Butyric acid glycerides ... and carcass composition

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ABSTRACT

Aim of the study was to verify the effects of butyric acid glycerides, as a supplemental ingredient in the diet, on live performance of broiler chickens and on the morphology of their small intestine, since short chain fatty acids are known as selective protection factors against intestinal microbial parasites, potent growth promoters of the gut wall tissues, also in terms of immune modulation response.
An experiment was carried out on 150 Ross 308 female chickens, allotted to 5 treatments, over a 35 days period: the control, with soybean oil as the energy supplement, and 4 treatments with increasing amounts (0.2, 0.35, 0.5, 1% mixed feed) of a mixture of butyric acid glycerides (mono-, di- and tri-glycerides).
Treated animals showed a higher live weight at slaughtering (P<0.05) with a better feed conversion rate. The carcase characteristics were not influenced, but the small intestine wall resulted slightly modified with shorter villi, longer microvilli (P<0.01) and larger crypts depth in jejunum (P<0.01), only with lowest concentration of the supplement (0.2%).
It is concluded that butyric acid glycerides are an efficient supplement to broilers' diets, deserving particular attention as a possible alternative to antimicrobial drugs, which have been banned in Europe.

Key Words: Butyrate, Glycerides, Broilers feeding.

RIASSUNTO

GLICERIDI DELL'ACIDO BUTIRRICO NELLE DIETA DEI BROILER: EFFETTI SULL'ISTOLOGIA DELLA MUCOSA INTESTINALE E SULLA COMPOSIZIONE DELLA CARCASSA

Scopo del lavoro è stato quello di studiare l'effetto dei gliceridi dell'acido butirrico, come integratori della dieta, sulle prestazioni produttive di broiler e sulle caratteristiche istologiche dell'intestino tenue, dal momento che gli acidi grassi a corta catena sono noti come fattori selettivi di protezione contro i parassiti intestinali, potenti fattori di crescita dei tessuti della parete intestinale, anche in termini di risposta immunitaria.

Si è condotta una prova su 150 broiler femmine Ross 308, assegnati a 5 trattamenti per un periodo di 35 giorni. I trattamenti erano: controllo con olio di soia come integratore energetico e 4 trattamenti con quantità crescenti (0,2, 0,35, 0,5 e 1% sul mangime) di una miscela di gliceridi (mono-, di- e tri-gliceri - di) dell'acido butirrico.
Gli animali trattati sono risultati più pesanti (P<0,05) e con migliori indici di conversione alimentare. Le caratteristiche delle carcasse non sono state influenzate dai trattamenti, ma la parete dell'intestino tenue ne è risultata leggermente modificata con villi più corti, ma microvilli più lunghi (P<0,01) e cripte più profonde nel duodeno (P<0,01), ma solo alla concentrazione più bassa di gliceridi (0,2%).
La conclusione è che i gliceridi dell'acido butirrico si sono dimostrati un efficace integratore delle diete per broiler, meritando una attenzione particolare come possibile alternativa all'uso di farmaci antimicrobici, nel momento in cui il loro impiego è stato messo al bando in Europa.

Parole chiave: *Butirrato, Gliceridi, Alimentazione dei broiler.*

Introduction

A recent review by Jósefiak et al. (2004) dealing with the fermentation of dietary structural carbohydrates in the avian gut, fermentation which leads to the production of short chain fatty acids (SCFA), acknowledges them as potent inhibiting factors of some pathogenic bacteria. Van Immerseel et al. (2004, 2005) tried SCFA as feed additives for the control of Salmonella enteritidis, with positive effects on the control of this pathogen. Isolauri et al. (2004) attributed to SCFA a growth promoting effect on the beneficial intestinal microflora, such as Lactobacillus and Bifidobacterium genera. Amongst SCFA, butyric acid (BA), is considered the prime enterocytes energy source and it is also necessary for the correct development of the gut associated lymphoid tissue (GALT) (Friedman et al., 2005). Moreover, Leeson et al. (2005) reported positive beneficial effects of BA on production performance traits of broiler chickens.

All these beneficial actions of BA in particular, make it a fatty acid deserving scientific and technical attention as a feed additive to poultry diets, particularly in concomitance with the banning of antibiotics in the EU.

The aim of the present trial was to study the effects of BA on the live performance of broiler chickens, looking also at the histological modifications of the gut tissues.

