EFFECT OF BACTERIAL INOCULATION ON NEUTRAL DETERGENT FIBRE DIGESTION AND ENERGY AVAILABILITY IN GERM-FREE CHICKENS

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Summary

The present study was done to examine whether inoculated and established bacteria in the digestive tract of germ-free (GF) chickens affect growth performance, energy availability, nitrogen utilization and neutral detergent fibre (NDF) digestibility of the host bird fed a high-fibre diet. Gnotobiotic (GB) chicks were made from GF birds by co-inoculating with *Ruminococcus albus*, and *Staphylococcus warneri*, only the latter of which was established in the chicken gut. No difference was detected among conventional (CV), GF and GB birds in body weight gain, food intake or food efficiency from 7 to 21 d of age. The amount of nitrogen retained was larger in CV than in GF and GB chicks. DE and ME values of the diet and NDF digestibility were higher in CV birds than in GF and GB counterparts. It was concluded, therefore, that the established bacterium *S. warneri* did not give any beneficial effects on the host bird as judged by growth performance, energy availability, nitrogen utilization, and NDF digestibility.

(Key Words: Bacterial Inoculation, *Ruminococcus albus*, *Staphylococcus warneri*, GF, Chickens, Protein and Energy Utilization)

Introduction

Hegde et al. (1982) suggested that in the chicken, the gut microflora could supply some, though relatively little, energy to the host from digestion of dietary fibre. This is shown by an increase in the ME value of a diet, being equivalent to about 17% of corn starch on the basis of ME. Although the amount of energy available from fibre digestion in the conventional (CV) state appears to be small, it may be useful for growth promotion particularly when the bird is deficient in energy (Muramatsu et al., 1991).

The ability of CV chickens to utilize dietary fibre as an energy source could be improved if bacteria like *Ruminococcus albus*, which have strong cellulase activity (Ohmiya et al., 1983), become established and dominant in the gut. Therefore, the present study was done to investigate if inoculated bacteria having cellulase activity can inhabit the chicken gut, and, if so, to examine whether the inhabitant bacteria can benefit the nutrient utilization of host birds.

Materials and Methods

Animals

The details of production of germ-free (GF) chicks and inspection of sterility status were described previously (Furuse and Yokota, 1984; Muramatsu et al., 1988). Briefly, fertilized eggs from Single Comb White Leghorn hens and cocks (from the Gifu Prefectural Poultry Experimental Station, Japan) were incubated at 37.8℃ for 18 d. Eggs for the production of GF and gnotobiotic (GB) chicks were placed in sterilized plastic isolators where conditions maintained were similar to those in the incubator and left there for 3 d to hatch. The remaining eggs were returned to the incubator and the chicks hatched there were used as CV controls. The GF and GB birds were reared individually in wire-mesh metabolism cages inside the isolators and the CV controls were housed individually in similar cages in a room where environmental conditions were matched to those maintained within the isolators. GB birds were made from GF chicks by co-inoculating with *Ruminococcus albus* and *Staphylococcus warneri* which were introduced into the

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Received August 12, 1991

Accepted December 24, 1991

lower gut at a dose of approximately 10^8/bird in 1 ml of the growing medium (Hoshino et al., 1991) from cloaca at 6, 13 and 18 d of age. Numbers of birds used for the experiment were 7, 8 and 16 in GF, GB and CV environments, respectively.

Experimental Procedures

The composition of the experimental diet mainly based on chick mash, isolated soybean protein and cellulose (Pulpflock, Sanyo Kokusaku Pulp Co. Ltd., Japan) is given in table 1. The

TABLE 1. COMPOSITION OF THE EXPERIMENTAL DIET

Ingredient	g/kg
Chick mash[1]	411.9
Isolated soybean protein[2]	165.9
Cellulose[3]	279.9
Corn oil	75.8
Vitamin mixture[4]	8.0
Mineral mixture[5]	58.5
Calculated value :	
Crude protein (g/kg)	197
Metabolizable energy (kJ/g)	10.0

[1] Diet No. 21 (Aichi-ken Agricultural Research Center, Japan; CP 140 g/kg; ME 11.1 kJ/g).

