MONOBUTYRIN : A NEW ANTIBACTERIAL MOLECULE FOR ANIMAL HEALTH, WITH GROWTH PROMOTING EFFECTS

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The EU Antibiotic Growth Promoters ban has led to an increased usage of antibiotics as therapeutics, including those that are of direct importance in human medicine. Antibiotic-resistance, increased diarrhoea, weight loss, mortality, human health hazards and other consequences of antibiotics prescriptions, urge feed producers and scientific communities to search for alternatives at a reasonable cost.

Many studies have shed a light on the positive properties and role of fatty acids (such as butyric acid) in managing animal gut health. Butyric acid has been proven to be particularly beneficial in the formation of intestinal micro-flora, the stimulation of villi growth and the integrity of the digestive track, thus increasing the resistance against pathogens (1).

It was also demonstrated that butyric acid acts directly on the genetic structure of *salmonella enteritidis* and *typhimurium* and inhibits its proliferation(2).

Furthermore, butyric acid, in its undissociated form, can have direct bacteriostatic effects on microorganisms like *E.coli* (3). As a matter of fact, in an acid environment (pH between 4 and 5.5), the butyric acid is found prevalently in its undissociated form, and therefore, it acts as an antibacterial agent(4). The intestinal acidity however varies between pH 6 and 7 in which the butyric acid dissociates resulting in a significant decrease in its antibacterial efficacy. In addition to that, the calcium bicarbonate present in the digestive track reacts with the acids creating a buffering effect. In a study of F. Boyen et al. (Ghent University) the efficacy of butyric acid against 54 porcine Salmonella Typhimurium strains was measured in vitro at different pH values. It was found that the MIC (minimum inhibitory concentration) of butyric acid at pH 4 is 5 mM, while at pH 6 the quantity of butyric acid required is 160 mM to inhibit *Salmonella* growth. The efficacy of butyric acid decreases by 32 times against *Salmonella* when pH increases from 4 to pH 6 (5).

The question is: how to combine the favorable effects of direct nutrition of the enterocytes, villi growth stimulation, intestinal mucosa integrity improvement, and at the same time, a strong antibacterial effect in the gut environment at a pH between 6 and 7?

Several studies demonstrated both in vitro as in vivo (5,6), that fatty acids monoglycerides have stronger antibacterial action than the fatty acids themselves.

The idea developed by SILO SpA was to produce and to use in the animal diets the Monobutyrin, which is a monoglyceride of the butyric acid, as a pH-independent antibacterial supplement and to carry butyric acid into the enterocytes as a nutrient and growth enhancer.

The Monobutyrin is obtained by combining a molecule of glycerol with a molecule of butyric acid (figure 1.)

The Monobutyrin is marketed by SILO SpA with the name Monobutyrin – Hydro C4.

Figure 1. Monobutyrin

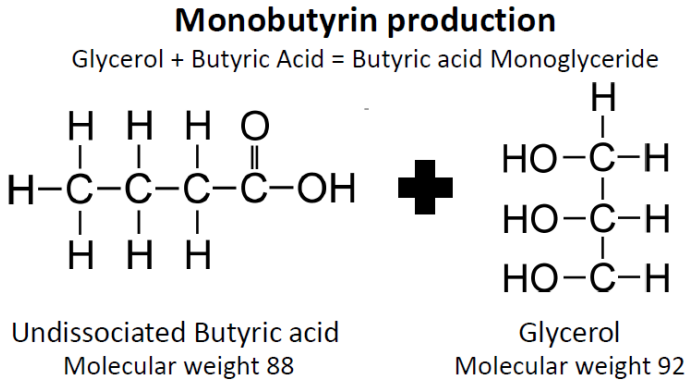
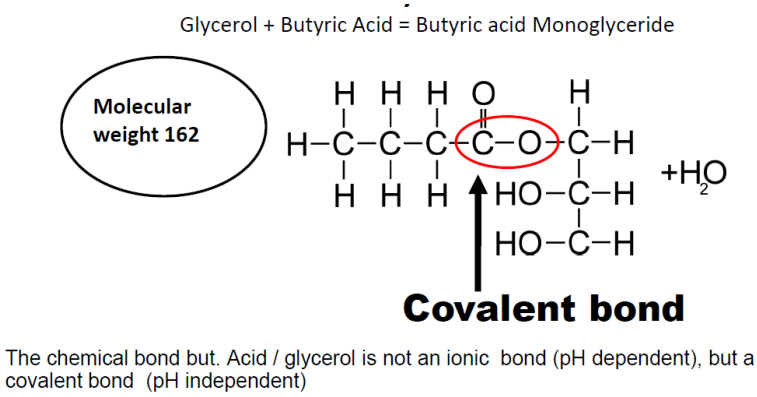


