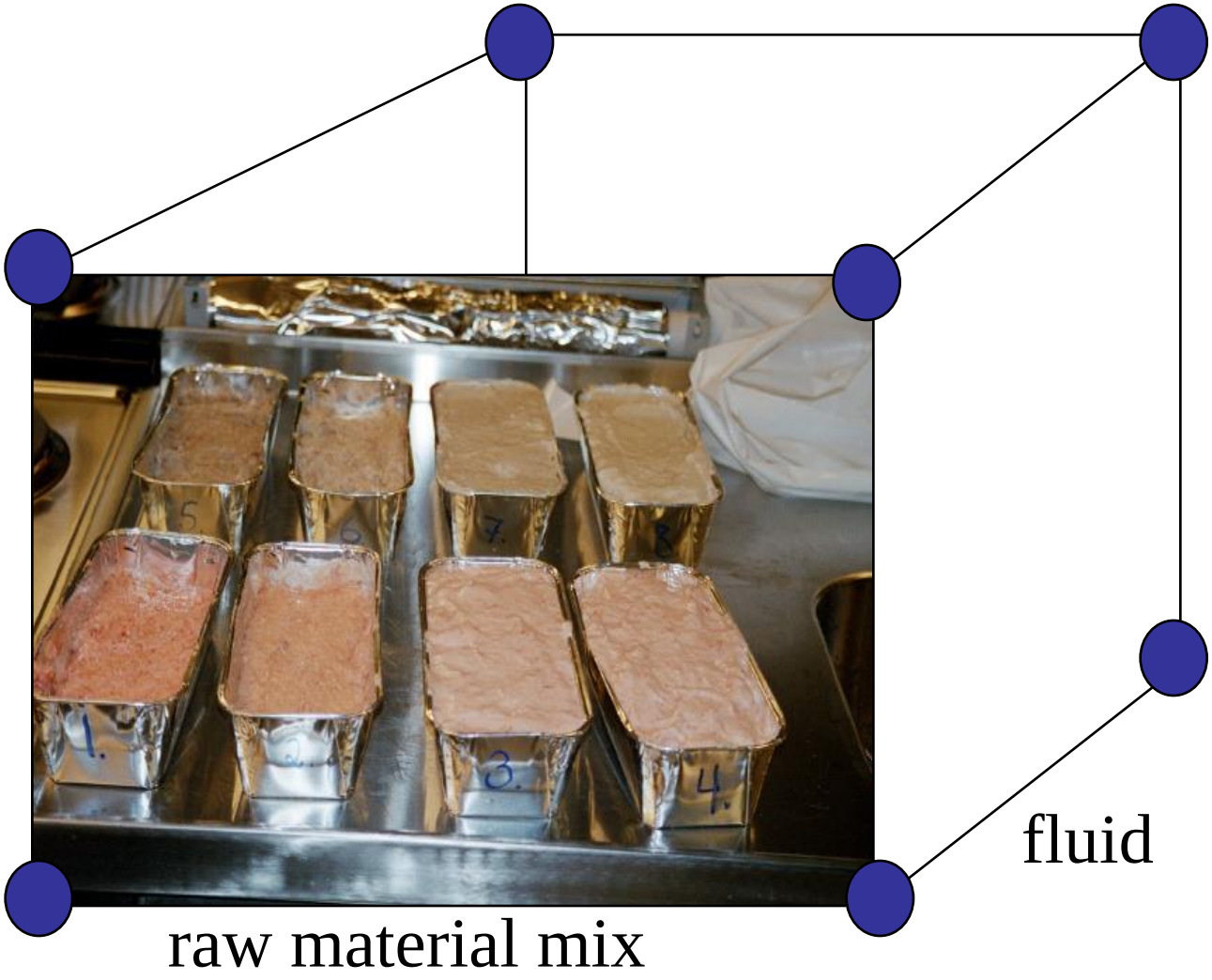
The Latin square design:



-treatments (colours) are squared (e.g. 5 x 5 here)
-rows might be working days of the week
-columns might be time of the day
-randomise whole rows and then whole columns
-best suited for exp. with 4 – 7 treatments

Factorial design:

Experimental design: fish pudding



Coded set-up and the taste response (Y1):

TESTS:	Potato starch	Milk	-1=Saith +1=Salmon	TASTE (Y1)
Test 1	-1	-1	1	8,3
Test 2	1	-1	1	6,3
Test 3	-1	1	1	3,8
Test 4	1	1	1	5,5
Test 5	-1	-1	-1	4
Test 6	1	-1	-1	2,8
Test 7	-1	1	-1	2,3
Test 8	1	1	-1	5,5

Preparation from the receipes

Process

Modelling:

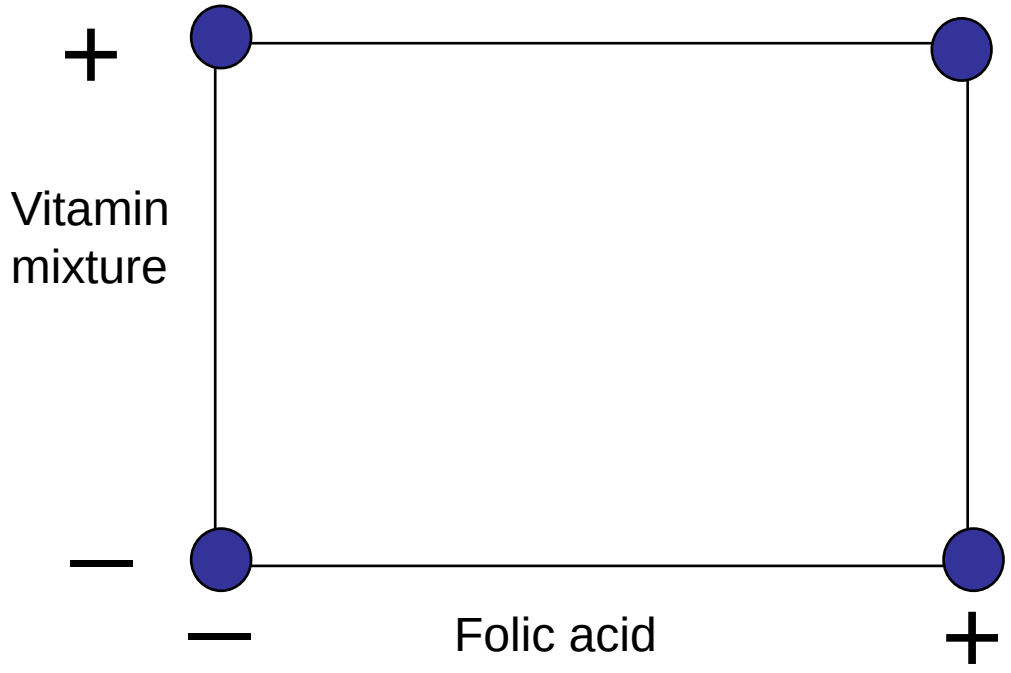
$$Y = b0 + b1\,X1 + b2\,X2 + \ldots\ldots + b1b2X1X2 + \ldots\ldots + \text{error}$$

$$\text{Taste} = 4{,}8 + 0{,}2\ \text{potato starch} - 0{,}5\ \text{milk} + 1{,}2\ \text{salmon}$$

Ref: MRC, 1991, Lancet, 338: 131-137.

