Direct-Fed Microbial PrimaLac and Salinomycin Modulate Whole-Body and Intestinal Oxygen Consumption and Intestinal Mucosal Cytokine Production in the Broiler Chick[1]

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ABSTRACT The current study investigated whole-body O_2 consumption, intestinal O_2 consumption, and intestinal inflammation status through mucosal cytokine production on broiler chicks fed the direct-fed microbial PrimaLac. One hundred twenty 1-d-old broiler chicks were randomly assigned to 1 of 3 experimental diets: standard starter diet (control), standard starter diet with added salinomycin (SAL), and standard starter diet with added PrimaLac (DFM). Birds were housed in 2 separate rooms, the control and SAL treatments in one room and the DFM in another. Intact ileal and cecal samples were collected on d 19, 20, and 21 after measuring whole-body O_2 consumption using indirect calorimetry. The O_2 uptake of ileal tissue was measured using an in vitro O_2 monitor. Analysis of intestinal immune status of broilers was measured by the relative differences in mRNA of both pro- and antiinflammatory cytokines: interleukin-(IL) 1β, IL-6, and IL-10 using real-time reverse transcription-PCR. Broilers exhibited a 6 to 16% decrease in whole-body energy expenditures and up to a 47% decrease ($P < 0.05$) in ileal energy expenditures in the DFM group compared with other treatments. The reverse transcription-PCR data demonstrated that DFM consortium numerically altered both pro- and antiinflammatory cytokines within the ileum of 19-d posthatch broilers. These data suggest that direct-fed microbials like PrimaLac increase metabolic efficiency via changes in intestinal physiology and metabolism.

Key words: broiler, direct-fed microbial, body energetics, cytokine

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INTRODUCTION

Due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in the United States (Patterson and Burkholder, 2003), there is an increasing interest in finding alternatives to antibiotics in poultry production. One choice could be direct-fed microbials (**DFM**), also called probiotics, which are live microbial feed supplements that beneficially affect the host animal by improving its intestinal health (Fuller, 1989). The DFM can be composed of one or several different species of microorganisms including bacteria and yeast (Patterson and Burkholder, 2003). The DFM colonization characteristics of bacterial species can differ (Isolauri et al., 2004). Additionally, different strains of the same species of DFM can have unique biological activity, such as different sites of adhesion, specific immunological effects, and fermentation characteristics (Isolauri et al., 2004). Despite advances in microbial molecular biology and the availability of significant amounts of genome sequences for most commensal intestinal bacteria, the current understanding of the biological actions of DFM microflora is incomplete (Mai, 2004). This is especially true of the effects of DFM on whole-body and organ tissue energy expenditures. No studies have described the effects of DFM on whole-animal or intestinal metabolism. Previous research has shown that intestinal inflammation is an energy-consuming process. The gastrointestinal (**GI**) tract of most animals has been estimated to consume 25% of total energy needs (Cant et al., 1996). The immune system has been estimated to account for approximately 1 to 3% of the basal metabolic rate in healthy vertebrates (Romanyukha et al., 2006). In contrast, immunologically challenged vertebrates can have resting metabolic rates that are increased from 8 to 27% (Martin et al., 2003), which might suggest that immunological challenges increase caloric demands.

Interaction between DFM bacteria and the intestinal epithelium is called cross-talk and is currently the object of intensive investigation (Kohler et al., 2003). The beneficial effects of DFM bacteria are dependent on the interaction with the innate immune system and possibly modu-

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lation of adaptive immunity (Kohler et al., 2003; Tien et al., 2006). Commensal bacteria have the ability to suppress inflammatory responses by inhibiting specific intracellular signal transduction pathways (Nusrat et al., 2001). Enteric commensal bacteria could be signaling epithelial cells to dampen host inflammatory responses via direct communication with cells of the innate intestinal immune system as well as enterocytes (Gaskins, 2003; Peters et al., 2005). It is well established that most immunoinflammatory effector genes, including interleukin- (**IL**) 8, IL-6, and others are controlled at the transcriptional level (Nusrat et al., 2001).