Figure 2. Monobutyrin structure



The end product is a low molecular weight (162) and water dispersible molecule. It has one lipophilic and one hydrophilic head, which make it compatible with hydrophilic and lipophilic cell walls / membranes of bacteria. The bond between butyric acid and glycerol is a covalent bond (Figure 2), stable from pH 1 to pH 7 and up to 230°C. This means that the molecule is stable in different environments: water, feed, gizzard, stomach, gut. It starts its activity already in the drinking water or in the feed, in the first digestive tract and in the gut, without losing its properties throughout the digestive tract.

Preliminary tests in vitro have been carried out on some gram-positive and gram-negative bacteria, at pH 4,5 (to reproduce the stomach pH) and at pH 7 (to reproduce the gut pH)

Trial 1 in vitro

The Public Animal Health Institute of Brescia, dr. Loris Alborali, carried out a test on *E.coli* O157, *Salmonella typhimurium* and *Salmonella cholerasuis*, in which the MIC of butyric acid or Monobutyrin have been compared. The MIC of the Monobutyrin resulted to be 2-4 times lower than that of the butyric acid at pH 7. (table 1)

Table 1. Trial 1 in vitro. Determination of the MIC of the free butyric acid or Monobutyrin on swine strains of *Salmonella ss.* and *E.coli* O 157 – Public Animal Health Institute of Brescia - 2010

Substance	pH	*S. typhimurium*	*S. choleraesuis*	*E. coli* O 157
Butyric acid	7	0,25%	0,25%	0,25%
Monobutirin – Hydro C4	7	0,06%	0,12%	0,12%

Trial 2 in vitro

An in vitro trial carried out by prof. Steven Leeson – Guelph University, demonstrated that Monobutyrin – Hydro C4 is 3 times stronger in inhibiting the growth of *Clostridium perfrigens CP 27* compared with the butyric acid. In the trial 1000 ppm (0,1%) of Monobutyrin - Hydro C4 was enough to inhibit the growth of *Clostridium perfrigens*, while 3000 ppm (0,3%) of butyric acid was necessary to achieve the same effect (Table 2).

Table 2. Trial 2 in vitro. Inoculation with 10^5 of *Clostridium perfrigens CP 27*. Medium: Brain Heart Infusion. Incubation time and growth that had been appeared : + for 24hr, ++ for 37hr and +++ for 96hr; 3 replication for each concentration – From a report by Steve Leeson – Guelph University - 2009

ppm	pure butyric acid	Monobutyrin - Hydro C4
500	+	+
	+	+
	+	+
1000	+	no growth
	+	no growth
	+	no growth
1500	+	no growth
	+	no growth
	+	no growth
2000	++	no growth
	++	no growth
	++	no growth
2500	++	no growth
	++	no growth
	++	no growth
3000	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

Trial 3 in vitro

The Public Animal Health Institute of Forlì (dr. Paola Massi and dr. Giovanni Tosi), carried out a test in vitro in which the efficacy of butyric acid or Monobutyrin - Hydro C4 in reducing the cfu of *Salmonella typhimurium* at ph 4,5 and pH 7 were compared. The data reported in the Table 3 show that at both pH values the efficacy of Monobutyrin – Hydro C4 was 2 times stronger than that of the butyric acid.