[2] Fujipro-R: CP 84% (Fuji Oil Co. Ltd., Osaka, Japan).

[3] Pulpflock W-1 (Sanyo Kokusaku Pulp Co. Ltd., Japan).

[4] Muramatsu et al. (1987).

[5] Nesheim et al. (1962), except for selenium which was included at twice the published value.

chick mash contained (g/kg): maize, 582; wheat bran, 126; fat-extracted rice bran, 63; corn-gluten feed, 63; rapeseed meal, 40; soybean meal, 23; fish meal, 23; alfalfa meal, 48; $CaCO_3$, 16; $CaHPO_4$, 10; NaCl, 2; vitamin premix, 4. The diet was placed in doubly-wrapped plastic bags and irradiated at 50 kGy from a ^{60}Co source. The sterilized diets were given to the CV birds as well as to the GF and GB birds. Birds were not fed for 2 d after hatching, and thereafter were given the experimental diet and water *ad libitum* until 21 d of age. The body weight gain and food intake were measured on alternate days from 7 to 21 d of age. From 18 to 21 d of age, droppings were collected into 100 ml of 0.06 M hydrochloric acid in a deep, stainless-steel tray located beneath each metabolism cage. The acid prevented further microbial action in the droppings and loss of ammonia. The droppings were dried in a forced-air oven at 55℃ for 48 h and were ground for analysis.

Chemical analysis

Total N in the droppings and the diet was analyzed by a Kjeldahl method. The combustion energy of the diet, cellulose and of droppings was determined with an automated bomb calorimeter (Shimadzu CA-4P, Shimadzu Co., Kyoto, Japan). Neutral detergent fibre (NDF) was determined according to the method of Van Soest and Wine (1967). Dietary ME value was calculated after the correction for retained N (Hill and Anderson, 1958).

For obtaining DE value of the diet, urinary energy was subtracted from the total energy excreted in the droppings. The urinary energy was estimated from urinary N compounds which were extracted chemically with saturated Li_2CO_3 as described previously (Muramatsu et al., 1991). Approximately 2 g of the ground droppings was weighed accurately and placed in a 200 ml flask to which about 80 ml of saturated Li_2CO_3 was added and homogenized. The flask was sealed and incubated at 37℃ overnight, and the Li_2CO_3 extract was then filtered and made to 100 ml with saturated Li_2CO_3.

Total N, protein and uric acid in the Li_2CO_3 extract were determined by the Kjeldahl method, the method of Lowry et al. (1951), and that of Pudelkiewicz et al. (1968), respectively. A Conway's micro-diffusion method was used for the determination of ammonia and urea in the extract. Total creatinine in the extract was determined by a Jaffe reaction as described previously (Muramatsu and Okumura, 1979). The losses of N were corrected for each compound from the recoveries which were measured by adding known amounts of the respective compounds to the collection tray with homogenized droppings.

Urinary N contents were defined as total non-protein N, which was calculated from the difference between total N and protein N in the Li_2CO_3 extract. As a result, fecal N was obtained by subtracting the urinary N thus calculated in the extract from total N in the droppings. Urinary energy was estimated by the sum of each

urinary N compound multiplied by the corresponding factors (Tasaki and Sakurai, 1963) as follows: uric acid, 34.3 kJ/g N; ammonia, 30.6 kJ/g N; urea, 22.6 kJ/g N; total creatinine, 52.0 kJ/g N. Since there was a small, but significant amount of unidentified N detected in the extract, its energetic value was tentatively given as 34.9 kJ/g N by taking the average combustion energy of other extracted compounds.

Statistical analysis

The data were treated statistically according to a completely randomized design by an analysis of variance. Although the numbers of replicates were not equal among treatments due to limitation on facilities, the significance of differences between means was assessed by Duncan's multiple range test (Duncan, 1955) by taking a harmonic mean of replicates as 9.08.

Results

The establishment of the inoculated bacterium and its identification was described in detail elsewhere (Hoshino et al., 1991). Briefly, at the end of the experiment, establishment of *S. warneri* in the digestive tract was confirmed whereas that of *R. albus* was not. The number of colonies of *S. warneri* grown on an agar plate was larger in the samples from the lower gut, i.e. caecum and colon than in those from the upper gut, i.e. crop, duodenum and ileum.