Prevention of neural tube defects in the fetus by vitamins

A randomised double-blind prevention trial based on factorial design was conducted at 33 centres in seven countries to determine whether supplementation of folic acid or a mixture of seven other vitamins around the time of conception can prevent neural tube defects.



A total of 1817 women at high risk were allo-cated at random to one of the four groups

Ref: MRC, 1991, Lancet, 338: 131-137.

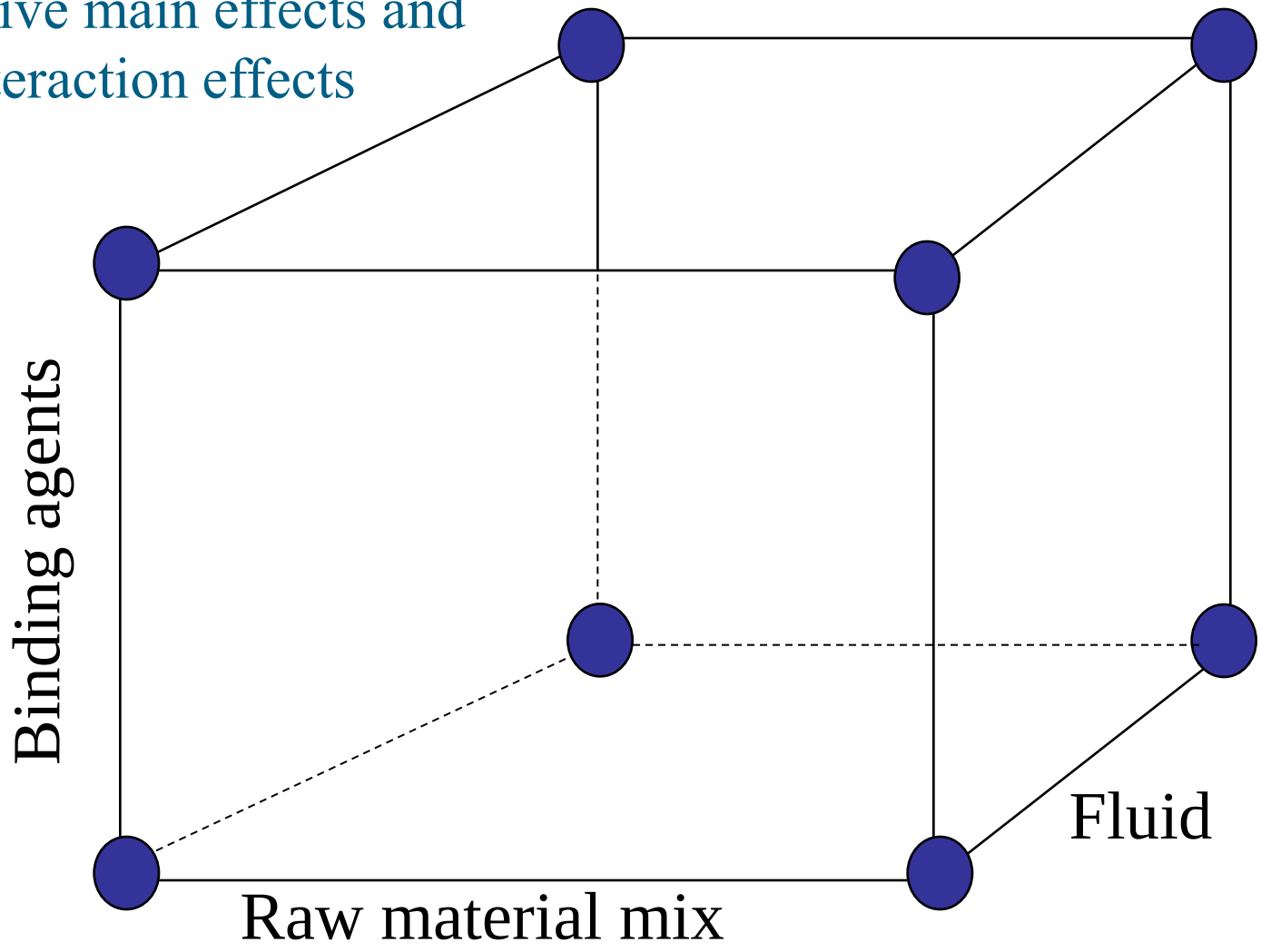
Results

Randomisation group			All women		
	Folic acid	Other vitamins	NTD/all		Relative risk: folic acid *vs* non-folic acid (95% CI)
A	+	−	2/298	6/593 (1·0%)	0·28 (0·12–0·71)
B	+	+	4/295		
C	−	−	13/300	21/602 (3·5%)	
D	−	+	8/302		

- Women given folic acid showed a 1,0 % risk for NTD in fetus
- Women in the other groups showed a 3,5% risk for NTD in fetus
- Women in the vitamin mix group showed no sign.diff. from other groups
- Folic acid supplementation before pregnancy is recommended

Full factorial design

-Give main effects and
Interaction effects



Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction

A randomised controlled trial with a factorial design (2^3) was done to examine the effects of dietary intervention in the secondary prevention of myocardial Infarction (MI).

2033 men under 70 years, admitted to 21 hospitals, and who had recovered from MI were allocated to receive or not to receive advice on each of the three dietary factors.

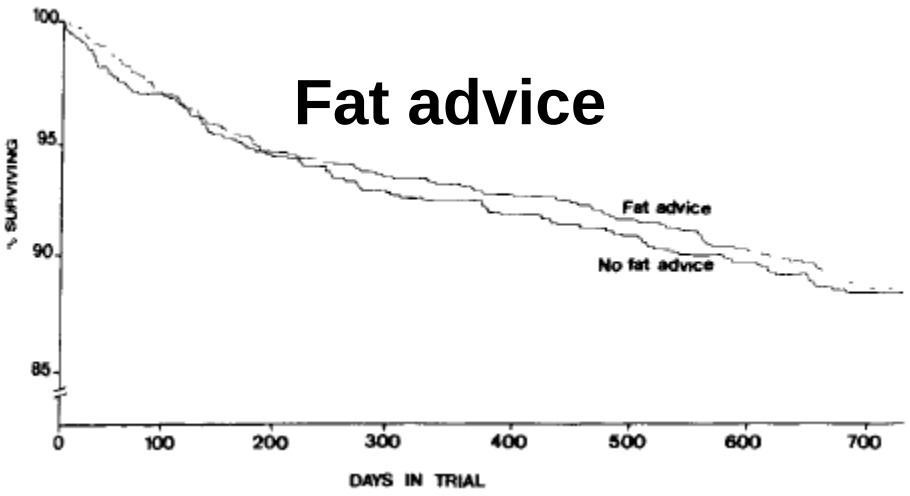
	Dietary intake At 6 months	Dietary intake At 2 years
% fat energy		
Fat advice	32·1 (6·0, 937)*	32·3 (5·9, 869)
No fat advice	35·3 (5·9, 942)	35·0 (5·8, 876)
P/S ratio		
Fat advice	0·78 (0·30, 937)	0·78 (0·32, 869)
No fat advice	0·40 (0·23, 942)	0·44 (0·25, 876)
EPA (g per week)		
Fish advice	2·3 (1·3, 947)	2·4 (1 4, 883)
No fish advice	0·7 (0·7, 932)	0 6 (0·7, 862)
Cereal fibre (g per day)		
Fibre advice	19 (8, 926)	17 (8, 849)
No fibre advice	9 (5, 953)	9 (5, 896)

