Effect of Genotype on Whole-body and Intestinal Metabolic Response to Monensin in Mice

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ABSTRACT : Two lines of mice, M16 selected for rapid growth and a randomly selected control ICR as well as their reciprocal crosses were used to study the effects of genotype on whole-body energetics and intestinal responses to monensin. Six mice, eight weeks of age, from each line or reciprocal cross were assigned to one of two treatments, 1) drinking water containing 20 mmol/L monensin dissolved in 0.5% V/V ethanol, and 2) drinking water containing 0.5% V/V ethanol (control) for two weeks. After 11 days (age of 9 weeks and 4 days), whole-body O_2 consumption was measured. At the end of two weeks, jejunal O_2 consumption, intestinal tissue composition and histomorphometrics as well as the rate and efficiency of glucose absorption were estimated. In comparison with the control, monensin administration in drinking water resulted in less daily water intake (13.4 vs. 15.5 ml/mouse, $p<0.01$), less protein to DNA ratio of jejunal mucosa (5.41 vs. 6.01 mg/mg, $p<0.05$), lower villus width (88 vs. 100 μm, $p<0.05$), and less jejunal tissue O_2 consumption enhancement by alcohol (7.2 vs. 10.5%, $p<0.01$) in mice. Other than those changes, monensin had little ($p>0.05$) effect on variables measured in either line of mice or their reciprocal cross. In contrast, the M16 line, selected for rapid growth, as compared to the ICR controls or the reciprocal crosses, had less initial (pre-monensin treatment) whole-body O_2 consumption per gram of body weight (1.68 vs. 2.11-2.34 μmol/min·g BW, $p<0.01$) as compared to the ICR and reciprocal crosses. In addition, the M16 mice exhibited greater growth (412 vs. 137-210 mg/d, $p<0.05$), better feed efficiency (41.7 vs. 19.9-29.3 mg gain/g feed, $p<0.05$), shorter small intestines adjusted for fasted body weight (1.00 vs. 1.22-1.44 cm/g FBW, $p<0.05$), wider villi (109 vs. 87-93 μm, $p<0.05$), more mature height of enterocytes (28.8 vs. 24.4-25.1 μm, $p<0.05$) and a lower rate (91 vs. 133-145 ηmol glucose/min·g jejunum, $p<0.05$) and less energetic efficiency (95 vs. 59-72 ηmol ATP expended/ηmol glucose uptake, $p<0.05$) of glucose absorption compared to the ICR line and the reciprocal cross. Monensin had little ($p>0.05$) effect on whole-body O_2 consumption and jejunal function, whilst selection for rapid growth resulted in an apparent down-regulation of intestinal function. These data suggest that genetic selection for increased growth does not result in concomitant changes in intestinal function. This asynchrony in the selection for production traits and intestinal function may hinder full phenotypic expression of genotypic growth potential. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 4 : 554-562)***

Key Words : Intestine, Metabolism, Monensin, Genotype, Growth, Mouse

INTRODUCTION

Antibiotics are used in animal industry, where allowed by law, usually for improving growth, feed efficiency, and general health. Monensin, one of the ionophore antibiotics, is used widely in beef cattle to cause a shift of more propionic acid relating to total volatile fatty acids by altering bacterial fermentation in rumen (Wang et. al., 2005) and consequently reducing energy loss as methane. The effect results in more efficient gain of growing animals. Initially, ionophores such as monensin and lasalocid were approved for use with poultry as cocciodiostats. Monensin is presumably a sodium ion carrier, which increases the permeability of membranes to the ion by binding it extracellularly, through the cell membrane, and releasing the ion on the intracellular side. The influx of sodium ions thus formed may serve as an energetic futile process to run short or even out of the energy needed for normal metabolic procedures in the microorganisms, and the intestinal epithelial cells of the host also, when they are administered with monensin. Few studies have been conducted to study the effects of antibiotics, especially ionophores, on intestinal absorption. Riley et al. (1986) found no effect of monensin administration on glucose or amino acid transport in chickens. In contrast, Raja et al. (1989) reported that the ionophore, valinomycin, increased glucose uptake in the mouse duodenum. There have been reports that monensin decreased oleic acid absorption in rat enterocytes (Husenet et al., 1990) and intestinal cholesterol uptake from cultured human intestine (Sviridov et al., 1993). Our laboratory failed to find any effects of the ionophore antibiotics, laidlomycin, laidlomycin propionate or monensin on whole-body energy metabolism or jejunal function of Swiss Webster mice (Fan et al., 2003).

