

Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks

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To investigate the effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity, female broiler chicks were fed on either no additive (control) or dried *B. subtilis*-culture-supplemented commercial diets (215 g crude protein/kg, 12.85 MJ metabolizable energy/kg) at 10 or 20 g/kg diet for 28 d from 14 to 42 d of age. Body weight, and moisture, fat, protein and ash contents of the body were not influenced by the *B. subtilis* culture. Feed efficiency, N utilization, the ratio of abdominal fat or liver to body weight, acetyl-coenzyme A carboxylase (EC 6.4.1.2) activity, liver and serum cholesterol contents were significantly lower in treatment groups, while fatty acid synthetase activity and serum cholesterol concentration were not significantly different, compared with the control group. Liver triacylglycerol concentration was decreased in chicks given 20 g culture/kg diet, while serum and carcass triacylglycerol concentrations were significantly lower in treatment groups than in the control group. Serum phospholipid concentration was increased but carcass phospholipid concentration was decreased in chicks given 20 g *B. subtilis*/kg diet, while liver phospholipid concentration was not significantly influenced. The advantages of inclusion of *B. subtilis* to the broiler diet included improved feed efficiency, less abdominal fat, reduced triacylglycerol concentrations in the liver, serum and carcass and reduced cholesterol concentrations in the liver and carcass.

Dried *Bacillus subtilis* culture: Feed efficiency: Abdominal fat: Cholesterol: Triacylglycerol

Recently, investigators have become interested in the advantages of substituting micro-organisms for antibiotics, because the continued use of subtherapeutic levels of antibiotics in animal feeds may result in the development of drug resistant strains of micro-organisms that are infectious to humans (Jiraphocakul *et al.* 1990). Moreover, increases in carcass fat and cholesterol contents of modern broiler chickens continue to be a health concern of consumers. In addition, abdominal and visceral fat are waste products to the poultry processor and add to waste management problems. Furthermore, accumulation of triacylglycerol in the liver results in fatty liver in broiler chicks.

Pertaining to these problems, recent investigations have shown that the inclusion of micro-organisms in animal diets prevents diarrhoea (Sissons, 1989), reduces serum cholesterol (Danielson *et al.* 1989; Imaizumi *et al.* 1992), reduces body fat (Chah *et al.* 1975), and improves growth and feed efficiency (Chah *et al.* 1975; Goodling *et al.* 1987; Jiraphocakul *et al.* 1990). There have been few investigations of the effects of *B. subtilis*

* For reprints.

Table 1. *Composition of the experimental diets (g/kg diet)**

	Control	<i>Bacillus subtilis</i> supplemented	
		+ 10 g	+ 20 g
Moisture	126	123	122
Protein	215	218	224
Fat	59	59	59
Fibre	50	50	50
Ash	80	80	80
Nitrogen-free extract†	470	470	465

* All variables were analysed four times.

† Nitrogen-free extract = 1000 - (moisture + protein + fat + fibre + ash).

culture in poultry. Therefore, the present study was conducted to evaluate the effect of a dried *B. subtilis* culture added to the diet on growth, body composition, lipogenic enzyme activities and various lipid fractions in liver, serum and carcass of broiler chicks.

MATERIALS AND METHODS

Animals and diets

Thirty 1-d-old female broiler chicks (Chunky) were raised in individual wire-floor cages and fed on a commercial starter diet with no antibiotics included (215 g crude protein/kg, 12.85 MJ metabolizable energy/kg). Feed and water were provided *ad lib*. House temperature was maintained at $25 \pm 3^\circ$ with a photoperiod of 14 h. At 14 d of age, female broiler chicks were weighed individually and divided into three treatment groups. One group was a control with no additive, and two treatment groups were given the commercial diet supplemented with 10 or 20 g dried *B. subtilis* culture/kg. The composition of the experimental diets after inclusion of the dried *B. subtilis* culture is presented in Table 1. Broilers were weighed individually on a weekly basis, and feed consumption was recorded daily.