To our knowledge, there have been no reports in the literature concerning the effects of DFM or the prophylactic ionophore, salinomycin (SAL), on whole-body or tissue energy utilization in any species. This study was designed to estimate changes in whole-body energy expenditures in chicks supplemented with a DFM. Additionally, because the GI tract and its immune system require much energy, of special interest was the effect of the DFM on intestinal energy consumption as well as its effect on concomitant changes in the innate immune system of the intestines of the broiler chick.

MATERIALS AND METHODS

Experimental Design

One hundred twenty (trial 1) and thirty-six (trial 2) 1-d-old broiler chicks were placed on a standard corn-soybean meal diet (17.08% CP, 2.4% fat, and 2,830 kcal of ME/kg). All of the broilers were housed, maintained, and euthanized under an approved protocol from the Institutional Animal Care and Use Committee at North Carolina State University. The objective of trial 1 was to determine whether DFM and salinomycin supplementation affect whole-body or intestinal tissue respiration. In trial 2, the objective was to measure treatment differences in intestinal length, weight, as well as serosal and mucosal DM and organ weight. Additionally, whole ileal cytokine expression was measured.

A completely randomized design was used for the 2 trials. Chicks from each treatment were randomly blocked by age for experimental measurements, so the average age of the chicks was 21 d at the time of measurements. Chicks were assigned to one of following treatments: control (no additives,), SAL (salinomycin, 50 ppm feed), and a DFM consortium (PrimaLac; 0.3% of a diet). PrimaLac was added as a lyophilized mix containing 1×10^8 cfu/g of *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium thermophilum, and Enterococcus faecium.* This level of DFM supplementation was chosen to ensure thorough colonization in the intestines of treated chicks. Salinomycin was chosen as our negative control because of its widespread use in the poultry industry as a coccidiostat and its antimicrobial properties against gram-negative organisms (Duffy et al., 2005).

Chicks were placed at hatch in Petersime batteries (1 battery per treatment); the control and SAL groups were housed in batteries in a separate room from the DFM group with single pass air. The birds were kept in the batteries for the whole duration of the experiment. To prevent cross-contamination, access to birds was restricted to essential personnel, and all personnel were required to enter a control room before entering the DFM room and were not allowed to reenter the control room without showering and changing clothes. Chickens were fed the respective treatment diets for 21 d and fasted for 12 h before sample collection. Water and feed were provided ad libitum and BW and feed intakes recorded.

Individual birds were regarded as the experimental units for estimates of all parameters except feed intake. Feed intake estimates were based on pen averages. The data from each trial were analyzed using a 1-way ANOVA statistical program, Statistix 8 (Analytical Software, Tallahassee, FL). Because sample sizes in these trials were small to moderate, Fisher's least significant difference was used to test differences between means only when the ANOVA indicated significance at $P \leq 0.05$ (Motulsky, 2005).

Sample Collection

On d 21, trial 1 birds were measured for whole-body O_2 consumption after a 12-h feed deprivation period. Thereafter, the chicks were euthanized by cervical dislocation, and ileal and cecal tissue samples were collected for O_2 consumption analysis. All tissue sampling was completed within 15 min after euthanasia. Ileal samples were obtained 2 cm above ileocecal colonic junction. In trial 2, birds were fasted for 12 h, weighed, and euthanized by cervical dislocation. The abdominal cavity was exposed, and 2 sections, one at the gizzard-duodenal junction and the other at the end of the colon, were made to excise the small and large intestine, cecum, and colon. Liver, crop, gizzard, bursa of Fabricius, and pancreas were also removed and weighed. All organ weights were expressed per gram of fasted BW. After blotting dry, the total GI tract was weighed, and its unstretched length was measured. The weight and length of the duodenum (pyloric sphincter to bile duct), jejunum (bile duct to yolk stalk), ileum (yolk stalk to cecum), cecum, and colon were also recorded. Portions of each intestinal segment were rinsed in ice-cold 0.9% NaCl (wt/vol), blotted dry, weighed, and the mucosa was gently removed by scraping with the edge of a glass microscope slide. The remaining muscularis externa and serosa were weighed, and the weight of the mucosa was calculated by difference. The amount of DM in the intestinal mucosa, serosa, or intact tissues was determined by drying at 80°C in a forced-air oven for 48 h. Approximately 100 g of the ileal tissue collected was placed in RNAlater (Ambion, Austin, TX) for subsequent analysis of cytokines.

