PAPER

Monobutyrine: a novel feed additive in the diet of broiler chickens

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Abstract

Purpose of the present work was to validate the monoglyceride of butyric acid (MB) as an alternative to antibiotics as growth promoters in the diet of broiler chickens. The approach is a kind of prolongation of previous works, in which a blend of mono-, di- and tri-glycerides of butyric acid have been previously tested.
The results indicated that MB was very efficient in limiting the mortality of birds challenged with *Eimeria spp*., but did not appreciably impair the performance of unchallenged birds. In conclusion, the metabolisable energy content of MB appeared comparable with that of soybean oil and MB a reliable coccidiostat.

Introduction

An adequately developed and healthy gastrointestinal tract (GIT) is the necessary condition to the best animal performance. In addition to the functions of proper feed digestion and nutrient absorption, the GIT performs the basic task of protection against microbial and viral infections which may attack the animal via its intestine. In chickens, also sub-clinical factors may negatively affect feed efficiency and growth rate, so representing an important cost to poultry industry. Since the GIT welfare depends greatly depends on a healthy symbiotic micro-population, the management of intestinal microbes is the key to success in poultry production.

The use of antibiotics in feeds, as growth promoters to prevent intestinal pathologies has proven inefficient over the years because of the well-known problem of acquired resistance of pathogenic microbes to specific antibiotics, leading to their banning in 2006 in the EU.

Butyric acid, a saturated fatty acid with four carbon atoms, is a natural product of fermentation of dietary fibre produced by the symbiotic cellulolytic microbes of the digestive tract of all superior animals.

Butyric acid has been acknowledged to be an important beneficial factor since it acts as: i) promoter of cell proliferation of colon epithelial cells (Sakata, 1987; Sharma *et al*., 1995); ii) stimulator of the gut immune system (Friedman and Bar-Shira, 2005); iii) modulator of the composition of intestinal micro-population against harmful microbes (Van Der Wielen *et al*., 2000; Isolauri *et al*., 2004; Jósefiac *et al*., 2004); iv) prevention factor against colon cancer (Hassig *et al*., 1997). As a consequence of the banning of antibiotics in the EU, valid alternative solutions to counteract the gut pathogenic microbes and the consequent enteric disease have been looked for. Butyric acid in the feed appears to be the key to solve the problem. However, it is not actually possible to introduce it as such into the diet, due to its unpleasant odour which limits, when doesn't completely waste, the palatability of feeds. Butyric derivatives of some kind of protection must therefore be used, like sodium butyrate or the expensive coating protection. Butyric glycerides may be an even better solution to the problem. The mono-, di- and tri-glycerides of butyric acid are to be preferred because easily absorbable and practically odourless. A blend of butyric glycerides produced very good results in previous trials with broilers (Leeson *et al*., 2005; Antongiovanni *et al*., 2007). The subject of the present study is the effect on the performance of broiler chickens of the monoglyceride of butyric acid (MB), as the unique butyric glyceride supplemented into the diet of broilers, possibly more efficient than the previously tested blend, because more easily absorbable.

Materials and methods

The experimental design consisted of two growth trials: experiment one with *Eimeria spp*. challenged birds and experiment two with unchallenged birds. Both experiments lasted 6 weeks. At the 21st day of experiment one, all the birds were orally challenged with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* (5×10^4; 8×10^3 and 1×10^3).

The additive containing prevalently MB was tested at two concentrations of butyric acid: 20% of the additive (MB 20) and 30% of the additive (MB 30), as illustrated in Table 1, and at different times of the experiments (see Table 2). The blend of glycerides, tested in the cited previous experiments and characterised by 60% butyric acid, was tested again in experiment one as a kind of blank test. One-day old male chicks Ross 708, vaccinated at the hatchery against coccidiosis (Paracox 5), were purchased from a commercial hatchery. One hundred and fifty birds were randomly allotted to 30 pens (5 birds per pen, 10 pens per treatment) in experiment one and another 150 birds were randomly allotted to 15 pens (10 birds per pen, 5 pens per treatment) in experiment two. The birds were fed usual maize-soybean non-medicated mixed feeds in both experiments. The composition and calculated nutritional characteristics of the diets are illustrated in Table 3. The starter diet was administered for the first two weeks, and then the animals were fed the grower-finisher one. As indicated in Table 2, soybean oil was partially replaced by the different glycerides, according to the rationale of the experiments.

In experiment #1 (Table 2), the birds were submitted to 3 treatments: C (control), with no glyceride added other than 5% soybean oil; T1, with MB 30 at the 1% level in the feed, replacing the same amount of soybean oil; and T2 with BG at the 0.2% level, in substitution for the same amount of oil.

In experiment #2 (Table 2) the birds received again 3 treatments: C (control), with 5% soybean oil and no other glyceride added; T1, with MB 20 replacing 1% oil all the time; T2 with MB 30 for the first week only, then with MB 20 for another two weeks and soybean only for the final three weeks. Live body weights and feed intakes were recorded weekly per single

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Key words: Butyric acid, Monobutyrine, Broilers, Coccidiostat.