General procedure

At 42 d of age, five female broiler chicks from each group were killed by decapitation and abdominal fat and liver were immediately removed and weighed. A 4 g portion of each liver was flushed with ice-cold saline (9 g NaCl/l) before assessment of lipogenic enzyme activity, and another 4 g portion was used for analysis of various lipid fractions; the remainder was returned to the carcass. The carcasses (the body without digestive contents and feathers) were minced five times to obtain uniform mixing.

The various lipid fractions were separated by thin-layer chromatography on silica-gel chromarods using hexane-diethyl ether-formic acid (60:10:0.1, by vol.) and hexane-benzene (1:1, v/v) as developing solvents, and quantified using an Iatroscan TH-10 TLC/FID Analyser (Iatron Laboratories Inc., Tokyo, Japan) (Tanaka *et al.* 1979). Carcass composition was determined by the method of the Association of Official Analytical Chemists (1980). For measuring nutrient utilization, faeces from five chicks of each group were collected 1 week before the experiment finished. Each sample was analysed three times, and results were accepted if the within-sample differences were less than 1%.

Table 2. *Effect of 10 or 20 g dried Bacillus subtilis/kg diet on performance characteristics in female broilers aged 42 d†*
(Mean values and standard deviations for ten chicks)

	Control (C)		+10 g		+20 g		C v. 10+20	10 v. 20
	Mean	SD	Mean	SD	Mean	SD		
Body wt (BW; g)	1835	28	1874	26	1874	30	NS	NS
Cumulative feed intake (g)	2819	43	2701	44	2734	14	NS	NS
Cumulative feed efficiency (g wt gain/g feed)	0.52	0.02	0.56	0.02	0.55	0.01	**	NS
Abdominal fat wt (g/kg BW)	18	3	16	2	15	1	*	NS
Liver wt (g/kg BW)	23	3	20	1	20	1	**	NS
Caecum length (mm/kg BW)	17	1	18	1	19	2	*	NS
Caecum weight‡ (g/kg BW)	4.5	1.5	5.2	1.0	4.6	1.2	NS	NS

* $P < 0.05$, ** $P < 0.01$.

† For details of diets and procedures, see Table 1 and p. 524.

‡ Caecum plus its contents.

Table 3. *Effects of 10 or 20 g Bacillus subtilis culture/kg diet on body composition (g/kg carcass weight) and nutrient utilization (g/g) in female broilers aged 42 d†*
(Mean values and standard deviations for five chicks)

	Control (C)		+ 10 g		+ 20 g		C v. 10+20	10 v. 20
	Mean	SD	Mean	SD	Mean	SD		
Moisture	652	11	663	8	670	5	NS	NS
Protein	164	7	162	6	163	6	NS	NS
Fat	145	16	137	9	134	6	NS	NS
Ash	39	6	38	4	33	2	NS	NS
Nutrient utilization								
Dry matter	0.73	0.02	0.73	0.01	0.73	0.02	NS	NS
Fat	0.81	0.02	0.81	0.01	0.82	0.01	NS	NS
Nitrogen	0.56	0.02	0.59	0.01	0.58	0.01	*	NS

* $P < 0.05$.

† For details of diets and procedures, see Table 1 and p. 524.

Table 4. *Effects of 10 or 20 g Bacillus subtilis/kg diet on lipogenic enzyme activity (nmol/min per mg protein†) in female broilers aged 42 d‡*
(Mean values and standard deviations for five chicks)

	Control (C)		+ 10 g		+ 20 g		C v. 10+20	10 v. 20
	Mean	SD	Mean	SD	Mean	SD		
Acetyl-CoA carboxylase (<i>EC</i> 6.4.1.2)	0.232	0.020	0.223	0.014	0.197	0.014	*	*
Fatty acid synthetase	4.26	0.77	4.78	0.65	3.94	0.45	NS	NS

* $P < 0.05$.

† Enzyme activities are expressed as nmol substrate converted to product/min per mg protein at 37°.

‡ For details of diets and procedures, see Table 1 and pp. 524–526.