Table 2 gives performance results and N utilization of GF, GB and CV chicks. Although there was no significant difference in body weight gain, food intake, food efficiency and N intake, CV birds tended to have better performance than GF and GB counterparts. Total N and urinary N excretions were lower in CV than in GF and GB birds, and thereby N retention and N utilization were significantly improved ($p < 0.05$) in the presence of the CV gut microflora.

Distribution of urinary N compounds is given in table 3. Total N, uric acid N and total creatinine N were significantly lower ($p < 0.05$) in CV birds than in GF and GB chickens, whereas the opposite was true for ammonia N. Although no significant difference was found in urea N, the value tended to be lower in the presence of the CV gut microflora.

The values of energy excretion, dietary energy and NDF digestibility are shown in table 4. No significant changes were found in total and urinary energy excretion among treatments. The values for DE, ME and NDF digestibility were significantly higher ($p < 0.05$) in CV birds than in GF and GB counterparts.

Discussion

Mitsuoka (1977) suggested that at least about one week is necessary for certain bacteria to be established in the chicken gut. In a preliminary experiment, in fact, the establishment of *S.*

TABLE 2. BODY WEIGHT GAIN, FOOD INTAKE, FOOD EFFICIENCY AND NITROGEN UTILIZATION OF GERM-FREE (GF), GNOTOBIOTIC (GB) AND CONVENTIONAL (CV) CHICKS[1]

	GF	GB	CV	SEM[2]
Body weight gain (g/14 d)	51.7	57.0	61.4	4.8
Food intake (g/14 d)	248	250	242	10
Food efficiency (g gain/g intake)	0.21	0.23	0.25	0.014
N intake (mg/d)[3]	589	569	640	34
Total N excretion (mg/d)[3]	443[a]	453[a]	368[b]	22
Urinary N excretion (mg/d)[3]	367[a]	352[a]	270[b]	19
N retained (mg/d)[3]	146[a]	116[a]	272[b]	35
N utilization[3,4]	0.24[a]	0.20[a]	0.41[b]	0.04

[ab] Means not sharing a common superscript letter are significantly different at $p < 0.05$.

[1] GB birds were mono-associated with *Staphylococcus warneri* which was inoculated and established in the gut.

[2] Standard error of mean.

[3] Measured for the last 3 d, from 18 to 21 d of age.

[4] Calculated as mg N retained/mg N intake.

TABLE 3. DISTRIBUTION OF NITROGENEOUS COMPOUNDS EXTRACTED WITH Li_2CO_3 IN DROPPINGS OF GERM-FREE (GF), GNOTOBIOTIC (GB) AND CONVENTIONAL (CV) CHICKS[1]

	GF	GB	CV	SEM[2]
Total N (mg/d)[3]	186[a]	184[a]	134[b]	16
Uric acid N (mg/d)[3]	112[a]	115[a]	83[b]	7
Ammonia N (mg/d)[3]	13.6[a]	16.7[a]	32.0[b]	3.5
Urea N (mg/d)[3]	37.8	32.0	29.0	4.8
Total creatinine N (mg/d)[3]	7.4[a]	7.0[a]	4.7[b]	0.4
Unidentified N (mg/d)[3]	15.2	13.3	30.6	8.9

[a,b] Means not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] See footnote to table 2.
[2] Standard error of means.
[3] Measured for the last 3 d, from 18 to 21 d of age.

TABLE 4. ENERGY EXCRETION, DIETARY ENERGY VALUE AND NEUTRAL DETERGENT FIBRE (NDF) DIGESTIBILITY OF GERM-FREE (GF), GNOTOBIOTIC (GB) AND CONVENTIONAL (CV) CHICKS[1]

	GF	GB	CV	SEM[2]
Total energy excretion (kJ/d)[3]	192	183	164	9
Urinary energy excretion (kJ/d)[3]	6.0	6.0	6.1	0.5
Digestible energy (kJ/g)[3]	7.6[a]	7.8[a]	9.5[b]	0.4
Metabolizable energy (kJ/g)[3]	7.0[a]	7.2[a]	9.0[b]	0.4
NDF digestibility (%)[3]	0.9[a]	1.0[a]	12.3[b]	2.7

[a,b] Means not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] See footnote to table 2.
[2] Standard error of mean.
[3] Measured for the last 3 d, from 18 to 21 d of age.

warneri, was confirmed already 2 d after the inoculation in the lower gut of GB chicks (data not shown). It follows, therefore, that detection more than 7 d after single inoculation should be sufficient to examine if the introduced bacteria can inhabit the chicken gut. In the present study, however, multiple inoculation of the bacteria was performed not only to ensure their establishment in the chicken gut but also to strengthen any possible effects on host growth and nutrient utilization.