To date, however, there have been no studies on the effects of ionophores on whole-body metabolism and intestinal absorption and function across genotypes within a species. The widespread use of ionophores, within the

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animal industry, makes ionophore-related effects on whole-body energetics and intestinal function amongst different genotypes of special interest such as a genetically improved rapid growth animal or poultry. New genetic lines are constantly emerging as a result of selection for enhanced production traits such as growth and body composition. The complex manner in which whole-body and intestinal function may be altered during genetic selection (Croom et al., 1998, 1999) in animal production systems makes it unlikely that one study using a genotype such as that reported by Fan et al. (2003), could reflect possible whole-body and intestinal response to an ionophore antibiotic over a diverse range of genotypes within a species.

Ionophore antibiotic such as monensin exerts its effect on coccidia and ruminal bacteria through reducing or even damaging sodium ion concentration gradients across cell membrane of the microorganisms. Will the effect of monensin on microorganism occur similarly on the epithelia of the digestive tract in the host animals? Mouse is an excellent animal model in terms of its small body size, readily to handle, and time cost to investigate the issue. The following study was designed to explore the impact of selection for growth in mice on intestinal and whole-body energetic response to monensin. Of special interests were monensin×genotype interactions presenting in the intestinal tract, the primary site of action of monensin as a coccidiostats in poultry.

MATERIALS AND METHODS

Animal care and diet

Lines of male mice used included M16 (selected for rapid growth), ICR (random bred controls) and their reciprocal crosses, M16♂×ICR♀ and ICR♂×M16♀. The genetic and phenotypic characteristics of the mice have been previously described by Eisen (1975) and Eisen and Leatherwood (1978a, b). Mice were treated and cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of North Carolina State University. Mice were born within a three-day period and weaned at three weeks of age. After weaning, the mice were housed in pairs in polypropylene cages (26×48×20 cm) in a climatically controlled room (23°C; 65% relative humidity) with a 12-h light:dark cycle, illumination beginning at 07:00 h. Mice had free access to drinking water and a commercial pelleted feed (Rodent Laboratory Chow #5001, Purina Mills, Inc., St. Louis, MO).

Experimental design

A randomized complete block design was used as described by Fan et al. (1996). There were two independent factors, one was the line of mice as described above; the second was the administration of monensin via drinking water. The treatment arrangement of this experiment was factorial, in which mice from the four genetic lines were treated with drinking water containing either monensin or control (ethanol plus water) to constitute eight treatments, in total. Monensin was dissolved in drinking water, for oral administration, using ethanol as a solvent such that the final concentrations of monensin and ethanol in water were 20 mmol/L and 0.5% V/V, respectively. The control was drinking water containing 0.5% V/V ethanol. For each of the four lines, six cages of mice (age 7 weeks), two males in each cage, were randomly assigned to one of the two drinking water treatments. Forty-eight mice were used for the experiment. The mice were allowed to adapt to the cages for one week. Feed intake, drinking water intake and body weight of the mice were recorded daily for two weeks beginning at 8 weeks of age. Whole-body oxygen consumption rate was measured 3 d prior to estimation of jejunal respiration and glucose transport, which were measured at 9 weeks of age.

Whole-body oxygen consumption

Mice were placed individually in an O_2-ECO system (Columbus Instruments International, Columbus, OH) to determine whole-mouse O_2 consumption rate as previously described (Fan et al., 1996). In brief, the mouse was allowed to adapt to the respiration chamber for 60 min. The air flow to the respiration chamber was 0.5 L/min. Oxygen sensors were calibrated by flushing with standard air (20.1% O_2) for 15 min before initial measurement. Subsequent measurements were conducted after flooding the chamber with room air for 15 min. The O_2 concentration in the room air was recorded as initial concentration. During the measurement, the air inside the chamber was maintained at a positive pressure. Behavioral activity of the mouse in the chamber during the measurement period was monitored. If the mouse slept through the entire measurement period, the activity was scored 0, otherwise 1. Whole-body O_2 consumption was measured twice for two 12-min periods, consecutively, and the average value used. Body weight of each mouse was measured immediately after repeated measurements of whole-body O_2 consumption.