Table 3. Trial 3. Anti-salmonella activity of butyric acid or Monobutyrin – Hydro C4 at pH 4,5 or 7

Substance	mM/liter	pH	Salmonella Typhimurium (cfu/ml)
Positive Control		7	$120x10^5$
Positive Control		4,5	$96x10^5$
Butyric acid	12,4	7	$65x10^4$
Butyric acid	12,4	4,5	$25x10^3$
Monobutyrin - Hydro C4	12,4	7	$74x10^3$
Monobutyirin - Hydro C4	12,4	4,5	$32x10^2$

The positive results of the in vitro trials have been also confirmed in the field.

Trial 1 in vivo (Poultry) – Preliminary data

At the Public Animal Health Institute of Forlì (dr. Paola Massi and dr. Giovanni Tosi) three scientific trials were carried out on broiler chickens, aimed at testing the efficacy of different dosages of Monobutyrin – Hydro C4 in reducing the *Salmonella* infection levels in caeca, faeces and livers of orally infected animals. The three studies were conducted in 2009 and 2010 and will be the subject to a dedicated publication. In one of the trials the chickens were orally infected at day 10 post-hatch with 10^3 CFU of *S.typhimurium* and the treatment (5 liters of Monobutyrin – Hydro C4 per ton of drinking water) started 5 days post-infection. It was really surprising that with post-infection treatments in the drinking water it was possible to reduce the numbers of positive caeca after enrichment by 80% with 10 days of treatment, and by 100% with 15 days of treatment. Not only 100% of the caeca resulted to be negative for *Salmonella* after enrichment, but also the 100% of the livers and faeces (shedding) showed the same result after the 15-days-treatment. The control group showed 100% of positive caeca until day 20 post-infection, with an average CFU amount of 2220 CFU (50% of the chickens showed CFU amounts between 1700 and 5500). The control group was then treated with 10 litres of Monobutyrin per ton of drinking water only during the last 5 days before slaughtering, to evaluate the possibility to reduce the infection level by treating the animals just some days before slaughtering. As result of the 5 days treatment the control group showed 80% of negative caeca after enrichment, and the average salmonella CFU amount in

the 20% of positive chickens was 250. Some figures are shown in Tables 4 and 5. The subsequent studies carried out by Public Animal Health Institute in Forlì managed to optimize the dosages for salmonella prevention and treatment. All data will be published.

Table 4. Trial 1 in vivo. Presence/absence of *S.typhimurium* in caeca and cfu count of *S.typhimurium* at day 15 post-infection. The chickens were orally infected with 10^3 cfu at day 10 of life. The trial was carried out by the Public Animal Health Institute of Forlì. 2010

Group	Caeca positive after enrichment	CFU counts in positive caeca (cfu / g)
Control No treatments	10/10	300, 500, 600, 900, 1400, 1700, 3000, 3500, 4800, 5500
T1 Treated for 10 days with Monobutyrin – Hydro C4. The treatment started 5 days post- infection	2/10	300, 500