Whole-Body O_2 Consumption

Whole-body O_2 consumption was measured using an O_2-ECO indirect calorimeter (Columbus Instruments, Co-

lumbus, OH). Birds (40 per treatment) were placed in measurement chambers with airflow of 4.0 L/min. Oxygen and CO_2 measurements were initiated after the bird was in the measuring chamber for 20 min to allow for behavioral adjustment. Whole-body O_2 consumption and CO_2 expiration were measured in 3 consecutive, 60-s periods, and the mean value was calculated. The BW of each bird was measured immediately after the repeated measurements of gas exchange. Whole-body O_2 consumption and CO_2 expiration were expressed as μM O_2/min per gram of BW or μM CO_2/min per gram of BW, respectively.

Ileal and Cecal O_2 Consumption

In trial 1, a 12-bird subsample from each treatment group was used to estimate whole ileal and cecal tissue O_2 consumption. Each ileal and cecal sample was longitudinally cut and divided into two 20- to 40-mg pieces. The O_2 consumption rates of intact ileal and cecal tissue were monitored in constantly stirred buffer containing 11 g of M199 (Sigma Chemical Co., St. Louis, MO), 5.96 g of *N*-2-hydroxyethylpiperazine-*N*'thanesulfonic acid, and 0.36 g of $NaHCO_3$ in 1 L of deionized water at 37°C using an incubation chamber (YSI, Yellow Springs, Ohio) fitted with an O_2 electrode as previously described (Fan et al., 1997). Tissue O_2 consumption was expressed as nM O_2/min per milligram.

Real Time Reverse Transcription-PCR

Real time reverse transcription-PCR was used to assess the level of gene expression for 3 cytokines. Relative levels of IL-1β and IL-6 (both proinflammatory cytokines) and IL-10 (an antiinflammatory cytokine) were determined as indicators of general mucosal innate immune activity. Total RNA was isolated from 100 mg of ileal tissue by Trizol (Invitrogen, Carlsbad, CA) and treated with DNase I (Promega Corporation, Madison, WI). Samples were incubated at 37°C for 30 min with DNase I. Equal mass from each sample (1 μg) of DNase-free total RNA was used to generate a cDNA library using M-MLV and random primers following manufacturer instructions (Promega Corporation). This mixture was then incubated for 60 min at 37°C. The relative level of each of the above genes was assessed in individual reactions using gene-specific primers and dual-labeled probes specific for IL-β, IL-6, IL-10, and 28S as previously described (Rothwell et al., 2004). A 5-μL quantity of cDNA template was used for a reaction mix (qPCR Core kit; Eurogentec, San Diego, CA). Expression of each gene was normalized within each treatment group by comparison to 28S. The reaction was carried out using a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA). The cycle profile was as follows: 1 cycle of 95°C for 10 min and 45 cycles of 94°C for 30 s (step 1) and 59°C for 1 min (step 2). The cycle threshold ($\mathbf{C_t}$), a cycle at which the change in the reporter dye passes a significant threshold, was calculated for each reaction, and changes in expression of the different cytokines are reported using the ΔC_t {$\Delta C_t = [C_{t\ (\text{gene of interest treatment n})} - C_{t\ (\text{28s treatment n})}]$}. Positive ΔC_t results reflect a decrease in gene expression relative to the control population, and negative ΔC_t results reflect an increase in gene expression relative to the controls.