*Mean (SD, no of subjects).
P/S = polyunsaturated / saturated ratio of fatty acids intake

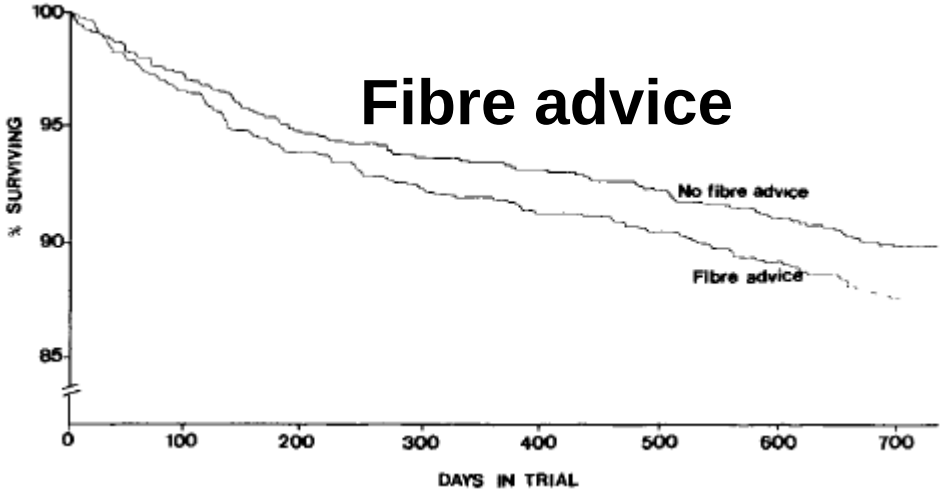
Ref. Burr, 1989, Lancet, Sept.: 757-761.

Survival

Fat advice

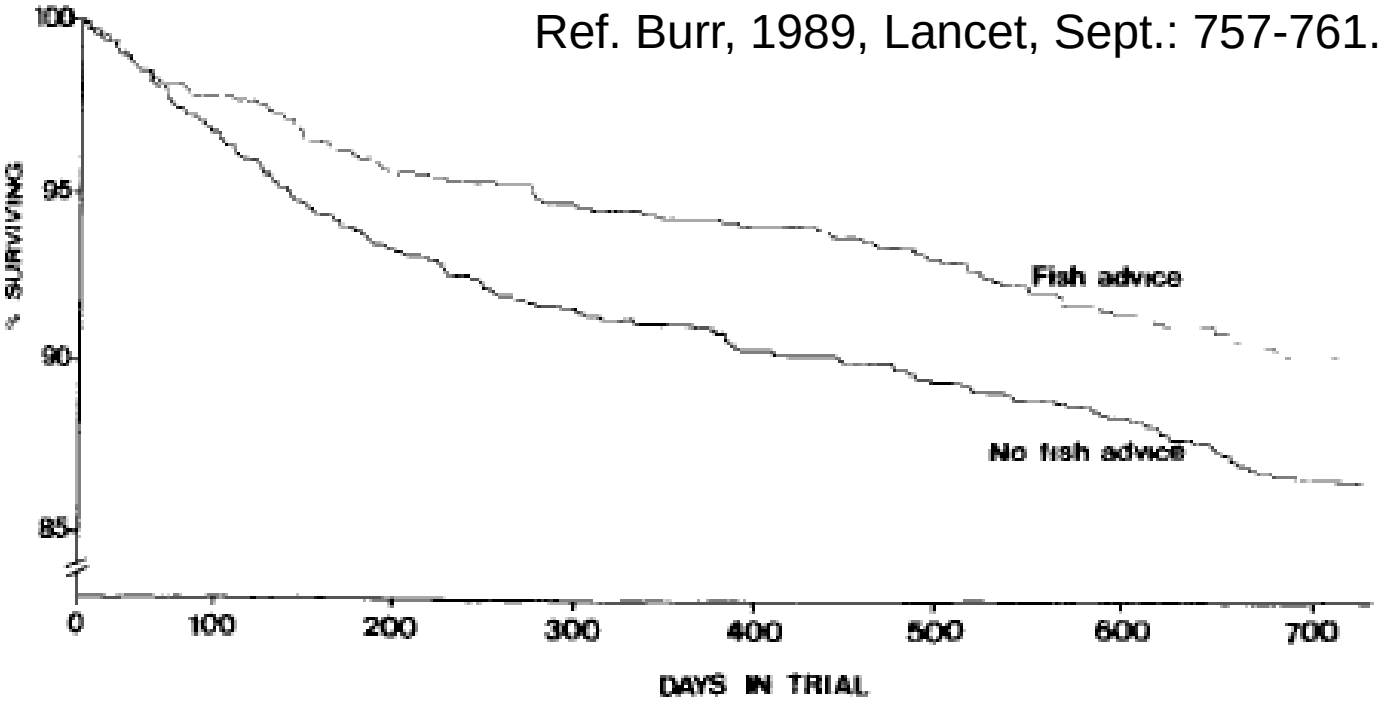


Fibre advice



Ref. Burr, 1989, Lancet, Sept.: 757-761.

Fish advice



Results

Diet group	All deaths
Fat advice	111 (10·9%)
No fat advice	113 (11·1%)
Fish advice	94 (9·3%)*
No fish advice	130 (12·8%)
Fibre advice	123 (12·1%)
No fibre advice	101 (9·9%)

The subjects advised to eat fatty fish had a 28 % reduction in 2 year all-cause mortality compared with those not so advised.

Ref. Burr, 1989, Lancet, Sept.: 757-761.

