
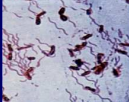


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The use of Monobutyryn to control Salmonella infection

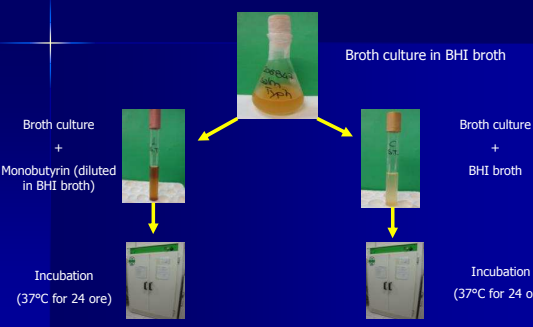
Results of *in vivo* and *in vitro* trials

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In vitro trials

(H.Thormar et al., Applied and Env.Microbiology 2006)



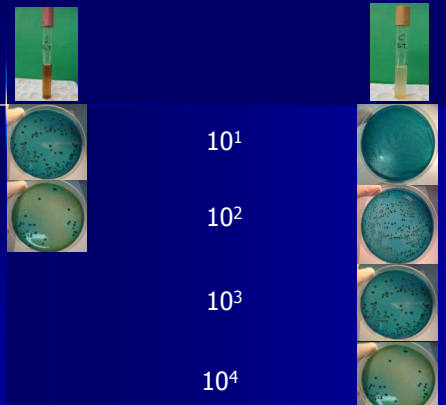
Broth culture in BHI broth

Broth culture + Monobutyryn (diluted in BHI broth)

Broth culture + BHI broth

Incubation (37°C for 24 ore)

Incubation (37°C for 24 ore)



10^1

10^2

10^3

10^4

Results

PRODUCT	mM/ litre	pH	Salmonella typhimurium (cfu/ml)
Positive control		7	120×10^5
Positive control		4.5	96×10^5
Butyric acid	12.4	7	65×10^4
Butyric acid	12.4	4.5	25×10^3
Monobutyryn Hydro C ₄	12.4	7	74×10^3
Monobutyryn Hydro C ₄	12.4	4.5	32×10^2

In vivo trials



Trial #1

High infecting dose of S.Typhimurium (10^7 cfu/head)

SPF chickens

Treatment in feed post-infection

Treatment in feed from day 1 of life

Determinations:
Intestinal Colonization

SPF birds (serum negative for S.Typhimurium)
30 birds/group

Infection at day 7 of life (via aesofagus)

Cloacal swabs 24 hours post-infection (to check the infection occurrence)

Treatment of feed from day 14 of life
(1.4% monobutyryn-Hydro C4 30)

Sacrifice of 10 birds/group at day 14, 24 and 34 of life
(counting S.Typhimurium in the caecal content)

Results/ Monobutyryn from day 14 of life

cfu/g of caecal content		
	Control	Group treated from day 7 g post-infection (14%)
Day 7 post-infection (14 days of life)	6,400,000	6,302,000 (start of treatment)
Day 17 post-infection (24 days of life)	25,120,000	171,120
Day 27 post-infection (34 days of life)	(high mortality)	1,000

SPF birds (serum negative for S.Typhimurium)
30 birds/group

Infection at day 7 of life (via aesofagus)

Cloacal swabs 24 hours post-infection (to check the infection occurrence)

Treatment of feed from day 1 of life
(0.3% e 1% monobutyryn-Hydro C4 30)

Sacrifice of 10 birds/group at day 14, 24 and 34 of life
(counts S.Typhimurium in caecal content)

Results/ Monobutyryn from day 1 of life

cfu/g of caecal content			
	Control	Group 0.3%	Group 1%
Day 7 post-infection (14 days of life)	6,400,000	2,226,000	770,000
Day 17 post-infection (24 days of life)	25,120,000	1,242,100	213,220
Day 27 post-infection (34 days of life)	--- (high mortality)	300	< 100