Since free BA is characterized by a strong unpleasant, penetrating smell, it is almost impossible to be coped with in the feed manufactory and results in poor intakes of the treated feed. For this reason the additive used in the present study was a mixture of mono-, di- tri-glycerides of BA, practically odorless.

Material and methods

Animals

One hundred and fifty Ross 308 female 1 day old chicks were randomly allotted to 5 pens on a peat litter, 30 birds per pen (treatment). Each bird was individually identifiable by a leg ring, changed as the bird grew.

Lighting program was 24 hours throughout the whole trial lasting 5 weeks.

Treatments

The additive used in the present study was a blend of mono-, di- and tri-glycerides of BA, produced to prevent the impact of the strong, unpleasant, penetrating smell of BA on feed intake. The animals were fed a starter, a grower and a finisher complete mixed feeds (Table 1). The 5 treatments differed in terms of quantity of glycerides blend added to the diet lipid fraction (see foot note of Table 1): plain soybean oil (SO) as the control treatment; SO integrated with a mixture of mono-, di- and tri-glycerides (MDT) of butyric acid (Table 2), 2 g/kg mixed feed, as treatment 1; SO with MDT, 3.5 g/kg mixed feed, as treatment 2; SO with MDT, 5 g/kg mixed feed, as treatment

Table 1. Ingredient composition (g/kg) of feed mixtures.

Ingredient	Starter (0-7 d)	Grower (8-21 d)	Finisher (22-35 d)
Maize meal	520.0	570.0	580.5
Soybean meal 48	355.5	331.0	310.0
Maize gluten feed	30.0	-	-
Dicalcium phosphate	19.0	19.0	19.0
Limestone	15.0	12.0	12.0
Sodium bicarbonate	2.5	2.5	2.5
Sodium chloride	2.5	2.5	2.5
DL-methionine	2.5	2.5	2.5
Lysine HCl	1.5	1.5	1.5
Choline chloride 50	1.5	1.5	1.5
Vitamin mineral premix	5.0	5.0	5.0
Lipid supplement [1]	45.0	52.5	63.0

[1] *Lipid supplement in the 5 different treatments:*
- treatment C, the control treatment, plain soybean oil;
- treatment 1, soybean oil containing 2.0 g butyric acid glycerides/kg mixed feed;
- treatment 2, soybean oil containing 3.5 g butyric acid glycerides/kg mixed feed;
- treatment 3, soybean oil containing 5.0 g butyric acid glycerides/kg mixed feed;
- treatment 4, soybean oil containing 10.0 g butyric acid glycerides/kg mixed feed, fed for 21 days and then replaced by plain soybean oil.

3 and SO with MDT, 10 g/kg mixed feed, fed for 21 days only and then plain SO, as treatment 4. It was decided to reduce treatment duration at 3 weeks in order to verify whether this short span of time was sufficient to modify and maintain the modifications in the gut mucosa. Each pen corresponded to a single treatment.

Measurements

Individual live body weights were recorded at 7 days, 15 days and at slaughter at 35 days. Group feed intakes were recorded daily.

Dressing percentages and weights of breasts, thighs and drumstick, separable fat and liver were measured on the slaughtered birds.

Samples of jejunum and ileum were collected from 3 birds per treatment at the slaughter house and histologically examined for villi, microvilli and crypts. The histological samples (collected from 5 replicates per bird) were prepared according to the current lab procedure: dehydration with ethyl alcohol, inclusion in paraffin, re-hydration and coloration with hematoxylin-eosin. The optical microscope Leitz “Dialux 20” was equipped with a digital camera JVC mod. TK-C 1380 set at 4 and 25 pixes magnification.

Table 2. Average composition of the mixture of glycerides of butyric acid (g/kg).

Dry matter	996
Free glycerol	1
Monoglyceride	245
Diglyceride	503
Triglyceride	248
Esterified butyric acid	597

Statistical analyses

All data referable to the single different treatments were analyses by means of the Statistical Analysis System (SAS, 1990) by the one-way ANOVA, keeping the factor "diet" as the fixed one.