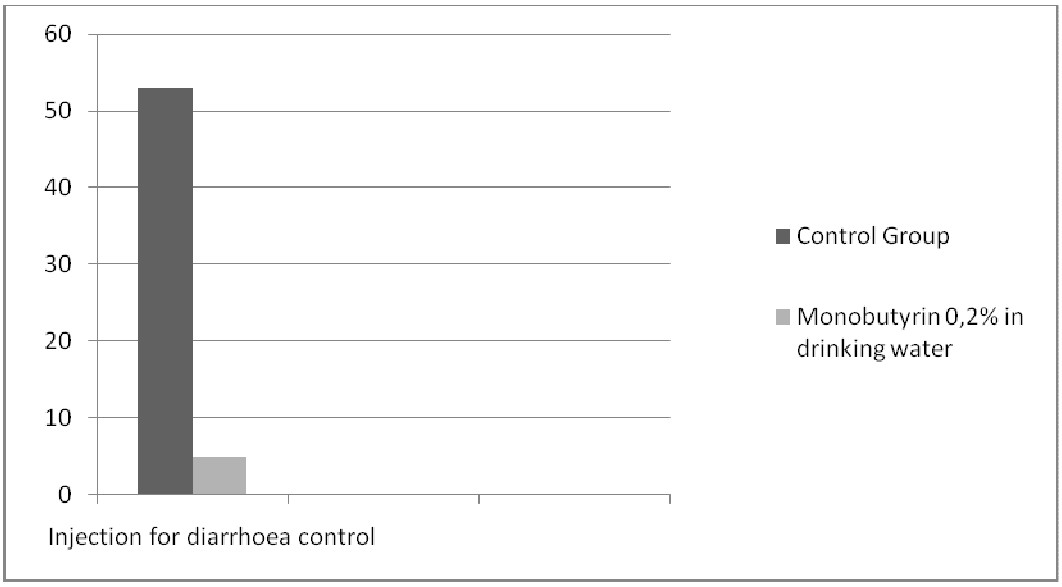
Table 5. Trial 1 in vivo. Presence/absence of *S.typhimurium* in caeca and cfu count of *S.typhimurium* at day 20 post-infection. The chickens were orally infected with 10^3 cfu at day 10 of life. The trial was carried out by the Public Animal Health Institute of Forlì. 2010

Group	Caeca positive after enrichment	CFU counts in positive caeca (cfu / g)
Control Treated with Monobutyrin – Hydro C4 only during the last 5 days before slaughtering	2/10	100, 300
T1 Ttreated for 15 days with Monobutyrin – Hydro C4. The treatment started 5 days post-infection	0/10	0

Trial 2 in vivo (Swine) – Preliminary data

Some preliminary results of a trial carried out under the supervision of the Public Animal Health Institute in Brescia, dr. Loris Alborali, show the efficacy of Monobutyrin – Hydro C4 in controlling the enteric pathologies. The trial is still ongoing and will be subject to a dedicated publication. 1332 animals are involved in the trial (678 animals in the control group and 653 animals in treated group). During the observed period from 23rd of June to August 1st 2010 (from day 41 to day 91 of age) the number of injection related to enteric diseases was 53 in the control group, and only 5 in the group treated with 0,2% of Monobutyrin – Hydro C4 in the drinking water. (Figure 3)

Figure 3. Trial 2 in vivo. Number of injections for diarrhea control in 2 groups of pigs from day 41 to day 91 of age. Trial carried out under the supervision of the Public Animal Health Institute of Brescia. 2010



This trend is commonly observed in the field where Monobutyrin – Hydro C4 is used in the drinking water or in the feed. In many cases Monobutyrin – Hydro C4 is used as substitution of zinc oxide and as alternative to antibiotics to control *E.coli*, *clostridium*, and other enteric pathologies like *Brachispira* and *Lawsonia*, specially in case of antibiotic-resistance in the farms. Some practical examples are reported here below.

Trial 3 in vivo – (Swine)

In a consulted by dr. Marian Porowski Polish farm of 1700 sows (positive for PRRS, Mhp, PCV2) with open production cycle, average weaning age at 28 days, 25,5 weaned piglets per sow per year, the main problem was Colibacteriosis in the after post-weaning period, in the feed transition phase and when animals entered into the fattening period. The following program was implemented: 3 liters of Monobutyrin – Hydro C4 per 1000 liters of drinking water were administered during the 4 days before and the 4 after the transition from pre-starter to starter feed, for 7 days before entering the fattening units and 7 day after the placement of the animal in the fattening units. Monobutyrin – Hydro C4 was used as zinc oxide and antibiotics replacement. The mortality was reduced after 6 months of Monobutyrin – Hydro C4 use from 3,5% to 1,9% in the fatteners and from 2,5% to 1,3% in the weaners (Figures 4 and 5).