Trial #2

Infesting dose of S.Typhimurium
5x10³ cfu/head

Broiler chickens

Treatment in the drinking water post-infection

DETERMINATIONS:
Intestinal colonization
Systemic diffusion
Fecal excretion
Infection of crop "pre-slaughtering"

Broilers ROSS 308 (serum negative for S.Typhimurium)
30 birds/group

Infection at day 10 of life (via aesofagus)

Cloacal swabs 24 hours post-infection (to check the infection occurrence)

Treatment in drinking water from day 15 of life
(0.5% di monobutyryn-Hydro C4 30)

In the positive control : treatment in water in the last 5 days
(1% monobutyryn-Hydro C4 30)

sacrifice of 10 birds/group at day 20, 25 and 30 of life
(counts of S.Typhimurium in liver and caecal content)

Bacteriological trials

Qualitative search (presence/absence) by ISO method 6579:2002/Amd 1:Annex D

On the positive samples:
quantitative search on the starting material
Cfu in 1 g of liver and of caecal content
Sensibility limit: 100 cfu/g

Counts in CAECA : day 10 post-infection (day 20 of life)

Positive control group (no treatment) Group treated with Monobutyryn Hydro C4-30
Start of treatment: 5 days post-infection
Treatment duration: 5 days
Dosage: 0.5% in drinking water

100% positive after enrichment 40% positive after enrichment
60% negative after enrichment

Control group			cfu/g caecal content		
30%	6000-8000	cfu/g			
20%	3000-6000	cfu/g			
10%	1500-3000	cfu/g			
10%	1000-1500	cfu/g			
30%	100-1000	cfu/g			

Monobutyryn group		
20%	1000-1500	cfu/g
20%	100-1000	cfu/g
60%	0	cfu/g

Counts in CAECA : day 15 post-infection (day 25 of life)

Positive control group (no treatment) Group treated with Monobutyryn Hydro C4-30
Start of treatment: 5 days post-infection
Treatment duration: 10 days
Dosage: 0.5% in drinking water

100% positive after enrichment 20% positive after enrichment
80% negative after enrichment

Control group			cfu/g caecal content		
30%	3000-6000	cfu/g			
20%	1500-3000	cfu/g			
10%	1000-1500	cfu/g			
40%	100-1000	cfu/g			

Monobutyryn group		
10%	300-500	cfu/g
10%	100-300	cfu/g
80%	0	cfu/g

Counts in CAECA : day 20 post-infection (day 30 of life)

Positive control Group treated with Monobutyryn Hydro C4-30 for 5 days only before slaughtering (from day 25 up to day 30 of life) Gruppo trattato con Monobutirina Hydro C4-30
Inizio del trattamento: 5 gg. dopo l'infezione
Durata del trattamento: 15 giorni
Dosaggio: 0,5% in acqua di bevanda

20% positive after enrichment 100% negative after enrichment
80% negative after enrichment

Control group			cfu/g caecal content		
20%	100-300	cfu/g			
80%	0	cfu/g			

Monobutyryn group		
100%	0	cfu/g

Counts in livers of the control group

Control group before treatment Control group after 5 days of treatment with 1% Monobutyryn in drinking water before slaughtering

10 days post-infection 15 days post-infection 20 days post-infection

50% positive after enrichment 60% positive after enrichment 100% negative after enrichment
50% negative after enrichment 40% negative after enrichment

Control group			cfu/g caecal content		
50%	100-500	cfu/g			
50%	0	cfu/g			

Control group		
60%	100-1000	cfu/g
40%	0	cfu/g

Control group after 5 days treatment		
100%	0	cfu/g

Counts in livers of the group treated with Monobutyryn – Hydro C4 - 30

5 days of treatment 10 days of treatment 15 days of treatment

20% positive after enrichment 10% positive after enrichment 100% negative after enrichment
80% negative after enrichment 90% negative after enrichment