Acknowledgements: the authors would like to thank the company SILO S.r.l., Firenze (Italy), both for the financial support and for providing the butyric glycerides.
The contribution of Mr. Silvano Lancini, animal technician of the Department, was essential to the accomplishment of the whole work.

Received for publication: 23 February 2010.
Accepted for publication: 17 July 2010.

Licensee PAGEPress, Italy
Italian Journal of Animal Science 2010; 9:e69
doi:10.4081/ijas.2010.e69

pen, as the statistical unit. At the end of the six weeks of the experiments, the birds were slaughtered by an authorized slaughter house, according to the Italian animal welfare standards. The chilled carcasses were then measured for carcass weights, i.e. the weight of the single bird after removal of feathers and abdominal viscera, except kidneys and for breast yields. All the birds dead during the experiment were submitted to autopsy in order to ascertain the cause of the death.

Statistical analysis: the design of the experiments was a completely randomized one with the pen as the experimental unit. The performance results were analysed by a one-way ANOVA using the procedure of SAS (1999), keeping the factor "diet" as the fixed one. The mortality figures were analysed by the χ^2 test.

Results

Performance results are detailed in table 4. Previous experiments of ours (Leeson *et al*., 2005; Antongiovanni *et al*., 2007) indicated that the effect of butyrate on the birds' gut is at the maximum level within the first two weeks. This is the reason why the results relative to 14 days are reported in Table 4 as the performance of the starter period.

Experiment #1

Live body weights at the end of the second week resulted statistically comparable, between 470 and 488 g. Feed/gain ratios were not too bad for challenged birds, around 1.8, again statistically not different from one another. On the contrary, at the end of the trial, the MB treated animals (T1) were lighter, even though not significantly. The T1 birds resulted with the worst feed/gain ratio but, again, statistically not different from that of the other experimental groups. The BG chickens (T2) exhibited practically the same performance as the control ones. Also the weights of the carcasses and the breast meat yields resulted statistically comparable in any case. Amid all these meaningless results, an important significant piece of information comes from the mortality rates: the incidence of mortality was quite heavy in the untreated birds, in comparison with the MB ones, 14% *vs.* 2% ($P<0.05$). The autopsy examination of the dead animals revealed the incidence of heavy coccidial infections.

Experiment #2

When the environment was strictly controlled in order to avoid contaminations from outside, the mortality of untreated unchallenged birds resulted very low and not significantly different from the mortality of the treated groups. The same situation was reported by Leeson *et al*. (2005) with 0.1% butyric acid in the diet. The autopsy examinations revealed no coccidial infection as the cause of death, but excessive intakes of feed in the crop (too palatable?). However, the χ^2 test did not attribute statistical significance to the differences.

In this second experiment, all the differences resulted devoid of statistical significance. The trend was that the growth rates of the T2 treated birds appeared poorer than those exhibited by both the control and the T1 treated chickens, together with the feed/gain ratios, decidedly good.

Table 1. Characterisation of butyric glycerides tested as feed additives.

	MB 20°	MB 30°	BG#
Composition			
Mono-butyrine	34.0	51.0	30
Di-butyrine	5.6	8.4	50
Tri-butyrine	0.4	0.6	20
Glycerol	60.0	40.0	-
Butyric acid in the mixture	20	30	60

°Glycerides of butyric acid as the only fatty acid, diluted in glycerol; #blend of glycerides with butyric acid in the 1 or 3 position (30%), in both 1 and 3 positions (50%) and in all the three positions (20%).

Table 2. Rationale of treatments (% of mixed feed).

	Control	T1	T2		
Experiment #1					
Soybean oil, %	5.0	4.0	4.8		
MB20, %	-	-	-		
MB30, %	-	1.0	-		
BG, %	-	-	0.2		
Weeks	0-6	0-6	0-6		
	Control	T1	T2	T2	T2
Experiment #2					
Soybean oil, %	5.0	4.0	4.0	4.0	5.0
MB20, %	-	1.0	-	1.0	-
MB30, %	-	-	1.0	-	-
Weeks	0-6	0-6	0-1	2-3	4-6

Table 3. Diet composition.