ELSEVIER

Chemometrics and Intelligent Laboratory Systems 29 (1995) 271–282

Chemometrics and intelligent laboratory systems

Example: Fish

Multivariate evaluation of feed for Atlantic halibut

Ragnar Nortvedt [a], Stig Tuene [b]

Table 1
Experimental design. The order of the experimental units was randomized. The first three variables (vars.) were varied according to 2^3 FD, whereas the other four vars. show the actual diet composition (in % of dry matter). Var. No. 2 is a qualitative description of floating feed (+1), sinking feed (−1) or a mix of the two types (0). Tank Nos. 1, 2, 5 and 7 represent the replicated center points in the design

Tank No.	Design variables			Diet composition (%)			
	1: (%) dry matter	2: floating vs. sinking	3: pellet size (mm)	4: carbo-hydrate	5: ash	6: protein	7: fat
9	95.0	+1	3.0	10.2	13.7	53.6	22.5
4	96.1	+1	8.0	8.9	14.2	53.3	23.6
10	95.6	−1	3.5	7.6	11.8	58.7	21.9
3	94.3	−1	7.0	8.2	11.3	53.4	27.1
11	32.2	+1	4.0	11.3	14.0	53.4	21.3
6	36.3	+1	9.0	8.8	13.0	55.3	22.9
12	32.3	−1	3.5	10.0	13.6	55.6	20.8
8	32.3	−1	9.0	10.9	12.6	55.1	21.4
1	63.6	0	5.0	10.8	12.2	54.1	22.9
2	63.6	0	5.0	10.8	12.2	54.1	22.9
5	63.6	0	5.0	10.8	12.2	54.1	22.9
7	63.6	0	5.0	10.8	12.2	54.1	22.9

Results

Table 4
Explained variance in PLS response models of the gross feed conversion efficiency (GFCE), protein efficiency ratio (PER), protein productive value (PPV), fat efficiency ratio (FER), fat productive value (FPV) and neutralized fat productive value (FPV_n) in the periods P_1 (2.07–7.08) and P_2 (10.08–5.09) and in the total experimental period (Tot: 12.06–5.09.93). The models were based upon the designed variables and their cross terms. The weighted regression coefficients of the significant variables are shown according to: $Y = \beta 0 + \beta 1 \times \text{dry matter} + \beta 2 \times \text{floating feed} + \beta 3 \times \text{pellet size} + \beta 4 \times (\text{dry matter})^2 + \beta 5 \times (\text{dry} \times \text{floating}) + \beta 6 \times (\text{dry} \times \text{pellet size}) + \beta 7 \times (\text{floating})^2 + \beta 8 \times (\text{floating} \times \text{size}) + \beta 9 \times (\text{pellet size})^2$

Response	Variance (%)	$\beta 0$	$\beta 1$	$\beta 2$	$\beta 3$	$\beta 4$	$\beta 5$	$\beta 6$	$\beta 7$	$\beta 8$	$\beta 9$
$GFCE_{P1}$	21.6	0.946	0.001	–	0.019	–	–	3.2×10^{-4}	–	–	0.001
$GFCE_{P2}$	72.2	1.014	–	0.018	0.018	–	1.9×10^{-4}	2.0×10^{-4}	–	0.003	0.001
$GFCE_{tot}$	51.5	0.856	–	–	0.023	–	–	1.5×10^{-4}	0.054	–	0.002
PER_{tot}	52.7	1.571	–	–	0.042	–	–	2.8×10^{-4}	0.086	–	0.003
PPV_{tot}	36.0	0.166	7.1×10^{-4}	0.014	0.005	5.0×10^{-6}	1.7×10^{-4}	1.0×10^{-4}	–	0.002	4.2×10^{-4}
FER_{tot}	44.7	4.413	−0.003	–	0.086	-1.5×10^{-5}	–	–	0.274	–	0.008
FPV_{tot}	83.5	−0.287	1.5×10^{-3}	–	–	4.3×10^{-6}	–	2.5×10^{-4}	0.056	–	–
FPV_n	58.3	3.449	−0.005	–	–	-4.1×10^{-5}	–	-7.5×10^{-4}	–	–	–

A few conclusions:

- The systematic variation in the designed parameters influenced fat retention stronger than growth rate over the total experimental period
- Pellet size (var. No. 3) and the combined effect from pellet size and high dry matter content (var. Nos. 1x3) were the most significant positive parameters upon GFCE and protein utilization
- High dry matter content (var. No. 1) and the combined effect from pellet size and high dry matter content (var. Nos. 1x3) were most important to fat utilization
- Future fish feeds should consequently maximize the parameters above, within biological reasonable limits, to achieve the best utilization of the feed

Example mice:

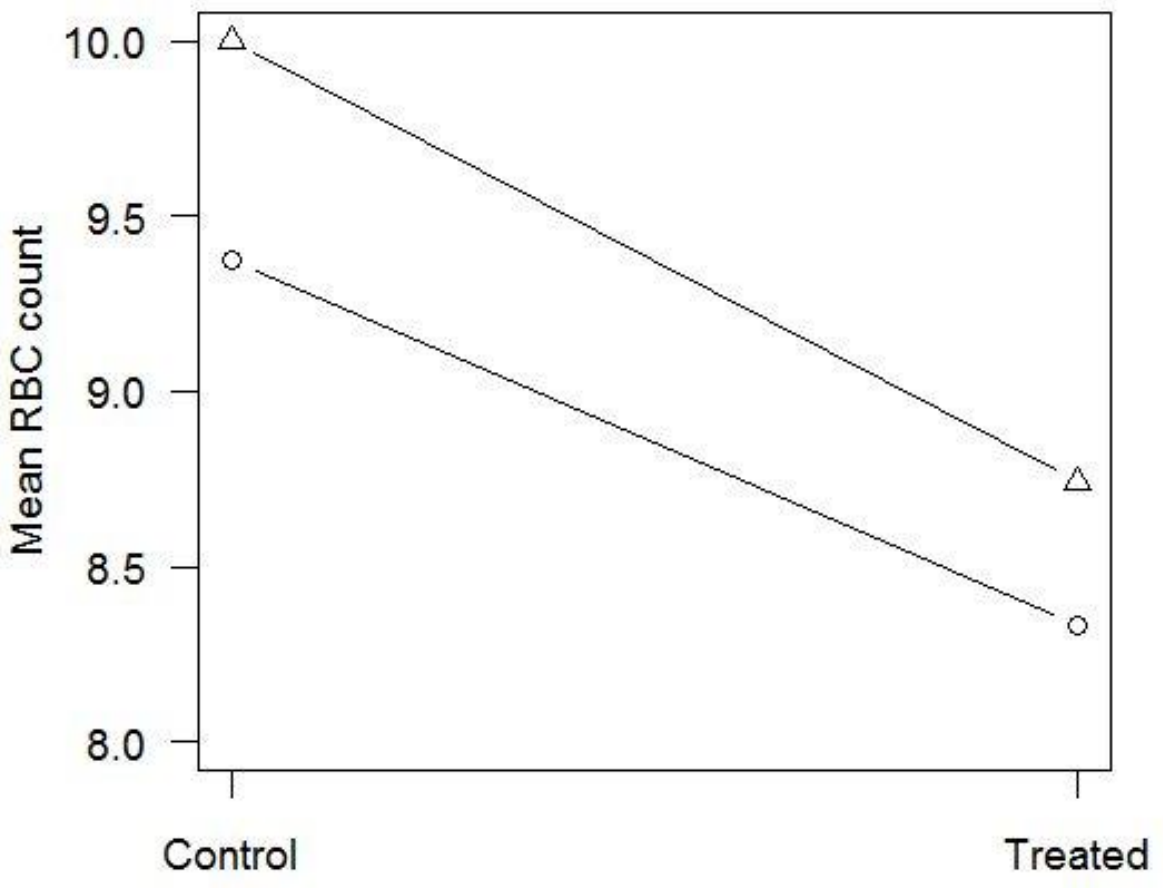
Red Blood Cell counts

Strain	Control	Treated	Strain means
BALB/c	10.10	8.95	
	10.08	8.45	
	9.73	8.68	
	10.09	8.89	9.37
C57BL	9.60	8.82	
	9.56	8.24	
	9.14	8.18	
	9.20	8.10	8.86
Treatment Mean	9.69	8.54	

In this study mice of two strains (BALB/c and C57BL) were dosed with with chloramphenicol at 2000mg/kg. This is a 2(strains) x 2(dose levels) factorial design.