Figure 4. Trial 3 in vivo.
Mortality in fatting pigs
6 months before and 6 months after the use of Monobutyrin – Hydro C4

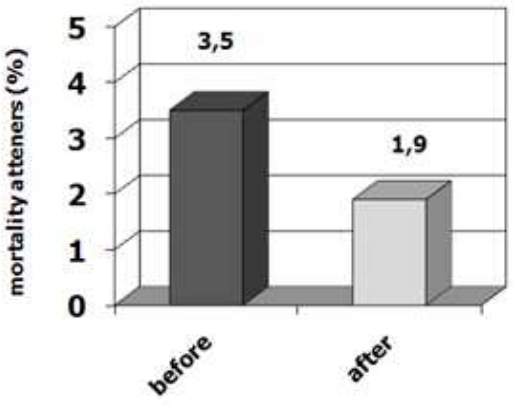
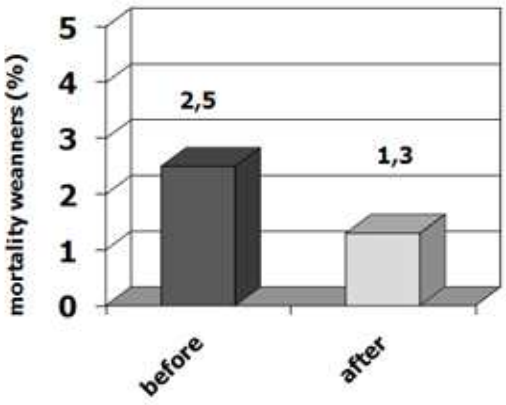


Figure 5. Trial 3 in vivo.
Mortality in weaning piglets 6 month before and 6 months after the use of Monobutyrin – Hydro C4



Trial 4 in vivo (Swine)

In a Spanish farm with 7000 finishing herd the mortality due to Sudden death (Clostridium like process) was a severe problem during the first 6 months of 2009. Up to 38% of total mortality was caused by sudden death syndrome. Monobutyrin – Hydro C4 was administered in the drinking water (2 liters per ton of water) in the second semester of 2009. After this, not only the mortality was reduced by half due to clostridiosis, but also productivity was improved (-15% in FCR) and the costs of medications was reduced by 47% (Tables 6 and 7)

Table 6. Trial 4 in vivo. Performance results, medication costs and costs for kg of produced meat in a farm 6 months before and 6 months after the use of Monobutyrin – Hydro C4

Measured parameters	1st semester 2009 before the use of Monobutyrin	2nd semester 2009 Use of Monobutyrin	Variation	Variation as %
FCR	2,77	2,35	-0,420	-15%
ADG	0,758	0,764	0,006	
Total costs of medications for each animal in Euros	3,14	1,65	-1,485	-47%
Cost per kg of produced meat in Euros	1,03	0,97	-0,060	-6%

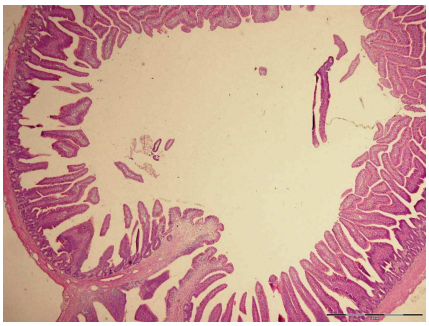
Table 7. Trial 4 in vivo. Percentage of mortality due to hemorrhagic syndrome and total medications cost in a farm 6 months before and 6 months after the use of Monobutyrin – Hydro C4

	% Dead hemorrhagic syndroms / total dead	Costs of medications in the feed for each animal	Total costs of medications
January-09	13,7	2,50	2,94
February-09	35,0	2,28	2,83
March-09	42,2	3,03	3,40
April-09	41,7	2,60	2,94
May-09	46,9	1,82	2,04
June-09	45,8	2,88	4,67
Start of treatment with Monobutyrin – Hydro C4			
July-09	34,6	0,67	3,05
August-09	24,8	0,20	0,38
September-09	25,5	0,15	1,94
October-09	20,4	0,01	0,65
November-09	22,4	0,00	1,95
December-09	21,4	0,00	1,94
January-10	15,0	0,00	

Trial 5 in vivo - (Poultry)