RESULTS

Body weight gain and feed intake in control, SAL, and DFM groups were calculated in trial 1 at 18 d of age (Table 1). The SAL treatment had significantly lower BW, gain, and feed intake compared with the control and DFM treatments ($P = 0.001$ and 0.02, respectively). There were no significant differences in BW gain or feed intake between the control and DFM-treated birds.

Average BW (g) used to calculate data in Tables 2 and 3 were 308, 593.4, and 531.6 for SAL, control, and DFM, respectively. Cecal, colonic and jejunal, mucosal, and serosal DM contents were not affected by treatment. The DFM birds had significantly lower ileal mucosa DM than control birds ($P < 0.02$; Table 2); the SAL group did not differ significantly from control or DFM. Differences between treatments in the weight of intestinal segments adjusted for fasted BW (mg/g of fasted BW) were significant only in the jejunum, where DFM-treated birds had the lowest weight ($P < 0.001$; Table 3). No differences were observed between control and DFM-treated birds, with the exception of colon weight, which was significantly higher in DFM-treated birds compared with control birds ($P < 0.02$; Table 3). The SAL birds had longer intestinal segments, total intestinal length, cecum, and colon when adjusted for BW ($P < 0.05$; Table 3) compared with control and DFM. Salinomycin decreased intestinal segment density (mg/cm) compared with control and DFM. The DFM decreased jejunal density compared with the control group ($P < 0.001$).

The DFM treatment decreased liver weight adjusted for fasting BW compared with the control birds ($P = 0.04$), whereas the proventriculus was smaller in the control group ($P < 0.004$). There were no significant differences among treatments in bursa, pancreas, or gizzard weight.

Whole-body O_2 consumption and CO_2 production values are listed in Table 4. Data presented herein are in the range of those reported previously (Fan et al., 1997). Whole-body O_2 consumption adjusted for fasted BW was 16% less ($P < 0.05$) in the DFM group than in the SAL treatment. The DFM-treated birds had the lowest numerical whole-body O_2 consumption of all treatments when adjusted to BW, although this difference was statistically significant ($P < 0.05$) only compared with the SAL treatment. There were no differences in whole-body CO_2 expiration among treatments. There were also no differences in respiratory quotient (CO_2 production:O_2 consumption) between the treatments.

Total ileal O_2 consumption was lower in the DFM group than in the SAL-treated birds ($P = 0.01$; Figure 1). Although there was a numerical decrease with DFM, no statistically significant differences were noted between the DFM and control groups.

Table 1. Body weight and feed intake of 18-d-old broiler chickens used in trial 1[1]

	Diet[2]			
Item	Control	SAL	DFM	Significance
BW[3] (g)	468 ± 11[a]	274 ± 11[b]	439 ± 11.2[a]	0.000
BW gain (g)	423 ± 11[a]	235 ± 11[b]	400 ± 11[a]	0.000
Feed intake (g) total per pen[4]	7,886 ± 242[a]	6,433 ± 242[b]	7,728 ± 242[a]	0.015

[a,b]Means in rows lacking a common superscript are significantly different ($P \leq 0.05$) as a result of least significant difference mean comparison.

[1]Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac).

[2]Least square means ± SEM.

[3]Ten birds per pen; 4 pens per treatment. n = 120.

[4]Feed intake was calculated on a battery pen basis. n = 12.

Interleukins measured in this experiment were selected as indicators of the inflammation state of the chicken gut (Isolauri et al., 2001). The DFM altered expression of IL-6 (decreased), a proinflammatory cytokine, and IL-10 (increased), an antiinflammatory cytokine. Because of the small sample size, the significance of those changes is unclear.