We want to know:

- does treatment have an effect on RBC counts (results above)
- do strains differ in RBC counts
- do strains differ in their response to chloramphenicol (the interaction).

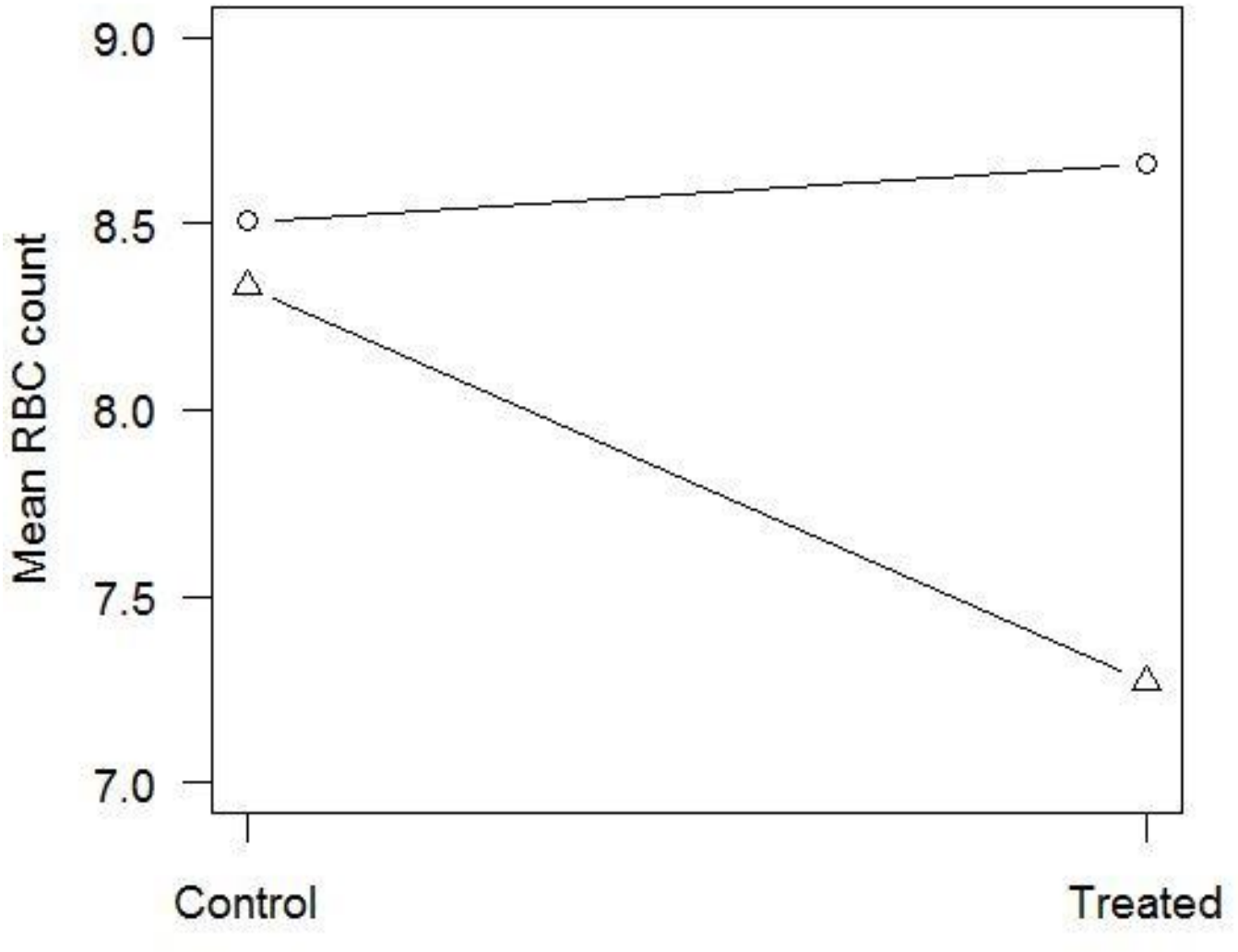


No interaction effect

Another mice example with different strains:

Red Blood Cell counts

Strain	Control	Treated	Strain means
C3H	7.85	7.81	
	8.77	7.21	
	8.48	6.96	
	8.22	7.10	7.80
CD-1	9.01	9.18	
	7.76	8.31	
	8.42	8.47	
	8.83	8.67	8.58
Treatment means	8.42	7.96	



Interaction effect! This means that results based on a single strain cannot be generalised.