Small intestinal tissue preparation

Preparation of intestinal tissue for weight and length measurements, oxygen consumption estimation and morphometric analyses was conducted according to Fan et al. (1996). After 18 h of feed deprivation, mice were killed by cervical dislocation and fasted body weights (FBW) were recorded. The small intestine was weighed and its unstretched length recorded. The mid-jejunum was removed at its midsection for glucose transport and respiration measurements as well as histological morphometric measurements and biochemical analyses (Fan et al., 2003).

Jejunal sections used for biochemical analyses were stored at -20 °C until analysis.

Jejunal histological morphometric analysis

The jejunal proximal sections were prepared for histological morphometric analyses as previously described (Bird et al., 1994a). After intestinal samples were fixed in Carnoy's solution for 4 h, they were dehydrated in graded concentrations of ethanol and embedded in paraffin. Tissue sections with thickness of 5 μm were cut and stained with Fuelgen reagent and counterstained with 0.05% Fast Green. A computer-generated microscopic image analyzer (Optimetric, BioScan, Edmons, WA) was used to measure histological morphometric parameters such as villus height, villus width at the base, villus planar perimeter length, crypt depth, external muscle layer thickness and height of enterocytes at mid-villus. Ten jejunal villi in which the lamina propria was intact and the villus cleanly sectioned through the villus midline were examined from each mouse.

Jejunal glucose transport assay

The mid-jejunal section was used to estimate glucose uptake according to the procedure of Black (1988), modified for mouse tissue (Bird, 1994a, b), by estimating the accumulation of (^{3}H)-O-methly-D-glucose (3OMG), a non-metabolizable glucose analogue, within the jejunal tissue in the presence and absence of phlorizin, an inhibitor of the intestinal Na^+-dependent glucose transporter. The jejunum was assumed to account for 50% of the small intestinal weight (Fan et al., 1996). Glucose transported by the entire jejunum was estimated by multiplying glucose transport per gram of jejunum times 50% of the small intestinal weight.

Jejunal tissue oxygen consumption

Oxygen uptake of intact jejunum and jejunal serosal was estimated using an oxygen monitor as described by (McBride et al., 1985; Gill et al., 1989) and modified by Fan et al. (1996). Tissue from the mid-jejunal section was prepared by rinsing in ice-cold media 199 (11 g M199, 5.96 g HEPES and 0.36 g $NaHCO_3$ in 1.0 L H_2O, pH 7.4; Sigma Chemical, St. Louis, MO) to remove digesta residues, and then divided into four 20- to 40-mg intact jejunal pieces. Mucosa was scraped from the remaining tissue samples with the edge of a glass microscope slide, leaving the jejunal serosa, including muscularis externa. The serosa was cut into four 20- to 40-mg pieces. Either intact jejunal tissue or serosal tissue pieces were placed in incubation chambers fitted with an O_2 electrode containing 4 ml of media 199 and constantly stirred at 37°C to measure O_2 consumption rate. The O_2 consumption rate of intestinal mucosa was estimated using the difference between the O_2 consumption rate of intact jejunal tissue and that of the serosa (Fan et al., 2003). Oxygen consumption rates of jejunal intact tissue and serosal tissue attributable to Na^+/K^+-ATPase were measured by the differences in O_2 consumption in the presence and absence of ouabain, an inhibitor of Na^+/K^+-ATPase (Sigma Chemical Co., St. Louis, MO), respectively.

The oxygen consumption rate of jejunal intact tissue attributable to monensin administration was measured by the differences of O_2 consumption in the presence of monensin (Sigma Chemical Co., St. Louis, MO) plus its solvent, ethanol, minus O_2 consumption in the presence of ethanol alone. The final concentrations of ethanol and monensin plus ethanol in media 199 were 0.5% V/V and 20 μmol/L plus 0.5% V/V, respectively. The concentration of ethanol in media containing ethanol alone was 0.5% V/V. Oxygen consumption of the entire jejunum was estimated by multiplying O_2 consumption rate per gram of jejunum and 50% of the small intestine weight.