DISCUSSION

To our knowledge, this is the first study to describe a link between the feeding of DFM or salinomycin with changes in whole-body O_2 consumption and with changes in the innate immune system of the gut. Feeding of the DFM, PrimaLac, decreased whole-body O_2 consumption by 17% when compared with the feeding of SAL. Marked differences were also noted in ileal O_2 consumption from the feeding of the DFM as compared with the feeding of SAL, in which the O_2 consumption rate was 47% less in the DFM- than in the SAL-fed birds. It should also be noted that differences in a broad array of parameters including BW gain and feed intake were adversely affected by the feeding of salinomycin in the present study. This is puzzling, given the common use of salinomycin as an anticoccidial treatment within the poultry industry (Scalzo et al., 2004) and the fact that the 50-ppm level utilized is well below the 80- to 160-ppm concentration for which salinomycin toxicity has been reported in chickens (Keshavarz and McDougald, 1982). However, there have been reports of salinomycin intoxication in turkeys at the level of 60 ppm of the feed (Van Assen, 2006). Salinomycin exerts its action by its ability to insert itself into membranes, thereby increasing the intracellular flux of K^+ and other cations such as Na^+ (Mitani et al., 1976). Ionophores, like salinomycin, do not discriminate between bacterial and mammalian membranes to affect cellular ion transport capacity (Butaye et al., 2003). In the mature bird, there are more microbial cells in the gut than cells within the body (Lin, 2003). In the present study, birds on all treatments were kept under clean conditions within properly maintained brooder batteries in well-ventilated bird rooms with filtered air. Under these conditions, the intestinal allochthonous bacteria load for SAL-treated birds may have been reduced such that excess salinomycin may have been available for binding directly to the intestinal enterocytes. Salinomycin is known to bind to cellular and mitochondrial membranes and ultimately increase intramitochondrial K^+, therefore disrupting cytoplasmic and mitochondrial redox potential and oxidative phosphorylation (Mitani et al., 1976; Williams, 2005). This may explain the increase in ileal O_2 consumption and subsequent increased whole-body O_2 consumption in the SAL birds compared with the DFM birds. Fan et al. (2003) reported that the polyether iono-

Table 2. Dry matter of serosa and mucosa in ileum, jejunum, and intact colon and cecum in 3-wk-old broiler chickens in trial 2[1]

	Diet[2]			
DM content (%)	Control	SAL	DFM	Significance
Jejunal serosa[3]	26.72 ± 0.53	27.01 ± 0.74	28.14 ± 0.74	0.30
Jejunal mucosa	23.96 ± 1.08	20.28 ± 1.53	24.59 ± 1.62	0.10
Ileal serosa	30.37 ± 0.80	30.44 ± 1.13	30.46 ± 1.14	0.99
Ileal mucosa[4]	21.08 ± 0.65[a]	18.67 ± 0.91[ab]	17.85 ± 0.97[b]	0.02
Intact cecum	22.51 ± 0.69	23.94 ± 0.98	24.82 ± 0.99	0.15
Intact colon	19.46 ± 0.37	19.51 ± 0.53	19.31 ± 0.53	0.96

[a,b]Means in rows lacking a common superscript are significantly different ($P \leq 0.05$) as a result of least significant difference mean comparison.

[1]n = 36.

[2]Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac).

[3]Least square means ± SEM.

[4]Means with different superscripts differ significantly ($P < 0.05$).

Table 3. Adjusted weight of intestinal segments in 3-wk-old broiler chickens in trial 2[1]