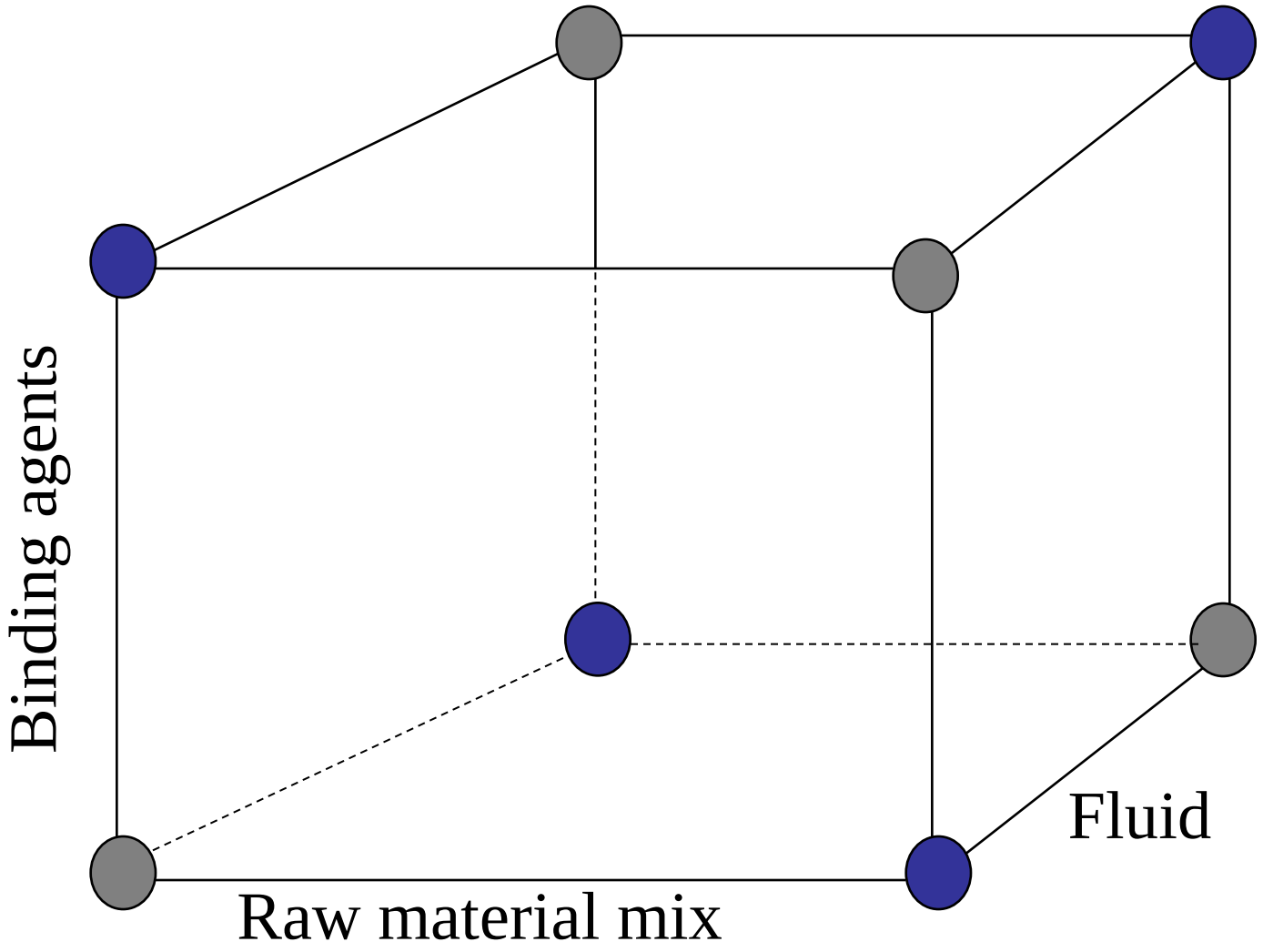
See also: Richter CA, Birnbaum LS, Farabollini F et al. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol 2007;24:199-224.

Test your calculations:

What is the calculated interaction effect between Factor 2 (milk) and Factor 3 (salmon) in the initial Fish pudding example?