Enzyme assay

Livers were homogenized in 0.25 M-sucrose solution containing 1 mM-EDTA-2Na, after which the homogenates were centrifuged at 600 g at 4° for 10 min. The supernatant fractions were recentrifuged at 105 000 g at 4° for 60 min and the resulting clear supernatant fractions (cytosolic fraction) were used for assaying lipogenic enzymes. Acetyl-CoA carboxylase (*EC* 6.4.1.2) activity was assayed by a $H^{14}CO_3$ -fixation method (Qureshi *et al.* 1980). Fatty acid synthetase activity was assayed by the $[1-^{14}C]CoA$ incorporation method (Hsu *et al.* 1965). The protein content of the solution used for each enzyme assay was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Enzyme activities are expressed as nmol substrate converted to product/min per mg protein at 37°.

Statistical analyses

Treatment effects were assessed for all response variables using one-way ANOVA in which the overall treatment differences were represented by two orthogonal contrasts: (1) control v. supplement and (2) low v. high levels of supplementation. Differences were considered statistically significant at the 5% level. Where appropriate, regression analysis was used to assess the statistical significance of the correlation between variables.

Table 5. Effects of 10 or 20 g *Bacillus subtilis* culture/kg diet on lipid fractions in the liver, serum and carcass of broiler chicks aged 42 d†

(Mean values and standard deviations for five chicks)

	Control (C)		+ 10 g		+ 20 g		C v. 10 + 20	10 v. 20
	Mean	SD	Mean	SD	Mean	SD		
Liver (mg/g)								
Triacylglycerol	21.6	5.6	18.9	4.1	12.6	3.2	**	*
Total cholesterol	3.4	0.2	3.2	0.1	3.1	0.1	*	NS
Phospholipid	9.4	0.6	10.6	0.3	9.3	0.4	NS	NS
Serum (mmol/l)								
Triacylglycerol	0.39	0.07	0.29	0.05	0.32	0.04	*	NS
Total cholesterol	4.62	0.15	4.60	0.22	4.33	0.15	NS	NS
Phospholipid (mg/l)	965	70	924	99	1738	103	**	**
Carcass (mg/g)								
Triacylglycerol	9.9	2.0	6.7	1.1	6.6	0.5	**	NS
Total cholesterol	2.2	0.2	1.8	0.1	1.5	0.1	**	NS
Phospholipid	0.7	0.2	0.7	0.05	0.3	0.1	**	**

* $P < 0.05$, ** $P < 0.01$.

† For details of diets and procedures, see Table 1 and p. 524.

RESULTS

Growth performance study

As shown in Table 2, statistically significant differences were not seen in body weight, feed intake and caecum weight among the groups. An improved feed efficiency was observed in the 10 and 20 g *B. subtilis*-supplemented groups compared with control chicks ($P < 0.01$). The ratios of liver and abdominal fat to body weight were significantly decreased in treatment groups, compared with the control. Caecum lengths of broiler chicks given 10 or 20 g *B. subtilis* were significantly greater than those of control chicks.

Body composition and nutrient utilization

None of the groups exhibited any changes in body moisture, protein, fat, ash, dry matter or fat utilization (Table 3). A significantly improved N utilization was observed in chicks given 10 or 20 g *B. subtilis*/kg diet, compared with the control.

Hepatic lipogenic enzyme activities

Hepatic acetyl-CoA carboxylase activity was significantly reduced when 20 g dried *B. subtilis* culture/kg was added to the diet (Table 4). Fatty acid synthetase activity, however, was not significantly different.

Lipid fractions study

The effects of dried *B. subtilis* culture on liver, serum and carcass lipid fractions are presented in Table 5. The liver triacylglycerol concentration was significantly reduced in 20 g/kg *B. subtilis*-culture-fed chicks, compared with other groups. Triacylglycerol concentrations in the serum and carcass were significantly reduced in the treatment groups compared with the controls. Cholesterol concentrations in the liver and carcass were significantly reduced in the treatment groups, compared with the control, whereas those in

the serum were not significantly different. In the present study dried *B. subtilis* culture did not influence liver phospholipids. However, broiler chicks given 20 g *B. subtilis* culture/kg diet had higher levels of serum phospholipids and lower levels of carcass phospholipids.

