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Direct Fed Microbial, Primalac®, Supplementation and Jejunal Glucose and Proline Transport in Broiler Chickens[1]

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Abstract: Direct fed microbials (DFM) are a putative alternative to the feeding of subtherapeutic levels of antibiotics in poultry production. Previous studies with a DFM, Primalac®, have suggested that DFM may decrease ileal energy expenditures in broilers. These changes might be related to nutrient transport in the gastrointestinal (GI) tract. The current study examined the effects of supplementing broiler diets with DFM on ileal glucose and proline absorption and their relationships to GI energy expenditures. Twenty-four broiler chickens were fed a standard starter diet (CON) and CON + DFM, (PrimaLac® 0.3% w/w) from hatch to 3 wk of age. On d 21, birds were euthanized, ileal tissue was dissected and glucose and proline uptake were estimated. In adjacent tissue, total O_2 (TO_2) and oubain (Na/K ATPase-sensitive) O_2 consumption were estimated. Primalac® had no effect ($P>0.05$) on ileal glucose and proline absorption transport rates as well as oubain sensitive and non-oubain sensitive oxygen consumption rates. Total passive transport of proline across the entire ileum was decreased by Primalac®.

Key words: Direct fed microbial, probiotic, broiler, intestinal function

INTRODUCTION

Direct fed microbials (DFM) are non-pathogenic microorganisms that may alter intestinal microbial colonization and function (Chichlowski *et al*., 2007c; Patterson and Burkholder, 2003). The use of DFM is considered to be a potential alternative to the feeding of antibiotic growth promoters in poultry production (Patterson and Burkholder, 2003). Potential mechanisms of DFM action include inhibition of pathogen growth in the gastrointestinal (GI) tract and alterations of the innate intestinal immune response (Fuller, 1989; Galdeano and Perdigon, 2006; McCracken and Gaskins, 1999; Simon and Jadamus, 2002; Vaughan and Mollet, 1999). Whilst DFM may protect broiler chickens against enteric bacterial infection (Dalloul *et al*., 2005), they may also contribute to nutrient digestion and absorption (Hooper *et al*., 2001; Lan *et al*., 2004).

There is a paucity of data regarding the influence of DFM on nutrient transport rates in the chicken small intestine; however, data from other animal models have been reported. One study suggested that gastrointestinal microflora may affect glucose transport (Hooper *et al*., 2001). In that study, colonization with commensal *Bacteroides thetaiotaomicron* in mice led to increased ileal levels of Na-dependent SGLT-1 glucose transporter mRNA. In another study, after oral treatment of rats with *Saccharomyces boulardii*, there was a marked stimulation of Na-dependent D-glucose uptake into jejunal enterocyte's brush border membrane vesicles with a corresponding increase in the membrane density of the SGLT-1 Na-dependent glucose transporter (Marteau *et al*., 2004).

The specific objectives of the present study were to investigate the effects of the DFM consortium, Primalac®, on the absorption rates and total absorption flux of glucose and proline across the ileum of broiler chicks as well as describe concomitant changes in ileal energy expenditures.

MATERIALS AND METHODS

Experimental design: Twenty-four mixed sex, one-day old broiler chickens were fed a standard corn-soybean meal broiler diet (17.08% CP, 2.4% fat, and 2830 kcal ME/kg). Chicks were assigned to one of the following treatments: CON (no additives) and DFM (0.3% w/w of the diet). DFM is a consortium of *Lactobacillus casei*, *L. acidophilus*, *Bifidobacterium thermophilium*, and *Enterococcus faecium* (Primalac®, Clarksdale, MO).

A completely randomized design was used. Individual bird measurements were the experimental units. The data were statistically analyzed using the ANOVA procedure of STATISTIX®8 (Tallahassee, FL). Each bird's body weight (BW) was used as a covariate for all intestinal glucose and proline transport analysis. Due to the relatively small total number of experimental units, Fisher's LSD test was used to test the significance of differences between the treatment means if overall significance was $P < 0.05$.

Animal care and biosecurity were as previously described (Chichlowski *et al*., 2007a,c). All experiments were conducted according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University.

Table 1: Ileal glucose and proline transport and efficiency in 21 day old broiler chicken[1]

	Treatment[2,3]		
Parameters	CON	DFM	Sig.
Ileal glucose uptake:	nano moles nutrient/minute per gram of intact ileum[4]		
Active	116±30[3,4]	62±30	0.11
Passive	140±27	165±27	0.36
Total	247±33	215±33	0.36
Ileal proline uptake:			
Active	72±17[a]	16±17[b]	0.02
Passive	146±26	173±26	0.32
Total	196±31	186±31	0.74

[a,b]Means in rows lacking a common superscript are significantly different (P = 0.05)[1] n= 24
[2]CON = no additives, DFM = Direct-Fed Microbial (Primalac®). [3]Least Square Means ± SEM
[4]All calculations performed with BW [g] as a covariate

Sample collection and analyses: On day 21, birds were euthanized by cervical dislocation after 12-h feed deprivation. Ileal tissue samples were immediately removed from each bird, longitudinally cut and divided into ten, 20 to 40 mg pieces for nutrient uptake and whole-tissue O_2 consumption analyses. Active, passive and total ileal uptake of glucose and proline were estimated using ^{3}H-3-O-methyl-D-Glucose and ^{14}C-proline as described previously (Fan *et al*., 1997). Whole ileal glucose and proline flux was estimated by multiplying the transport value (nano moles/minute per gram of intact ileum) by total ileal tissue weight. The ileal tissue weights used were means of ileums collected in each treatment goup, 7.26 g (CON) and 6.37 g (DFM). These treatment means were different (P<0.05).