Treated group			cfu/g caecal content		
10%	100	cfu/g			
10%	< 100	cfu/g			
80%	0	cfu/g			

Treated group		
10%	100	cfu/g
90%	0	cfu/g

Treated group		
100%	0	cfu/g

Evaluation of fecal excretion

Qualitative search (presence/absence)
with ISO method 6579:2002/Amd 1:Annex D

On the pool of feces collected from sterile sheets of paper located on the insulators' floor 12 hours before each sampling

Treatment of the control group from day 25 up to day 30 with Monobutyryn

	20 days	25 days	30 days
Control group	Presence	Presence	Absence
Monobutyryn group	Presence	Absence	Absence

Evaluation of crop infection

Qualitative search (presence/absence)
with ISO method 6579:2002/Amd 1:Annex D

on the crop swabs after 4 hours of fasting pre-slaughtering (30 days of life)

Control group after treatment with 1% di Monobutyryn 5 days before slaughtering	Absence
Monobutyryn group	Absence

Trial #3

Infesting dose of *S.Typhimurium*
5X10³ cfu/head

Broiler chickens

Combined treatment (feed+water) pre and post-infection

DETERMINATIONS:
Intestinal colonization
Systemic diffusion
Fecal excretion
Infection of crop "pre-slaughtering"

Broilers ROSS 708 males
Infection at 12 days of life (via aesophagus)

Coecal swabs 24 hours post-infection (to check the infection occurrence)

The infection was made at day 12. The use of Monobutyryn at higher levels finished 2 days post-infection

	TREATMENTS
1 Control group	==
2	0.4% Monobutyryn in feed from 0 up to 14 days 0.025% Monobutyryn in drinking water from 15 up to 39 days 0.4% Monobutyryn in drinking water from 40 up to 42 days
3	0.8% Monobutyryn in feed from 0 up to 14 days 0.025% Monobutyryn in drinking water from 15 up to 39 days 0.2% Monobutyryn in drinking water from 40 up to 42 days
4	0.4% Monobutyryn in drinking water from 0 up to 14 days 0.025% Monobutyryn in drinking water from 15 up to 42 days
5	1.2% Monobutyryn in feed from 0 up to 7 days 0.025% Monobutyryn in drinking water from 8 up to 42 days

Sacrifice of 10 birds/group at day 22, 23 e 43 of life (counts of *S.Typhimurium* in liver and caecal content)

Presence/absence and counts of *S.Typhimurium* in the caeca and livers 10 days post-infection

GROUP	POSITIVE CAECA AFTER ENRICHMENT	COUNTS IN POSITIVE CAECA (cfu/g)	POSITIVE LIVERS AFTER ENRICHMENT	COUNTS IN POSITIVE LIVERS (cfu/g)
1	10/10	2000, 3500, 3200, 1800, 750, 1200, 2000, 2500, 3100, 2800	10/10	600, 800, 800, 1100, 800, 700, 600, 800, 500, 800.
2	9/10	400, 800, 550, 700, 500, 600, 900, 1000, 1200.	6/10	100, 200, 100, 100, 300, 200
3	8/10	800, 1000, 700, 400, 500, 600, 300, 400	5/10	100, 100, 100, 200, 300
4	5/10	200, 300, 400, 600, 500	3/10	100, 100, 200
5	9/10	500, 500, 700, 600, 450, 600, 800, 800, 900	7/10	200, 100, 300, 300, 200, 300, 400

Presence/absence and counts of *S.Typhimurium* in the caeca and livers 21 days post-infection