Results and discussion

Live performance traits

Body weights, growth rate, feed intake and feed conversion rate are summarized in Tables 3 and 4. Treatment 2 resulted in the best weight gain at the end of the first week (P<0.05), but this significant difference was lost in the following periods. If the whole growth period is considered, the best growth performance was achieved with treatment 1, even though statistically better than the control birds only (P<0.05). Final weights resulted statistically different (P<0.05) from the control in any case but those relative to treatment 2: about 57 g..with treatment 1 (+ 3.2 %) and about 30 g with treatments 3 and 4 (+ 1.8 %). The figures of Table 4 seem to demonstrate that the supplementation with MDT determined higher growth rates with lower feed intakes during the first week: feed conversion rate of treated groups appeared lower (P<0.05) than the control one. Again, the better performance was lost during the following periods and in the whole period the chickens supplemented with MDT became more efficient than the control ones.

It appears that the presence of important amounts of BA in the gut may have worked significantly during the first days of life of the chicks only, but not afterwards. This is in complete agreement with the most recent literature (Friedman *et al.*, 2005). In fact, the lowest feed conversion rate at 7 days was registered in treatment 4, supplemented with the highest amount of MDT (10 g/kg), even though not for the whole growth period.

There are several bibliographic evidences, as highlighted by Friedman *et al.* (2005), that the development of adult-type GALT in the chicken occurs in early life, mostly in the first week, and

Table 3. Body weights and weight gains (30 replicates per treatment).

	Live weight (g)			Weight gain (g)		
	7 d	15 d	35 d	0-7 d	8-15 d	0-35 d
Control	127.3[ac]	374.8[a]	1824.6[a]	88.6[a]	248.4[a]	1785.9[a]
Treatment 1	126.1	355.8[bc]	1881.5[b]	88.3[a]	230.8	1843.7[b]
Treatment 2	132.1[a]	363.8[ac]	1806.2[a]	94.9[b]	232.5	1769.0
Treatment 3	120.4[b]	341.1[b]	1856.4[b]	83.2[a]	221.3[b]	1819.2
Treatment 4	124.2[bc]	368.7[ac]	1855.0[b]	86.7[a]	246.0[a]	1817.5
SE	2.3	6.8	32.8	2.3	7.1	22.1

Means within a column lacking a common superscript differ (P<0.05)
- control, plain soybean oil;
- treatment 1, soybean oil containing 2.0 g butyric acid glycerides/kg mixed feed;
- treatment 2, soybean oil containing 3.5 g butyric acid glycerides/kg mixed feed;
- treatment 3, soybean oil containing 5.0 g butyric acid glycerides/kg mixed feed;
- treatment 4, soybean oil containing 10.0 g butyric acid glycerides/kg mixed feed, fed for 21 days and then replaced by plain soybean oil.

Table 4. Average feed intakes and feed intake/weight gain ratios.

	Intakes (g)			Feed/gain		
	7 d	15 d	35 d	0-7 d	8-15 d	0-35 d
Control	104.1	326.7	2870.7	1.20	1.35	1.61
Treatment 1	93.2	320.0	2896.6	1.07	1.43	1.57
Treatment 2	98.7	306.7	2817.5	1.10	1.38	1.59
Treatment 3	87.8	317.2	2880.0	1.07	1.46	1.58
Treatment 4	87.8	306.7	2816.2	1.04	1.27	1.54

- control, plain soybean oil;
- treatment 1, soybean oil containing 2.0 g butyric acid glycerides/kg mixed feed;
- treatment 2, soybean oil containing 3.5 g butyric acid glycerides/kg mixed feed;
- treatment 3, soybean oil containing 5.0 g butyric acid glycerides/kg mixed feed;
- treatment 4, soybean oil containing 10.0 g butyric acid glycerides/kg mixed feed, fed for 21 days and then replaced by plain soybean oil.

that the functional development of the intestine as a digestive and absorptive organ is closely related to the maturity of GALT. In particular, Friedman *et al.* (2005) report several evidences that the functional maturation of enterocytes is driven by diet characteristics and that the development of functional GALT is consequent to the exposure to microflora antigens. Actually, the two aspects are strictly correlated: specific microbial populations degrade the feed structural carbohydrates in the gut, producing SCFA: of these butyric acid is known as the most effi-

Table 5. Carcass traits (30 replicates per treatment).

		Treatments					
		control	1	2	3	4	SE
Dressing out	%	84.3	85.3	84.6	85.0	85.2	0.4
Breast	g	335	369	355	349	355	18
Thigh and drumstick	"	409	427	427	405	427	9
Abdominal fat	"	28	29	38	39	28	5
Liver	"	41	41	43	41	37	3

- control, plain soybean oil;
- treatment 1, soybean oil containing 2.0 g butyric acid glycerides/kg mixed feed;
- treatment 2, soybean oil containing 3.5 g butyric acid glycerides/kg mixed feed;
- treatment 3, soybean oil containing 5.0 g butyric acid glycerides/kg mixed feed;
- treatment 4, soybean oil containing 10.0 g butyric acid glycerides/kg mixed feed, fed for 21 days and then replaced by plain soybean oil.