DISCUSSION

The purpose of the present experiment was to determine the advantages of dried *B. subtilis* culture on growth performance, body composition and lipid metabolism in broiler chicks. Broilers receiving the bacterial culture showed slightly greater weight gain and significantly improved feed efficiency. Similar results have been obtained using calves (Jenny *et al.* 1991) and turkeys (Jiraphocakul *et al.* 1990). Continuous feeding of *B. subtilis* to animals provides a constant inoculation of the organism in the alimentary tract (Jiraphocakul *et al.* 1990). Furthermore, this organism may associate with the gut wall and favour an increase in numbers of natural lactobacilli, which, in turn, will suppress the growth of undesirable enteric micro-organisms such as *Escherichia coli*, thus improving feed efficiency.

The effect of *B. subtilis* culture supplementation was more pronounced in decreasing abdominal fat than body fat content. It has been suggested that considerable changes in abdominal fat sometimes occur without large changes in body fat (Ricard *et al.* 1983). The decreased acetyl-CoA carboxylase activity observed in the present study may partly explain the decreased abdominal fat observed in the treatment groups (Hasegawa *et al.* 1994). This was confirmed by the regression analysis (Table 6). Decreased abdominal fat would constitute an important advantage in broiler production, because this fat is a waste product to the poultry processor and adds to waste management problems. It is of interest to note that there was a relationship between body fat content and caecum length, feed efficiency and body weight (Table 6). It is generally accepted that there is a relationship between body fat and body moisture, and between abdominal fat and body fat. The present study confirms this view.

In the present study *B. subtilis* culture influenced fatty acid synthesis in the liver of female broilers as indicated by a decrease in the activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis. Decreased fatty acid synthesis is of major importance for the triacylglycerol-lowering effect (Skorve *et al.* 1993). Thus, it is not surprising that liver, serum and carcass triacylglycerol concentrations were decreased. A

Table 6. Regression analysis results
(Observations from fifteen chicks)

<i>x</i>	<i>y</i>	<i>a</i>	<i>b</i>	<i>r</i> ²	<i>P</i>
Body moisture	Body fat	1.175	-0.005	0.998	< 0.01
Body fat	Caecum length	4.159	-0.170	0.9356	< 0.01
Body fat	ACC	0.148	+0.004	0.998	< 0.01
Body fat	Abdominal fat	-2.084	+0.268	0.996	< 0.01
Body fat	Feed efficiency	0.987	-0.032	0.762	< 0.01
Body weight	Body fat	59.198	-0.024	0.930	< 0.01
Liver TG	Serum TG	13.821	+0.890	0.811	< 0.01
Liver TG	Carcass TG	2.344	+0.305	0.561	< 0.05
Liver FC	Serum FC	71.464	+31.929	0.627	< 0.05
Liver FC	Carcass FC	-5.557	+2.286	0.988	< 0.01
Abdominal fat	ACC	-0.165	+0.028	0.762	< 0.01
Liver weight	Liver TG	-23.250	+19.500	0.535	< 0.05

ACC, acetyl CoA carboxylase (*EC* 6.4.1.2) activity; TG, triacylglycerol; FC, free cholesterol.

reduction in hepatic triacylglycerol concentration is known to be beneficial for chicks, inhibiting the development of fatty liver, whilst a lower triacylglycerol content in the carcass would result in a healthier meat for the consumer. Our results also indicate that *B. subtilis* might have anticholesterolaemic properties. It is unclear, however, by which mechanism *B. subtilis* culture decreases the cholesterol concentration. Youn *et al.* (1993) stated that there was a positive relationship between the activity of hepatic 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase (*EC* 1.1.1.88), the rate limiting enzyme of cholesterologenesis, and the hepatic cholesterol content of growing chicks. Therefore, reduced hepatic cholesterol synthesis may be one of the factors contributing to the decreased liver cholesterol concentration. A lower cholesterol concentration in the liver would result in less cholesterol in the serum and carcass (Table 6). In the present study there was also a relationship between liver triacylglycerol and serum triacylglycerol or carcass triacylglycerol (Table 6). Inclusion of 20 g *B. subtilis*/kg diet increased the serum phospholipid concentration and decreased carcass phospholipid, but it did not affect liver phospholipid. It is difficult from the present study to discuss this result. It would be logical to conclude that dried *B. subtilis* culture added to the broiler diet had a beneficial impact on broiler production and consumer health.

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