GB technique has been proved to be a useful tool to study interaction between specific gut microflora and host nutrition, metabolism and physiology in chickens. Fuller et al. (1978), for example, employed mono-contaminated GB chicks to identify a growth inhibitory factor produced in the gut. Szylit and Charlet (1981) attempted to improve growth of GF chickens by mono-association with *Lactobacillus spp*, although the results were equivocal.

The present study was conducted in the hope to improve the ability of degrading dietary fibre by introducing new bacterial species having cellulase activity into the chicken gut, and thereby to release energy which could then become available to the host GB bird. Cellulose was chosen for this purpose as dietary fibre because in CV chickens cellulose was a better energy source than hemicellulose, although the latter is more soluble than the former (our unpublished observation). As bacteria to be inoculated, *R. albus* which has a cellulose-degrading enzyme, and *S. warneri* which helps proliferation of *R. albus* (Hoshino et al., 1991) were used. Unfortunately, the established bacterium was not *R. albus* but was *S. warneri*. The inhabiting *S. warneri* did not give any beneficial effects to the host bird as judged by the growth performance, energy availability and fibre digestibility. Likewise, by comparing

GF and GB birds no significant effect of established *S. warneri* was found in either N utilization or excretion pattern of urinary N compounds.

In contrast to the little influence of the inoculated bacteria, effects of CV microflora were evident. Urinary N excretion and hence total N excretion were significantly lower in the CV chicks than the rest, resulting in higher N retention and N utilization in CV than in GF and GB environments. Growth and food efficiency showed a similar tendency, although no statistical significance was reached. In addition, DE and ME values of the diet and NDF digestibility were significantly higher in CV birds than in GF and GB counterparts. All these results confirmed our previous findings (Muramatsu et al., 1991). Furthermore, the excretion of urinary N compounds indicated a pattern typically found in the GF and CV environments, i.e. high ammonia in CV birds and high urea in GF counterparts. Since urease activity detected in the gut of the chick is originating from bacteria (Delluva et al., 1968), higher ammonia in the CV state and higher urea in the GF environment were in good agreement with the previous findings (Visek, 1974; Salter et al., 1974; Okumura et al., 1978).

Thus, although the present attempt to improve chicken growth by bacterial inoculation was unsuccessful, the results (tables 2 and 4) support the idea that with a diet high in dietary fibre, the chick reared in the CV environment could utilize dietary fibre as an energy source probably through bacterial carbohydrase activity (Hegde et al., 1982). Whether the released energy from dietary fibre could be reflected in growth or protein and energy gains of the bird may primarily be determined by its need for energy: in energy deficient birds, energy released from fibre fermentation by bacterial actions, though small in quantity, may well be utilized for growth and body protein accretion (Muramatsu et al., 1991). This suggests that under certain circumstances there is still some room for chicken growth to be improved by manipulating bacterial population or by changing inherent characteristics of inhabitant bacteria *per se*.

The ME and DE values of the diet in the CV state were considerably higher than those found in the GF and GB states (table 4). Assuming that the combustion energy of cellulose is 16.77 kJ/g as determined by a bomb calorimeter, only 30-35% of the differences in ME and DE values found between the CV and GF or GB environments could be accounted for by the energy arising from NDF digestion. The rest of the unaccountable difference in ME or DE values between CV and GF or GB states would, therefore, have to be explained by the differences in digestibility of other nutrients, although the true reason for this discrepancy remained unknown.

Acknowledgements

The present study was supported by a Grant-in-Aid (No. 6360038) for Developmental Scientific Research from the Ministry of Education, Science and Culture, Japan.

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