Mucosal biochemical analyses

Frozen jejunal sections were thawed and rinsed in ice-cold 0.9% NaCl (w/v), blotted dry, weighed and the mucosa gently removed by scraping with the edge of a glass microscope slide. Jejunal serosal and mucosal proportions and their respective dry matter contents were determined (Fan et al., 1996). Mucosal DNA content was measured using 20 mg of scraped mucosa. After homogenizing (Techmar, Cincinnati, OH) for 30 s in cold buffer (2.3 ml, pH 7.4, 10 mmol/L Tris, 1 mmol/L EDTA and 1 mol/L NaCl), the DNA content of the mucosal homogenate was measured by using a TKO 100 fluorimeter (Hoefer Scientific Instruments, San Francisco, CA) that used calf thymus DNA as a standard (Hoefer Scientific Instruments). Mucosal protein content was measured using 20 mg of scraped mucosa. Total protein was determined by measuring the absorbance of bicinchoninic acid complexed with Cu^+ at 550 nm (Pierce Biochemicals, Rockford, IL) using bovine serum albumin as a standard.

Calculations and statistical analyses

The ouabain sensitive proportion and the monensin sensitive proportion in jejunal intact tissue respiration were calculated by subtraction of total jejunal tissue respiration from those inhibited by ouabain and monensin, respectively (Fan et al., 2003). Glucose active transport was calculated as the difference between 3OMG accumulation in the media with and without phlorizin (Fan et al., 1996). The apparent energetic efficiency of active glucose transport of intact intestinal tissue (nmol ATP expended/nmol glucose uptake; APEE) was calculated assuming 5 nmol of ATP synthesized per ηmol of O_2 consumed divided by active glucose transport (Bird et al., 1994a). The theory and limitations of use of this scalar for relating glucose transport to energy expenditure by intestinal mucosa have been discussed, in

Table 1. Growth and performance of 8-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR×M16	M16×ICR	SEM	Control	Monensin	SEM	
Body weight (g)									
Initial	33.7^{C}	59.4^{A}	41.2^{B}	41.7^{B}	1.13	43.8	44.2	0.80	NS
Final	35.8^{C}	65.1^{A}	44.1^{B}	43.6^{B}	1.07	47.4	47.0	0.75	NS
Daily gain (mg)	149^{B}	412^{A}	210^{B}	137^{B}	36	258	196	25.4	NS
Feed intake (g/d)	6.0^{b}	10.5^{A}	7.2^{B}	6.8^{B}	0.43	7.44	7.75	0.31	NS
Gain/feed (mg/g)	24.8^{ab}	41.7^{a}	29.3^{ab}	19.9^{b}	4.55	33.8	24.1	3.22	NS
Water intake (ml/d)	11.1^{C}	19.4^{A}	14.3^{B}	13.0^{BC}	0.65	15.5^{M}	13.4^{N}	0.46	NS
Epididymal fat pad (per g)									
Weight (g/mouse)	0.43^{C}	2.05^{A}	0.84^{BC}	0.89^{B}	0.11	1.04	1.06	0.08	NS
Adjusted weight (mg/g FBW)	13.2^{C}	33.2^{A}	20.5^{B}	21.9^{B}	1.60	21.8	22.6	1.13	NS

$^{A, B, C}$ Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).
$^{a, b}$ Means in the same row without the same superscripts are significantly different between the lines ($p<0.05$).
$^{M, N}$ Means in the same row without the same superscripts are significantly different between the treatments ($p<0.01$).
SEM = Standard error of mean. L×T: Interaction between line and treatment. NS indicates $p>0.05$.
FBW = Body weight after feed-deprivation for 18 h.

Table 2. Small intestinal weight and length of 9-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR×M16	M16×ICR	SEM	Control	Monensin	SEM	
Feed-deprived body weight (g/mouse)	32.8^{C}	60.9^{A}	40.7^{B}	40.3^{B}	1.02	43.5	43.8	0.72	NS
Small intestine									
Weight (g)	2.64^{B}	4.83^{A}	2.99^{B}	3.10^{B}	0.163	3.40	3.38	0.12	NS
Length (cm)	46.9^{C}	60.7^{A}	49.6^{BC}	52.7^{B}	1.38	52.4	52.6	0.98	NS
Density (mg/cm)	56.3^{B}	79.1^{A}	60.2^{B}	58.9^{B}	1.98	64.1	63.2	1.40	NS
Adjusted small intestine									
Weight (mg/g FBW)	80.2	79.0	73.3	76.8	2.46	78.0	76.7	1.74	NS
Length (cm/g FBW)	1.44^{A}	1.00^{C}	1.22^{B}	1.31^{B}	0.031	1.25	1.25	0.02	NS

$^{A, B, C}$ Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).
SEM = Standard error of mean. L×T: Interaction between line and treatment. NS indicates $p>0.05$.
FBW = Body weight after feed-deprivation for 18 h.

detail, by Croom et al. (1998).