Item	Diet[2]			Significance
	Control	SAL	DFM	
Adjusted weight (mg/g of FBW)[3]				
Duodenum[4]	7.21 ± 0.32	7.56 ± 0.45	7.22 ± 0.45	0.808
Jejunum	16.16 ± 0.48[a]	14.33 ± 0.68[ab]	12.86 ± 0.68[b]	0.001
Ileum	12.294 ± 0.50	10.69 ± 0.71	11.88 ± 0.71	0.201
Cecum	5.88 ± 0.40	5.28 ± 0.56	5.69 ± 0.57	0.70
Colon	1.08 ± 0.13[b]	1.81 ± 0.18[a]	1.76 ± 0.18[a]	0.002
Total weight	42.63 ± 1.16	39.72 ± 1.65	39.39 ± 1.65	0.188
Adjusted length (mm/g of FBW)				
Duodenum	0.32 ± 0.02[b]	0.50 ± 0.02[a]	0.33 ± 0.02[b]	0.001
Jejunum	0.78 ± 0.03[b]	1.187 ± 0.05[a]	0.77 ± 0.05[b]	0.001
Ileum	0.76 ± 0.03[b]	1.05 ± 0.04[a]	0.80 ± 0.04[b]	0.001
Cecum	0.19 ± 0.01[b]	0.28 ± 0.01[a]	0.19 ± 0.01[b]	0.001
Colon	0.08 ± 0.01[b]	0.12 ± 0.01[a]	0.09 ± 0.01[ab]	0.005
Total length	2.13 ± 0.08[b]	3.14 ± 0.11[a]	2.19 ± 0.11[b]	0.001
Density (mg/cm)				
Duodenum	228.85 ± 9.30[a]	150.42 ± 12.79[b]	219.99 ± 12.79[a]	0.001
Jejunum	204.60 ± 6.10[a]	123.74 ± 8.38[c]	170.30 ± 8.38[b]	0.001
Ileum	165.05 ± 5.78[a]	104.23 ± 7.95[b]	149.14 ± 7.95[a]	0.001
Cecum	321.12 ± 14.96[a]	190.07 ± 20.55[b]	300.53 ± 20.55[a]	0.001
Colon	122.91 ± 14.83[b]	156.40 ± 20.39[ab]	196.82 ± 20.39[a]	0.0213
Total density	201.14 ± 5.13[a]	128.26 ± 7.057[b]	181.41 ± 7.06[a]	0.001
Adjusted weight (mg/g of FBW)				
Proventriculus	4.66 ± 0.16[b]	5.69 ± 0.23[a]	4.90 ± 0.23[ab]	0.0036
Gizzard	28.63 ± 0.79	30.24 ± 1.12	26.80 ± 1.12	0.1108
Bursa	2.10 ± 0.28	2.15 ± 0.41	2.12 ± 0.39	0.9949
Liver	26.15 ± 0.87[a]	23.81 ± 1.23[ab]	22.28 ± 1.23[b]	0.0392
Pancreas	2.46 ± 0.18	3.21 ± 0.25	2.92 ± 0.25	0.0553

[a–c]Means in rows lacking a common superscript are significantly different ($P \leq 0.05$) as a result of least significant difference mean comparison.

[1]n = 36.

[2]Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac).

[3]FBW = feed-deprived BW.

[4]Least square means ± SEM.

phores monensin, laidlomycin, and laidlomycin propionate had no effects on intestinal absorption and energetics. The mice in that study were housed in cages on corn cob litter. The results of the present study suggest it is possible that the SAL treatment resulted in an induced salinomycin toxic state under the animal care conditions used as evidenced by the more physiological O_2 consumption estimates in the control and DFM birds. Histological and scanning electron microscopy studies in this laboratory showing unusual changes in intestinal architecture as well as a paucity of bacterial colonization in all segments of the lower intestinal tract of birds support this interpretation (Chichlowski et al., 2007).

Changes in relative organ weights support a change in intestinal function rather than in intestinal mass as an explanation for the decreased whole-body and ileal O_2 consumption from the DFM compared with the SAL treatment. In general, all adjusted intestinal segment weights (Table 3) were the same for the SAL and DFM treatments. The SAL intestinal lengths were, however, longer than in both the control and DFM birds (Table 3). This resulted in a uniformly lower intestinal weight (mg/cm) for SAL-treated as compared with control and DFM-treated birds. In addition, no significant changes were noted in intestinal serosal and mucosal DM percentage between treatments. Our laboratory has noted a positive relationship

Table 4. Whole-body O_2 consumption and CO_2 production in 3-wk-old broiler chickens in trial 1[1]

Item	Diet[2]			SEM	Significance
	Control	SAL	DFM		
BW (g)	521[a]	304.9[c]	478.04[b]	11.15	0.001
Respiratory quotient[3]	0.85	0.81	0.82	0.02	0.48
Adjusted whole-body O_2 consumption (μM O_2/min per g of BW)	1.24[ab]	1.39[a]	1.17[b]	0.06	0.027
Adjusted whole-body CO_2 production (μM CO_2/min per g of BW)	1.05	1.15	1.002	0.07	0.29

[a–c]Means in rows lacking a common superscript are significantly different ($P \leq 0.05$) as a result of least significant difference mean comparison.