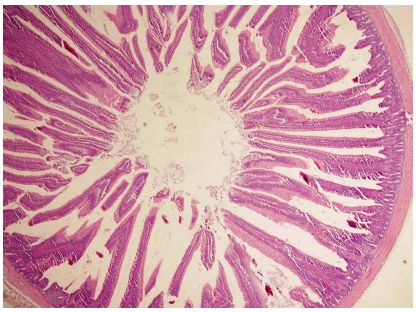
It is further interesting to observe that also the butyric acid in the monobutyrin molecular form (Monobutyrin – Hydro C4) is able to enhance villi growth. In a study conducted on broiler chickens by Prof. Roman Halouzka - University of Veterinary and Pharmaceutical Sciences of Brno, Institute of Pathological Morphology (2008) it was observed that the administration of 0,2% of the product Monobutyrin - Hydro C4 in the drinking water increased villi length in Meckels diverticulum area (+13,75%), in the ileum zone (+12,37%) and in the ileocecal valve zone (+1,84%) (Figure 8 and Figure 9). Concerning the effects on villi in the ileocaecal valve zone, Prof. Roman Halouzka wrote the following comment in his scientific report: *„The results show, and you can see it clearly, that the height of villi in some chicken groups increases towards the large intestine, which is a surprising finding.“*

Figure 8. Trial 5 in vivo. Data from the Report made by the University of Veterinary and Pharmaceutical Sciences Brno Institute of Pathological Morphology - Prof. Roman Halouzka. 2008. Villi hight in chickens fed unsupplemented diet or the product Monobutyrin - Hydro C4 in the drinking water