The data were statistically analyzed using the General Linear Model procedure of SAS® (1988). The model consisted of the fixed effect of line (L including two parental lines, M16 and ICR, and reciprocal crosses, M16×ICR and ICR×M16), monensin treatment (T), interaction of line by treatment (L×T) and the random residual term (Fan et al., 1996). Effects of the L, T, and L×T were tested using the residual error mean square as the denominator to calculate the F-value. Pairwise comparisons of means were conducted using Tukey's test with a minimum significant difference. Statistically significant differences were accepted at $p<0.05$.

RESULTS

Effects of monensin on energetics and jejunal function

Monensin had minimal effects regardless of mouse line used. Mice consuming monensin had decreased daily water intake ($p<0.01$; Table 1). No line×monensin difference was noted. Monensin had no significant effect on jejunal weight or length (Table 2). Monensin decreased protein/DNA of intestinal mucosa by 10% ($p<0.05$; Table 3). There were significant monensin×line effects on mucosal protein ($p<0.05$) and protein/DNA ($p<0.01$). Monensin decreased villus width by 12% ($p<0.05$; Table 3).

Treatment with monensin had no effect on whole-body oxygen consumption (Table 4). Similarly, no differences were noted in jejunal tissue oxygen consumption, including that due to ouabain sensitive respiration, as a result of monensin treatment (Table 5). Controls designed to measure effects of both ethanol, as well as ethanol plus monensin on jejunal respiration showed that ethanol did decrease intact tissue respiration ($p<0.01$; Table 5) compared to monensin but not ethanol plus monensin. Monensin had no effect on glucose transport (Table 6).

Effect of genotype on energetics and jejunal function

The M16 high growth line, at 9 weeks of age, gained weight at a greater rate (412 vs. 137-210 mg/d, $p<0.01$), consumed more feed (10.5 vs. 6.8-7.2 g/d, $p<0.01$) and

Table 3. Jejunal components and jejunal mucosal characteristics of 9-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR×M16	M16×ICR	SEM	Control	Monensin	SEM	
Dry matter (g/100 g)									
Intact	16.7	16.8	16.2	16.4	0.34	16.5	16.5	0.24	NS
Serosa	13.8	14.1	13.8	14.1	0.32	13.8	14.1	0.23	NS
Mucosa	18.0	18.5	17.8	18.4	0.53	18.1	18.2	0.38	NS
Mucosa									
Ratio (g/100 g)	63.0	67.0	64.0	64.2	1.16	65.5	63.6	0.82	NS
Protein (mg/g)	44.5	42.9	42.7	47.9	2.36	45.7	43.3	1.67	*
DNA (mg/g)	8.85	7.58	7.60	7.71	0.35	7.77	8.10	0.25	NS
Protein/DNA (mg/mg)	5.06	5.78	5.68	6.32	0.295	6.01^{m}	5.41^{n}	0.21	**
Villus (μm)									
Height	310	336	301	307	12.9	308	319	9.1	NS
Width	88^{b}	109^{a}	87^{b}	93^{ab}	5.4	100^{m}	88^{n}	3.8	NS
Perimeter	659	726	645	655	23	664	679	16	NS
Crypt depth (μm)	101^{b}	131^{a}	109^{b}	118^{ab}	6.6	110	119	4.6	NS
Muscle thickness (μm)	64.7	70.4	65.2	66.6	5.87	66.8	66.6	4.2	NS
Villus height/crypt depth ratio	3.28^{a}	2.67^{b}	2.88^{ab}	2.74^{b}	0.16	2.98	2.80	0.11	NS
Enterocyte height (μm)	25.1^{B}	28.8^{A}	24.4^{B}	24.9^{B}	0.63	26.1	25.5	0.44	NS

$^{A, B}$ Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).