GROUP	POSITIVE CAECA AFTER ENRICHMENT	COUNTS IN POSITIVE CAECA (cfu/g)	POSITIVE LIVERS AFTER ENRICHMENT	COUNTS IN POSITIVE LIVERS (cfu/g)
1	10/10	1600, 1700, 3800, 1000, 2200, 2800, 2000, 1900, 3500, 3200	5/10	700, 900, 650, 500, 300, 800, 900, 600, 900
2	8/10	500, 400, 400, 400, 300, 500, 300, 500	4/10	100, 100, 200, 200
3	6/10	100, 200, 300, 300, 300, 300	2/10	100, 100
4	4/10	100, 100, 200, 100	0/10	//
5	8/10	400, 600, 300, 300, 500, 200, 300, 300	4/10	100, 100, 100, 100

Presence/absence and counts of *S.Typhimurium* in the caeca and livers 31 days post-infection

GROUP	POSITIVE CAECA AFTER ENRICHMENT	COUNTS IN POSITIVE CAECA (cfu/g)	POSITIVE LIVERS AFTER ENRICHMENT	COUNTS IN POSITIVE LIVERS (cfu/g)
1	9/10	1200, 1000, 1500, 1350, 2000, 2500, 2200, 2300, 2200	8/10	500, 450, 700, 400, 500, 550, 700, 600
2	1/10	200	0/10	//
3	2/10	400, 300	0/10	//
4	3/10	100, 200, 200	0/10	//
5	6/10	300, 300, 400, 400, 400, 400	2/10	100, 100

Counts in CAECA

	cfu/g 10 days post-infection (day 22 of life)	% reduction of infection, made 100 the control group	cfu/g 21 days post-infection (day 33 of life)	% reduction of infection, made 100 the control group	cfu/g 31 days post-infection (day 43 of life)	% reduction of infection, made 100 the control group
control	2285		2370		1405	
group 2	665	71%	330	86%	20	99%
group 3	470	79%	150	94%	70	95%
group 4	200	91%	50	98%	50	96%
group 5	585	74%	290	88%	220	84%

Counts in the LIVERS

	cfu/g 10 giorni post-infection (22 ^a -day of life)	Reduction in % of infection made 100 the control group	cfu / g 21 days post-infection (33 ^a -day of life)	Reduction in % of infection made 100 the control group	cfu / g 31 days post-infection (43 ^a -day of life)	% reduction of infection, made 100 the control group
controllo	750		705		440	
gruppo 2	100	84%	60	91%	-	100%
gruppo 3	80	89%	20	97%	-	100%
gruppo 4	40	95%	-	100%	-	100%
gruppo 5	180	74%	40	94%	20	96%

Evaluation of fecal excretion

	22 days	33 days	43 days
Control Group	Presence	Presence	Presence
Group 2	Presence	Presence	Absence
Group 3	Presence	Absence	Absence
Group 4	Absence	Absence	Absence
Gruppo 5	Presence	Presence	Presence

Evaluation of the infection level in the CROPS (43 days of life)

GROUP	
control	Positive 8/10
2	Positive 0/10*
3	Positive 2/10
4	Positive 2/10
5	Positive 3/10

* 0.4% monobutyryn in water at 40-42 days of life



S. LEESON (Guelph University, personal communication)

Inoculum = 10⁸

Positive Control	ppm	%	Butyric acid	Monobutyryn Hydro C4 - 30
+	500	0.05%	+	+
+			+	+
++			+	+
	1000	0.1%	+	No growth
			+	No growth
	1500	0.15%	+	No growth
			+	No growth
			+	No growth
	2000	0.2%	++	No growth
			++	No growth
			++	No growth

Bacteria Growth
 + = 24 h
 ++ = 36 h
 +++ = 96 h

S. Leeson (Guelph University, personal communication)

Inoculum = 10^8

Positive Control	ppm	%	Butyric acid	Monobutyryl Hydro C4 - 30
+	2500	0.25%	++	No growth
+			++	No growth
++			++	No growth
	3000	0.3%	No growth	No growth
			No growth	No growth
			No growth	No growth
	4000		No growth	No growth
			No growth	No growth
			No growth	No growth
Bacteria Growth			No growth	No growth
+ = 24 h			No growth	No growth
++ = 36 h				
+++ = 96 h				