Table 6. Major histological traits of samples of jejunum and ileum of examined birds (3 replicates per treatment).

		Treatments					
		control	1	2	3	4	SE
Villus length jejunum	nm	1462^{Aa}	1337	1220^{B}	1305^{b}	1167^{Bc}	59
Villus length ileum	"	1101^{A}	857^{B}	856^{B}	697^{C}	787^{BC}	44
Villi jejunum, n./1250	"	6.28	5.75	8.83	7.33	7.50	0.61
Villi ileum n./1250	"	7.75^{a}	7.25^{a}	7.50^{a}	8.28	9.25^{b}	0.59
Microvillus jejunum	"	2.8^{A}	4.4^{B}	2.6^{A}	3.0^{A}	2.8^{A}	0.25
Microvillus ileum	"	3.4	3.8	3.1	3.3	3.5	0.23
Crypt depth jejunum		171^{A}	217^{B}	188	158^{A}	155^{A}	13
Crypt depth ileum	"	212^{Aa}	161^{B}	171^{bc}	185^{ab}	132^{Bc}	14

Means within a row lacking a common superscript differ (capital P<0.01; small P <0.05)
- control, plain soybean oil;
- treatment 1, soybean oil containing 2.0 g butyric acid glycerides/kg mixed feed;
- treatment 2, soybean oil containing 3.5 g butyric acid glycerides/kg mixed feed;
- treatment 3, soybean oil containing 5.0 g butyric acid glycerides/kg mixed feed;
- treatment 4, soybean oil containing 10.0 g butyric acid glycerides/kg mixed feed, fed for 21 days and then replaced by plain soybean oil.

cient one. As already reported by other authors (Isolauri *et al.*, 2004; Jósefiac *et al.*, 2004; Van Immerseel *et al.*, 2004, 2005; Friedman *et al.*, 2005; Leeson *et al.*, 2005) butyric acid is the major development promoter of the gut wall tissues and an important growth modulator of symbiotic intestinal microflora, as confirmed by our results at day 7.

Carcass traits

Carcass traits are summarized in Table 5. No statistically significant differences were found between treatments. Nevertheless, there was a trend towards higher dressing out percentages and heavier breasts and thighs in birds fed MDT, compared to SO. Treatments 2 and 3 appeared to increase the amount of abdominal fat. Lower liver weight was registered in 10 g MDT/kg feed.

Actually, all the treatments did not result significantly different from one another in terms of carcass yield. The not different size of the livers appears to mean that MDT was metabolized not differently from SO.

Histological samples

The major histological traits of the examined samples are summarized in Table 6.

Villus length appeared to be depressed by the presence of BA, both in jejunum and in ileum, while the number of villi per length unit (1250 nm) did not appear to be influenced, with the only exception of treatment 4 in the ileum.

The size of jejunum microvilli of treatment 1 birds was larger (P<0.01) than that of the other treatments, control included.

The crypt depth in the jejunum of birds receiving treatment 1 was significantly the largest (P<0.01).

The results of a previous trial have been confirmed (Leeson *et al.*, 2005).

The results lead to the following comments:

- the supplementation of the diet with butyric acid glycerides produced higher average body weights at 35 days : about 57 g with treatment 1 (+3.2%) and about 30 g with treatments 3 and 4 (+1.8%).
- feed intakes were not different, so that feed efficiencies were improved;
- the glyceride supplement had no influence on the carcass characteristics;
- the glyceride supplement had an influence on the morphology of the small intestine wall, with smaller villi, the density of which was increased, but larger crypt depth in jejunum, a sign of higher cell turn-over (Zhang *et al.*, 2005).

Further studies are needed to investigate the effect on GALT, which that is responsible for the immune modulation response of the gut, as suggested by the cited Friedman *et al.* (2005).

Conclusions

The blend of butyrate glycerides, supplemented to broilers' diets confirmed its beneficial effects on live performance and carcass traits of treated birds at the level of 2 g/kg mixed feed.

The morphology of the gut wall was slightly modified with shorter villi, longer microvilli and deeper crypts, again at the lowest level of 2 g/kg mixed feed.

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