Lowest height of villi at the
level of Meckel's diverticulumin group

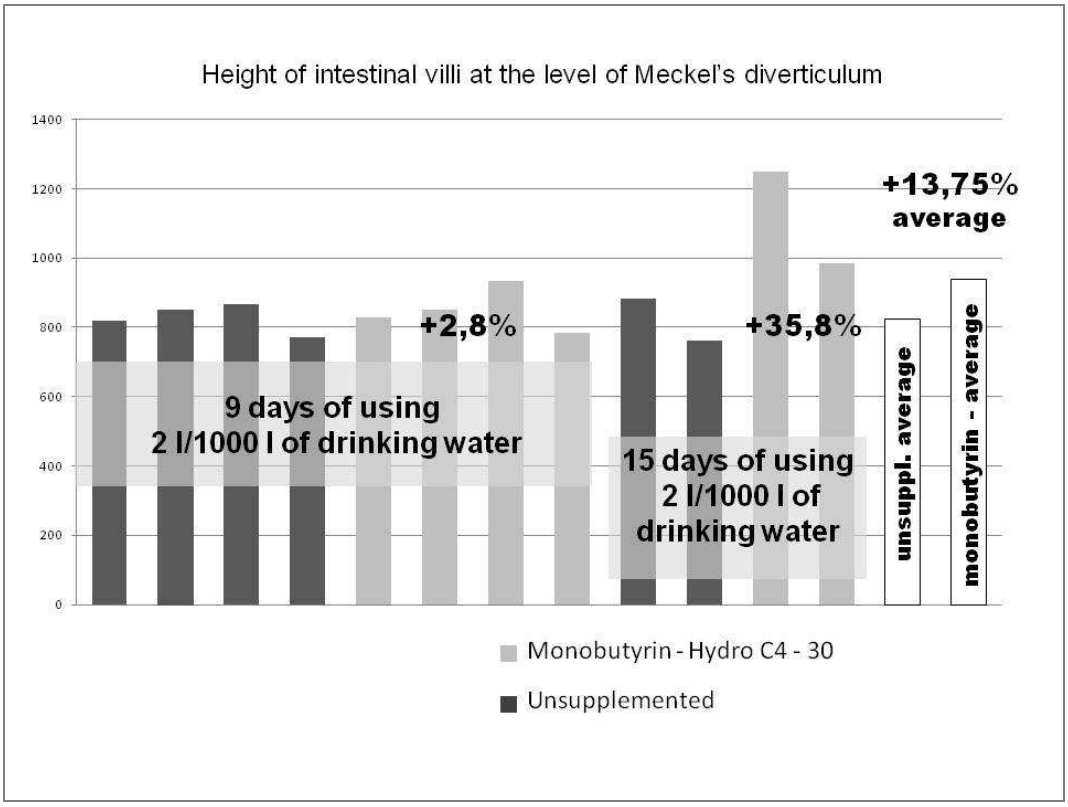
Control group

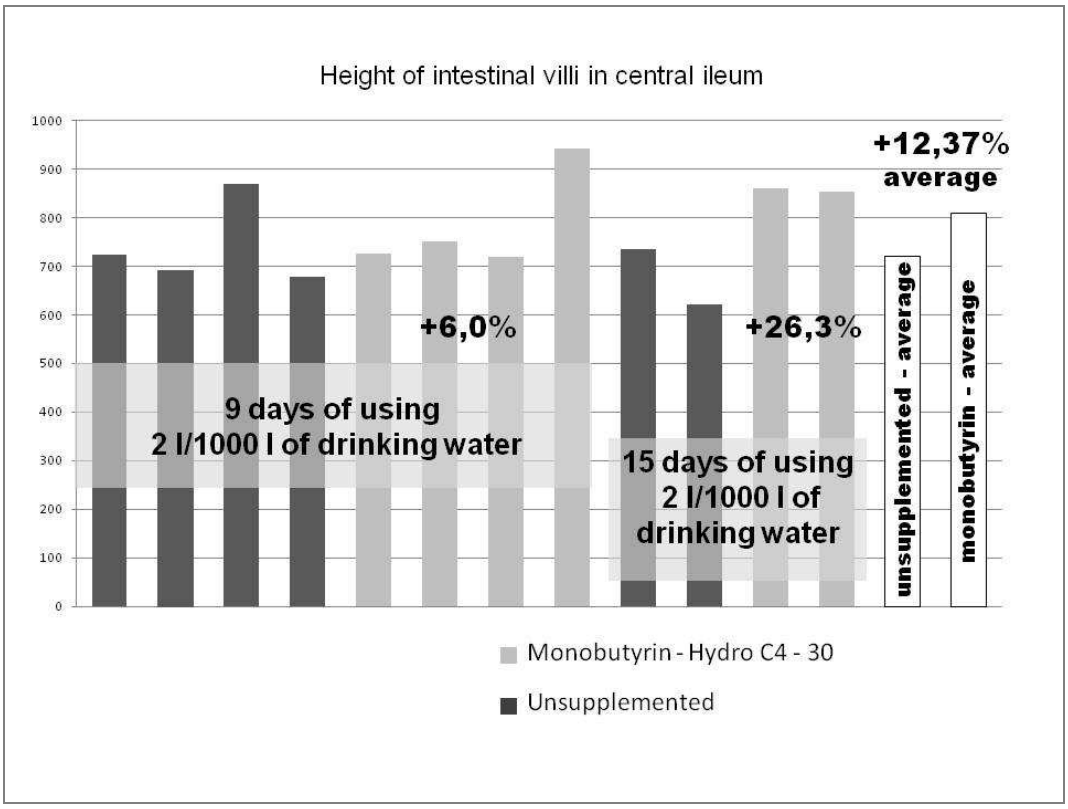


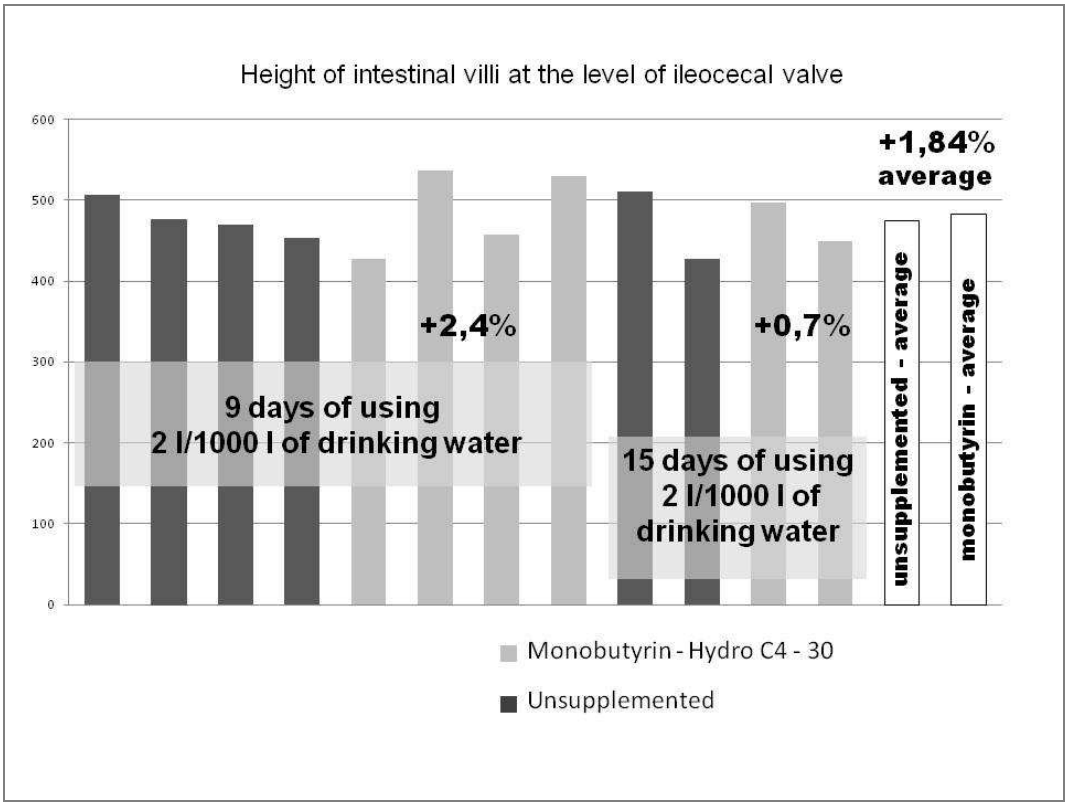
Heighest height of villi at the
level of Meckel's diverticulumin group

Group treated with Monobutyrin

Figure 9. trial 5 in vivo. Data from the Report made by the University of Veterinary and Pharmaceutical Sciences Brno Institute of Pathological Morphology - Prof. Roman Halouzka. 2008 Gut morphology of chickens fed unsupplemented diet or Monobutyrin – Hydro C4-30 in the drinking water




Height of intestinal villi in central ileum
1000
900
800
700
600
500
400
300
200
100
0
+12,37%
average
+6,0%
+26,3%
9 days of using
2 l/1000 l of drinking water
15 days of using
2 l/1000 l of
drinking water
unsupplemented - average
monobutyrin - average
Monobutyrin - Hydro C4 - 30
Unsupplemented


Height of intestinal villi at the level of ileocecal valve
600
500
400
300
200
100
0
+1,84%
average
+2,4%
+0,7%
9 days of using
2 l/1000 l of drinking water
15 days of using
2 l/1000 l of
drinking water
unsupplemented - average
monobutyrin - average
Monobutyrin - Hydro C4 - 30
Unsupplemented

Conclusion

In the everyday field practice and the scientific trials Monobutyrin – Hydro C4 proved to have strong pH-independent antibacterial properties, particularly against *E.coli*, *Salmonella spp.*, *Clostridium spp*., *Brachispira hyodysenteriae*, *Lawsonia intracellularis*. Since it is water dispersible, it can be administered in the drinking water or in the feed. The effects against pathogens are performed in water and throughout the whole digestive tract (stomach, gizzard, gut).

The positive effects of Monobutyrin – Hydro C4 on villi growth and gut integrity, combined with its antibacterial action, allow to improve performance with an economical return both in terms of FCR improvement and lower costs of medications. These characteristics of the Monobutyrin – Hydro C4 will fit very well in antibiotic reduction programs to be applied in animal husbandry.

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