[1]n = 118 (2 observations are missing).

[2]Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac).

[3]Respiratory quotient = CO_2 production:O_2 consumption.

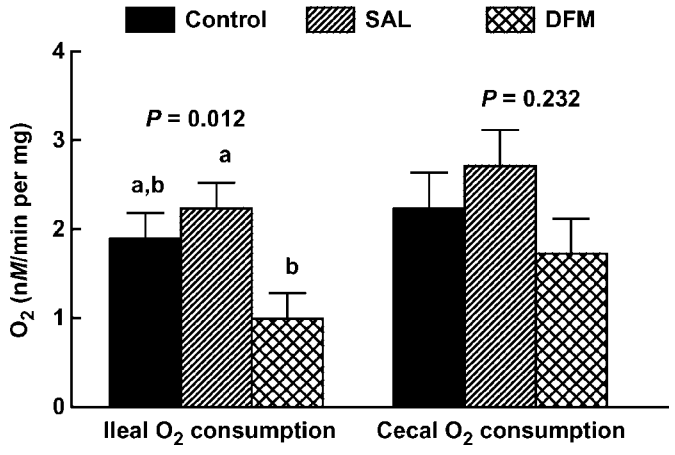

Figure 1. Ileal and cecal O_2 consumption in 3-wk-old broiler chickens. Values are means ± SEM. Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac). [a,b]Means with different letters differ significantly ($P < 0.05$).

among tissue mass, DM, and O_2 consumption. The lighter, more metabolically active intestinal tissue observed with SAL in the present study suggests fundamental changes in intracellular metabolism of intestinal tissues of SAL-treated birds. It is of interest to note that no statistically significant differences between the control and DFM treatments were found for whole-body O_2 consumption rates despite a previous report that this DFM increases growth and feed efficiency in chickens (Davis and Anderson, 2002). The present study found that the DFM decreased adjusted liver weight by 15%. Similar decreases in the liver weight of birds treated with this DFM, PrimaLac, have been reported previously (Mohan, 1991). The GI tract and the liver account for 42% of total body energy expenditures in the sheep (McBride and Early, 1989). No consistent differences between the control and DFM treatments were noted in adjusted GI weight and length in the present study (Table 3).

This study demonstrates the potential effects of SAL and DFM on intestinal and whole-body metabolism. The mechanisms are not clearly understood; however, alterations in intestinal and liver function as well as in intestinal immune function may explain, in part, this phenomenon. The cytokine data (Figure 2) clearly illustrate the need for further study of how the immune system is affected by DFM. In the present study, the biosecurity protocol involved with the housing of the birds under conditions that are cleaner and atypical to those found in modern production systems may have caused an induced case of salinomycin toxicity. It is not clear whether all DFM or probiotic consortia will evoke similar changes in energy consumption or immune function. Allochthonous bacteria, even strains within the same species, have been shown to vary in their ability to colonize sections of the GI tract as well as in their ability to affect change in the intestinal immune system (Garriga et al., 1998). The beneficial effects on growth and feed conversion reported in many studies using DFM are likely due to a complex and highly integrated cascade of alterations in the physio-