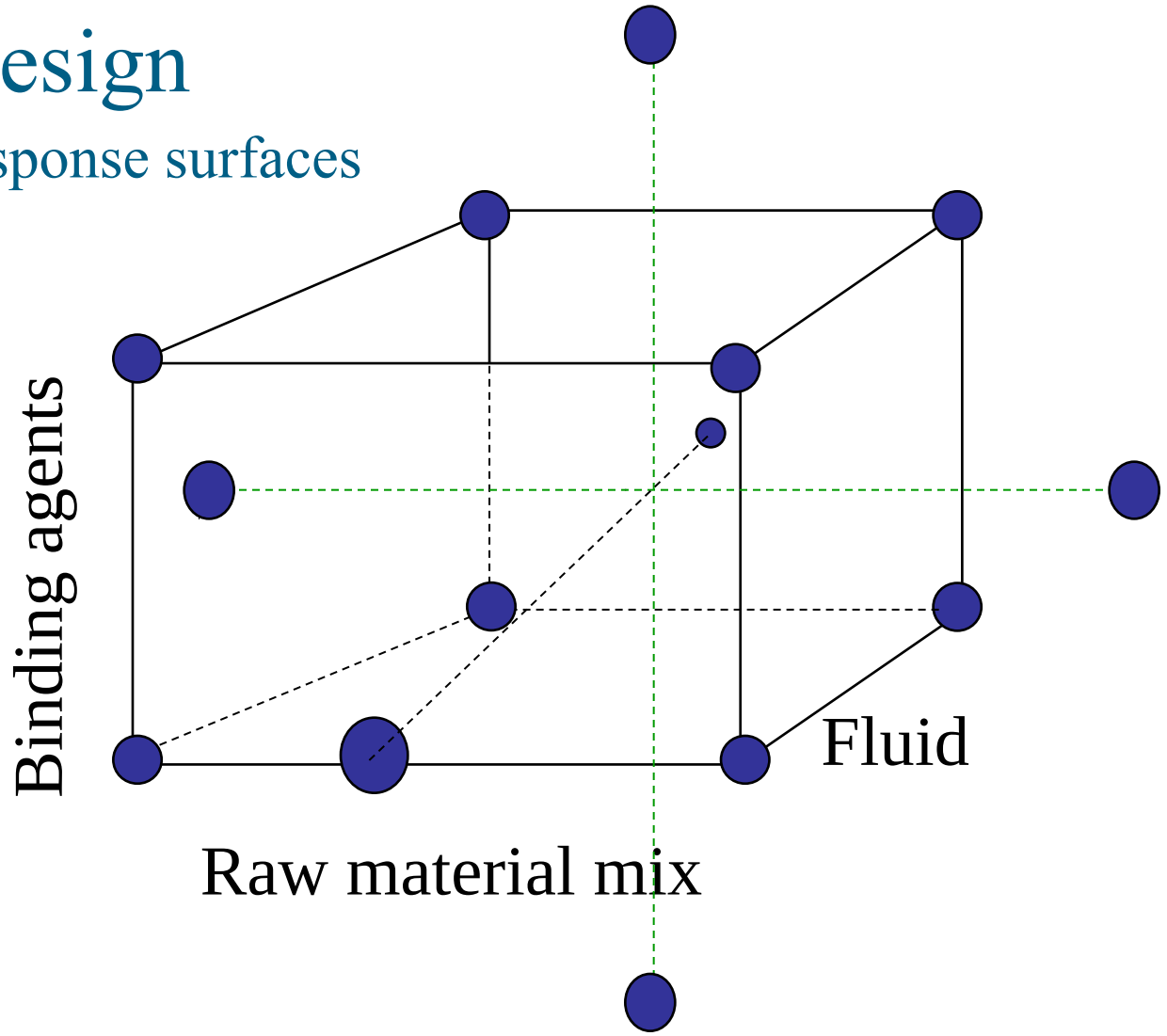
Reduced factorial design

- Give main effects, more cost effective in the initial test phase

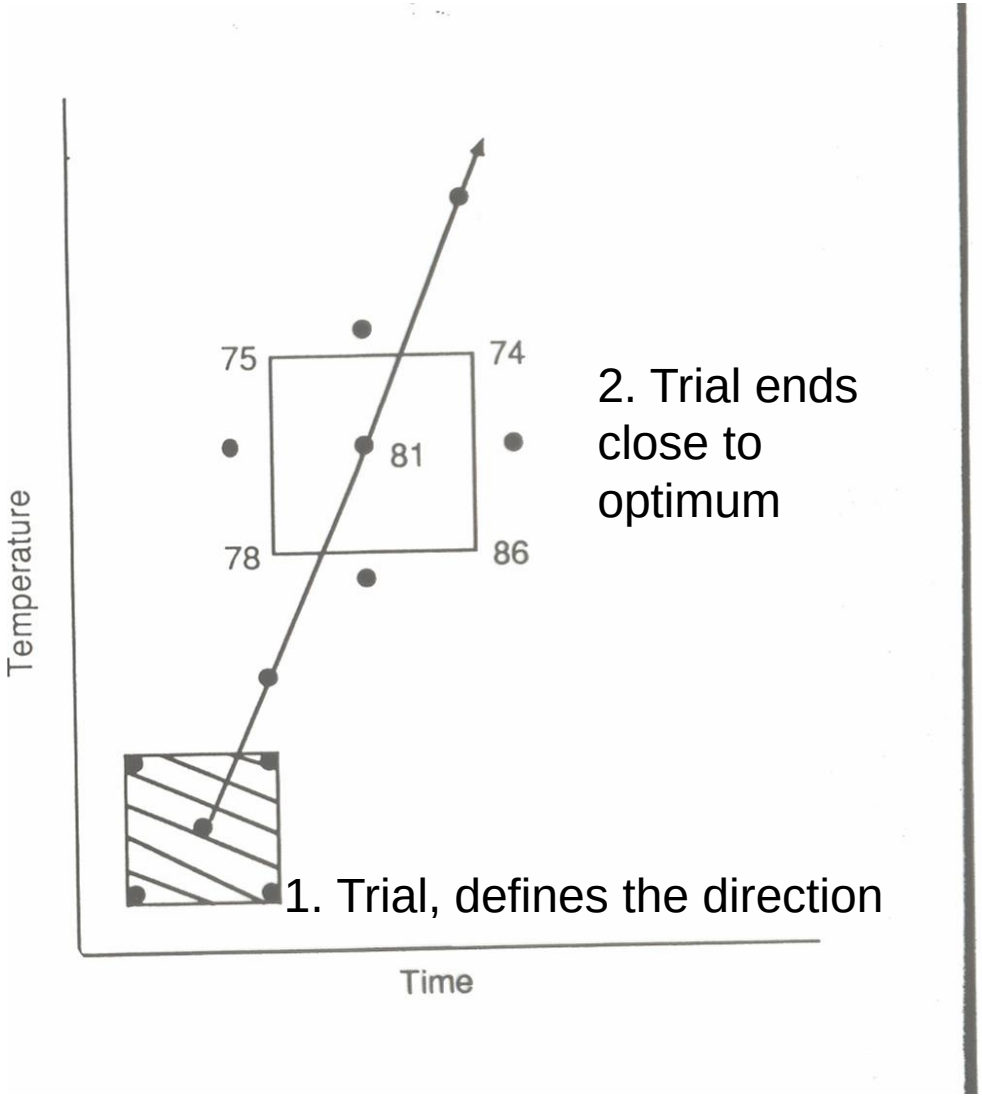


Star design

- Give response surfaces

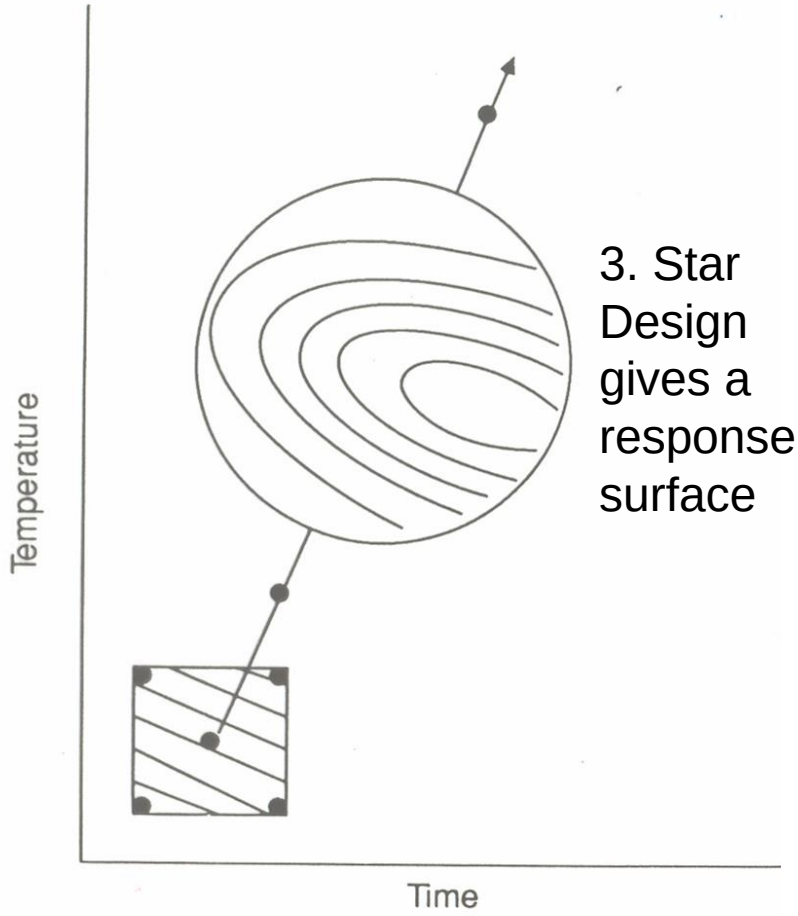


Strategic approach towards optimum, here with two variables:



2. Trial ends close to optimum

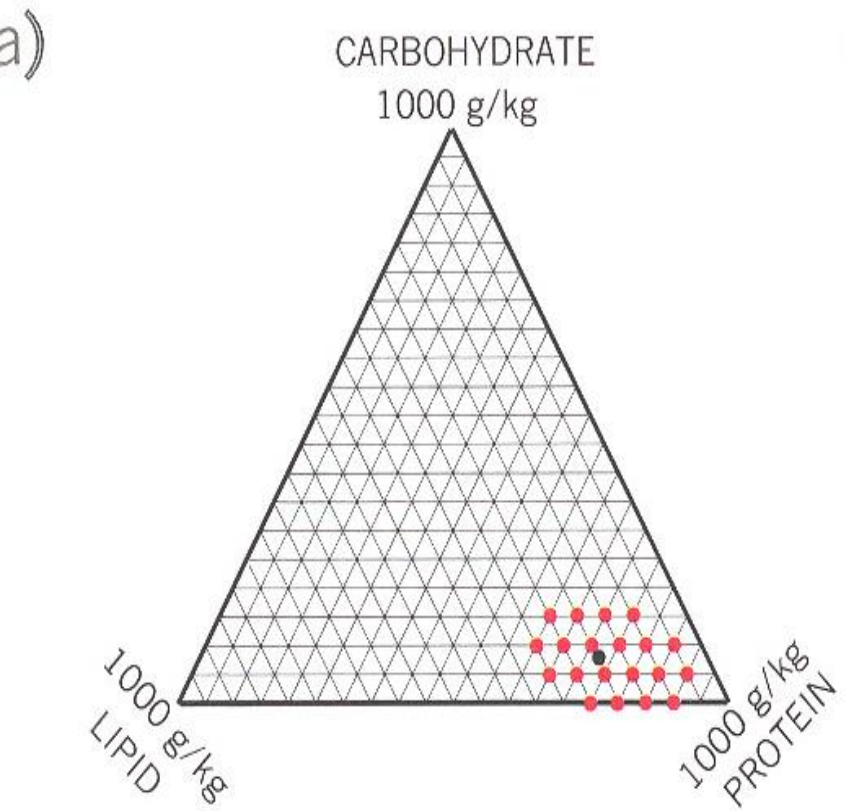
1. Trial, defines the direction

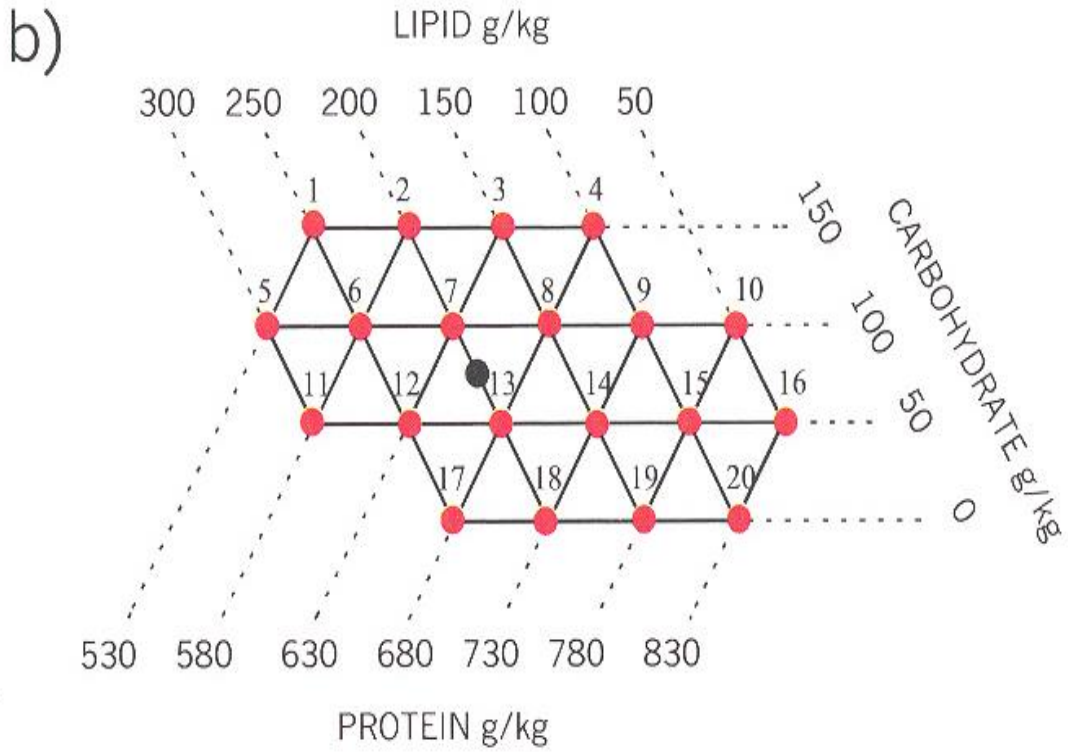


3. Star Design gives a response surface

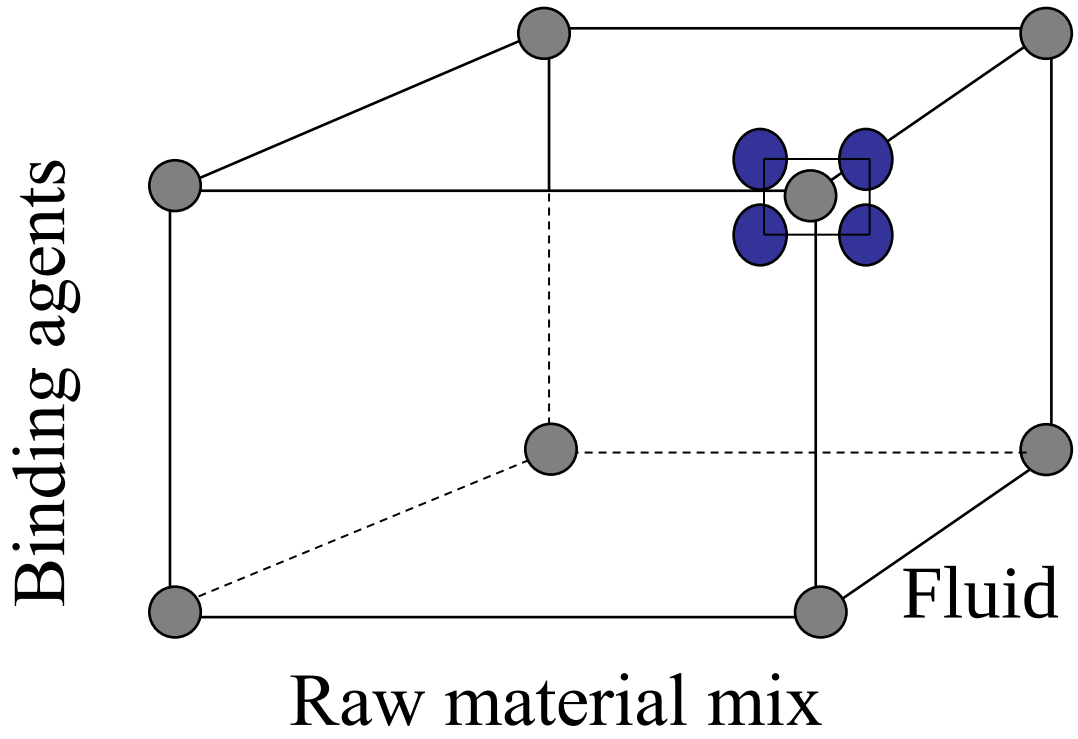
Mixture design

- Applied when the combination opportunities are ’closed’





Check the variability around optimum!



Robustness in product testing

Taguchi design

Design variable			Variables representing the customers' handling of the product					Average
Raw mix	fluid	Bind. agent	**Temp.**	-1	-1	1	1	**response**
			Tid	-1	1	-1	1	**Yw**
-1	-1	-1		Y1	Y9	Y17	Y25	5,6
1	-1	-1		Y2	Y10	Y18	Y26	7
-1	1	-1		Y3	Y11	Y19	Y27	7,1
1	1	-1		Y4	Y12	Y20	Y28	8
-1	-1	1		Y5	Y13	Y21	Y29	4
1	-1	1		Y6	Y14	Y22	Y30	2,3
-1	1	1		Y7	Y15	Y23	Y31	4
1	1	1		Y8	Y16	Y24	Y32	7

Experimental strategy towards optimum (approx. 20% of costs in each stage)

- 1st stage: Reduced FD, several relevant variables and wide variation
- 2nd stage: Focus on the most important variables and limited variation. Apply FD or a randomized block design
- 3rd stage: Apply a star design or a mixture design (D-optimal) near optimum to get response surfaces
- 4th stage: Test variability near optimum
- 5th stage: Test robustness by Taguchi design