The O_2 consumption rates of intact ileal and cecal tissue were estimated using an incubation chamber (YSI, Yellow Springs, OH) fitted with an O_2 electrode as described by Fan *et al*. (1997). The O_2 consumption rates of intact ileal tissue attributable to Na^+/K^+ ATPase and cytoplasmic protein synthesis were measured by the difference in O_2 consumption in the absence and presence of ouabain (2.0 μM; Fisher Scientific Co., Pittsburgh, PA). The percentage of ouabain sensitive O_2 consumption rate was expressed as the O_2 consumption rate of intact ileal or cecal tissue in the presence of ouabain divided by the O_2 consumption rate of the same tissue in the absence of ouabain and then multiplied by 100. No arcsin transformations were used in the analysis of the percentages of types of oxygen consumption since the range of percentage values was less than 40 (Little and Hills 1978).

RESULTS

Ileal uptake rates for glucose and proline are listed in Table 1, while Table 2 contains the estimated total, active and passive glucose and proline flux across the entire ileum. Whole ileal tissue oxygen consumption as well as oubain sensitive and non-oubain sensitive oxygen consumption values are listed in Table 3. Primalac® had little effect on glucose transport rates, although it significantly decreased ileal (P<0.05) active proline transport rate (Table 1) and decreased (P<0.05) total ileal passive proline flux (P<0.05; Table 3). No significant (P>0.05) effects were noted on whole ileal tissue total oxygen consumption as well as both oubain and non-oubain sensitive oxygen consumption rates (Table 3).

DISCUSSION

Previous studies using various DFM consortia, including Primalac®, have demonstrated enhanced growth performance in poultry; however the mechanism of this enhanced growth is not understood. The current study tested the hypothesis that this increased growth is the result of enhanced nutrient absorption. The results of the present study were inconclusive in demonstrating any biologically significant effect of the DFM consortium, Primalac®, on glucose or proline absorption from the ileum of the broiler chick. Interestingly the DFM was associated with a decrease in the rate of active proline transport as well as total passive glucose flux in the ileum. It is difficult to assign any functional significance to these numbers since absorption rates from the duodenum or jejunum were not measured. Indeed, the effects of DFM on transport rates in the more proximal sites of the broiler intestinal tract could be quite different. Another puzzling finding in this study is the failure of glucose and proline transport rates to change in a synchronous manner. In previous studies from this laboratory, we have noted that Na-dependent transporters seem to up-regulate in the same direction in the duodenum of the sheep (Bird *et al*., 1996).

Similar to previous studies in this laboratory (Chichlowski *et al*., 2007c), these results demonstrated no difference in total whole ileal tissue respiration between CON and DFM fed birds. Interestingly, subsequent studies, in this laboratory, have shown a decrease in total whole tissue ileal oxygen consumption. The conspicuous difference between the two studies was that Chichlowski *et al*. (2007c) utilized unsexed broiler chicks, presumably in a gender ratio of 1/1 male to female. More recent studies using all male broilers

Table 2: Analysis of estimated total ileal glucose and proline in 21 day old broiler chick[1]

	Treatment[2,3]		
Parameters	CON	DFM	Sig.
TGTI	1794 ± 244	1355 ± 244	0.09
TPTI	1432 ± 225	1170 ± 225	0.26
AGTI	834 ± 218	375 ± 120	0.06
PGTI	1014 ± 174	1050 ± 174	0.84
APTI	506 ± 109[a]	102 ± 109[b]	0.01
PPTI	1059 ± 171	1100 ± 171	0.81

Acronyms:
TGTI = Total Glucose Flux for Total Ileum (nM/min) TPTI = Total Proline Flux for Total Ileum (nM/min)
AGTI = Active Glucose Flux for Total Ileum (nM/min) PGTI = Passive Glucose Flux for Total Ileum (nM/min)
APTI = Active Proline Flux for Total Ileum (nM/min) PPTI = Passive Proline Flux for Total Ileum (nM/min)
[a,b]Means in rows lacking a common superscript are significantly different (P = 0.05)[-1] n= 24,
[2]CON = no additives, DFM = Direct-Fed Microbial (Primalac®). [3]Least Square Means ± SEM

Table 3: Ileal oxygen consumption in 21 day old broiler chicken[1]

	Treatment[2,3]		
Parameters	CON	DFM	Sig.
Intact tissue, μM O_2/min/g	2.75±0.85	2.16±0.85	0.50
Ouabain sensitive	1.00±0.37	0.88±0.37	0.70
Non-ouabain	1.81±0.42	0.92±0.42	0.06
Percentage:	---------- % ----------		
Ouabain sensitive	36.57±6.76	40.66±6.76	0.56
Non-ouabain	63.43±6.76	59.35±6.76	0.56

[a,b]Means in rows lacking a common superscript are significantly different (P = 0.05)[-1] n= 24
[2]CON = no additives, DFM = Direct-Fed Microbial (Primalac®). [3]Least Square Means ± SEM

have shown a marked decrease in total whole tissue ileal oxygen consumption (Qiu *et al*., manuscript in preparation). This difference indicates that gender may be, yet another, of many factors that affect broiler response to DFM.

The results of the present study suggest that the DFM does not increase nutrient transport in the ileum suggesting the growth promoting affect previously demonstrated in Primalac® fed birds is not due an increase in nutrient absorption by enterocytes. Further studies are needed to understand relevance of the decreased proline transport and determine the affects DFM treatment have on nutrient transport in other regions of the intestine.

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Abbreviation Key: DFM - direct-fed microbial, GI – gastrointestinal