	Diets	
	Starter, 0-14 days	Grower-finisher, 15-42 days
Ingredients		
Maize, %	51.90	55.35
Soybean meal 48, %	38.00	35.50
Soybean oil°, %	5.00	5.00
Dicalcium phosphate, %	2.50	2.00
Limestone , %	0.80	0.80
Premix#, %	0.50	0.50
Salt, %	0.30	0.30
Lysine HCl, %	0.30	0.10
DL Methionine, %	0.30	0.25
Choline Cl, %	0.20	-
Sodium bicarbonate, %	0.20	0.20
Calculated analysis		
Crude protein, %	22.0	20.5
Metabolizable energy, MJ/kg	12.23	12.44
Calcium, %	1.06	0.93
Available phosphorus, %	0.51	0.42
Sodium, %	0.18	0.18
Lysine, g/kg	14.2	12.0
Methionine + cystine, g/kg	9.9	9.2

°Partially replaced by glycerides, as indicated in Table 2. #Supplied per kg diet: Vitamin A, 3000 U; cholecalciferol, 1600 U; α-tocopherol acetate, 15 mg; riboflavin, 7 mg; panthotenic acid, 7 mg; cobalamin, 8 g; niacin, 20 mg; menadione, 1.5 mg; biotin, 0.25 mg; Mn, 50 mg; Zn, 50 mg; Cu, 5 mg; Fe, 30 mg; Se, 0.3 mg.

Discussion

Short chain fatty acids acetic, propionic and butyric, have been used as water sanitizers in the poultry industry. Unlike acetic and propionic, free butyric acid is quickly absorbed in the upper small intestine (Bolton and Dewar, 1965). As a consequence, it may be of little use to the gut environment if not stabilized in some way. If administered as butyric glyceride, its absorption rate is reduced and it is allowed to proceed further down the digestive tract and exert it antimicrobial action where required. Moreover, while free butyric acid is characterized by a strong unpleasant odour, which possibly makes the feed in which it is incorporated poorly palatable, supplemented butyric glycerides do not depress feed intake, so confirming the results of the cited previous works of ours (Leeson *et al*., 2005; Antongiovanni *et al*., 2007). Aim of the present work was a further step forward with reference to previous experiments by studying the effect on the chickens' performance of a nove form of butyric glyceride: the monoglyceride diluted in glycerol. The new form was expected to be more easily, even not too rapidly, absorbed and to enter quickly into the pathogenic microbial cell, due to its hydrophilic behaviour. Finally, to be even more palatable, due to the sweet taste of glycerol.

The results confirmed no negative effects of feeding butyric acid even throughout the whole 6 weeks growth period and at much higher dosages than in our previous experiments: in experiment one 1% MB 30 is equivalent to 0.3% butyric acid and in experiment two 1% MB 20 is equivalent to 0.2% butyric acid, as compared to 0.12% only in the case of the BG blend. The antimicrobial effect upon the pathogenic microbes was confirmed by the mortality rate, significantly reduced in experiment one in challenged birds. It doesn't seem necessary to provide monobutyrine throughout the whole growth period. In fact, in experiment 2 MB 30 was restricted to the first week only and MB 20 to the following two weeks, with no statistically appreciable differences in the chickens' performance.

Conclusions

The protective effect of monobutyrine on the gut environment has been confirmed with the experiment with Eimeria challenged birds. In fact, the mortality recorded in the group of birds treated with 1% MB 30 resulted significantly much lower than in the group of untreated chickens. In both experiments, when MB 20 and MB 30 partially replaced soybean oil in the diet, the final body weights resulted statistically not different, with comparable feed/gain ratios, with respect to those of the control group. The following speculation may then be proposed: since the MB supplements tested in the two experiments of the present work are characterized by a heat of combustion which is about half the heat of combustion of soybean oil (19-20 vs. 39 MJ/kg), but there was no depression in the birds' performance, we can assume that MB 20 and MB 30 must have the same apparent metabolizable energy content of soybean oil (associative effect?) if, when replacing the same amount of oil, no statistically different performance traits were observed. The speculation could be expressed in these terms: if the tabulated metabolizable energy content of soybean oil is 38 MJ/kg (Sauvant *et al*., 2002), that is 97% of its heat of combustion, MB 20 and MB 30 appear to have paradoxically the same metabolizable energy content (38 MJ/kg), that is about 210% their heat of combustion!

Table 4. Performance traits of broiler chickens.

	Control	T1	T2	SEM
Experiment #1				
Live body weight, 14 d, g	470	475	488	10
Feed/gain, 0-14 d	1.78	1.84	1.81	0.06
Live body weight, 42 d, g	2,777	2,691	2,782	89
Feed/gain, 0-42 d	1.93	2.04	1.96	0.06
Carcass weight°, g	2,385	2,419	2,499	89
Breast meat, g	512	563	579	46
Breast meat, % carcass	20.6	21.3	22.1	1.1
Mortality, %	14[a]	2[b]	4[b]	-
Experiment #2				
Live body weight, 14 d, g	472	476	457	7
Feed/gain, 0-14 d	1.34	1.35	1.39	0.03
Live body weight, 42 d, g	2,751	2,799	2,711	48
Feed/gain, 0-42 d	1.74	1.73	1.75	0.02
Carcass weight°, g	2,319	2,326	2,231	91
Breast meat, g	566	517	512	49
Breast meat, % carcass	24.5	22.2	23.0	2.9
Mortality, %	2	6	2	-

°Weight of the sacrificed bird after removal of feathers and abdominal viscera except kidneys; [a,b]different superscripts show significant differences (P<0.05).

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