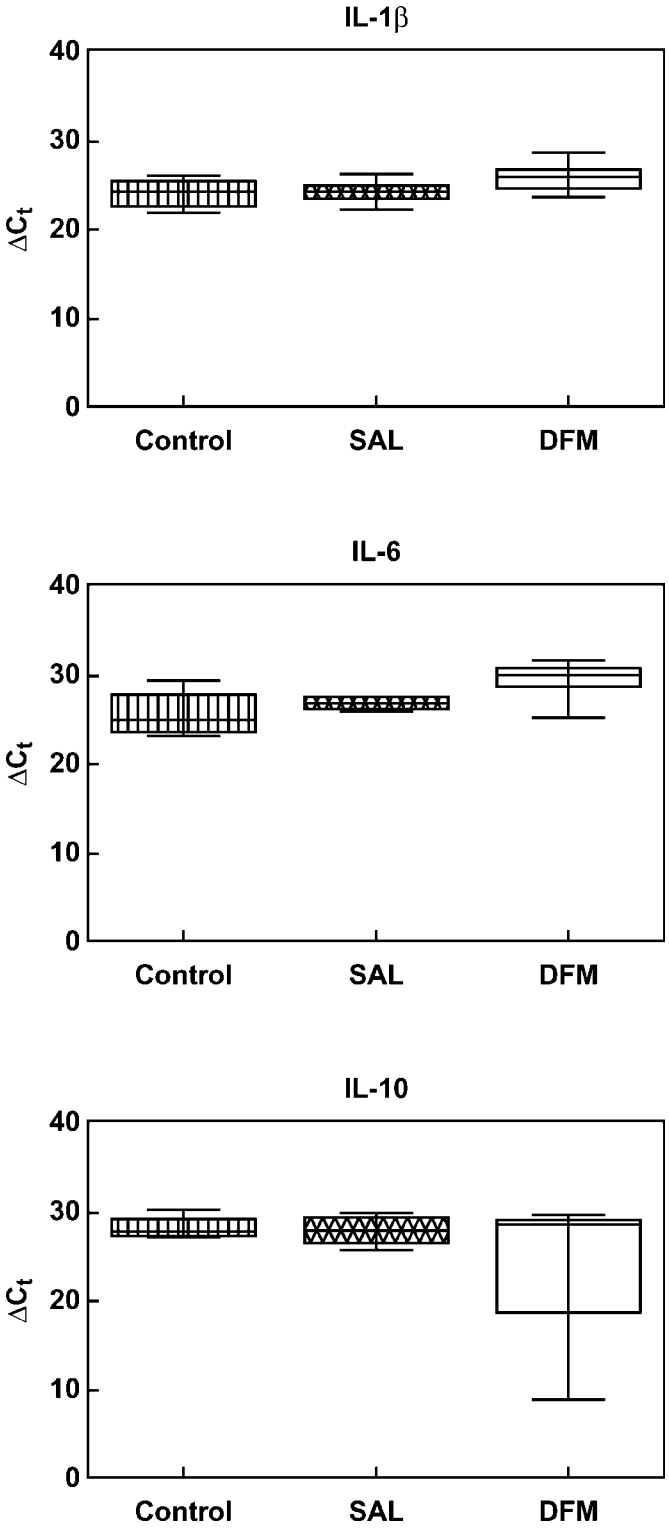

Figure 2. Whisker plot of cytokine production in the broiler chicken ileum; n = 18. Control = no additives; SAL = salinomycin (50 ppm); DFM = direct-fed microbial (PrimaLac). Cytokines expressed (IL-1β, IL-6, and IL-10) were determined using real time reverse transcription-PCR and presented as $\Delta C_t = (C_{t\ (\text{gene of interest treatment n})} - C_{t\ (\text{28s treatment n})})$, where C_t = cycle threshold and increasing ΔC_t represents decreased gene expression. The box represents a percentile range; the median is marked as a vertical line inside the box, and the lines outside the box extend to the highest and the lowest observations.

logical mechanisms of the bird. More studies on the effects of DFM and probiotics on physiological, biochemical, and immune processes are needed to develop a more dynamic understanding of the beneficial actions of DFM and probiotic bacteria in the intestinal tract and their subsequent effects on whole-body metabolism.

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