$^{a, b}$ Means in the same row without the same superscripts are significantly different between the lines ($p<0.05$).

$^{m, n}$ Means in the same row without the same superscripts are significantly different between the treatments ($p<0.05$)

SEM = Standard error of mean. L×T: Interaction between line and treatment. **, * and NS indicate $p<0.01$, $p<0.05$, and $p>0.05$, respectively.

Table 4. Whole body oxygen consumption of 9-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR× M16	M16× ICR	SEM	Control	Monensin	SEM	
Initial									
Activity	0.436	0.131	0.305	0.131	0.125	0.196	0.305	0.088	NS
Whole-mouse O_2 consumption									
μmol O_2/min·mouse	78.1	95.1	84.1	83.1	4.6	83.4	86.8	3.23	NS
Adjusted whole-mouse O_2 consumption									
μmol O_2/(min·g BW)	2.34^{A}	1.68^{B}	2.12^{AB}	2.11^{AB}	0.128	2.02	2.11	0.09	NS
After treating with monensin for 2 weeks									
Activity	0.70	0.44	0.13	0.48	0.16	0.37	0.50	0.11	NS
Whole-mouse O_2 consumption									
μmol O_2/min·mouse	39.3^{B}	57.3^{A}	40.1^{B}	43.4^{B}	2.83	46.9	43.1	2.00	NS
Adjusted whole-mouse O_2 consumption									
μmol O_2/(min·g BW)	1.11	0.90	0.92	1.00	0.064	1.02	0.95	0.045	NS

$^{A, B}$ Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).

SEM = Standard error of Mean. L×T: Interaction between line and treatment. NS indicates $p>0.05$.

BW = Body weight measured immediately after measuring whole-mouse oxygen consumption.

more water (19.4 vs. 11.1-14.3 ml/d, $p<0.05$) and had a greater feed efficiency (41.7 mg/g in M16 vs. 19.9 mg/g in M16×ICR, $p<0.05$) than the ICR randomly selected control or the reciprocal crosses (Table 1). Epididymal fat pad weight, an indicator of whole body fat content (Eisen and Leatherwood et al., 1981), in M16 was heavier (2.05 vs. 0.43-0.89 g/mouse) than those in ICR and the reciprocal crosses. Similarly, adjusted epididymal fat pad weight by body weight after feed-deprivation for 18 h was heavier in M16 (33.2 vs. 13.2-21.9 mg/g FBW; $p<0.01$) than those in ICR and the reciprocal crosses.

The reciprocal crosses (ICR×M16 and M16×ICR) had heavier initial (41.2-41.7 g/mouse) and final body weights (43.6-44.1 g/mouse) than the randomly selected controls ($p<0.01$) but were lighter than the M16 line (59.4 and 65.1 g/mouse, respectively, $p<0.01$). Daily gain and feed intake followed similar patterns, whilst the ICR×M16 line consumed less ($p<0.01$) water (14.3 ml/d) than the M16 line (19.4 ml/d) but more ($p<0.01$) water than the ICR line (11.1 ml/d). The adjusted weights of epididymal fat pads expressed as per gram of FBW of both reciprocal crosses were intermediate (20.5-21.9 mg/g FBW; $p<0.01$) between those of the ICR (13.2 mg/g FBW) and the M16 lines (33.2 mg/g FBW) as shown in Table 1.

Table 5. Jejunal oxygen consumption of 9-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR×M16	M16×ICR	SEM	Control	Monensin	SEM	
Intact tissue O_2 consumption μmol O_2/(min·g)	1.74	1.61	1.73	1.73	0.071	1.65	1.75	0.050	NS
Ouabain inhibited proportion (%)	18.8	18.2	9.6	17.4	4.3	13.9	18.1	3.1	NS
Alcohol enhanced proportion (%)	9.4^{A}	5.8^{B}	10.7^{A}	9.6^{A}	1.00	10.5^{M}	7.2^{N}	0.70	NS
Alcohol+monensin enhanced proportion (%)	9.17^{a}	2.98^{b}	6.13^{ab}	9.01^{a}	1.61	8.45	5.19	1.14	NS
Monensin inhibited proportion (%)	0.25	2.78	4.56	0.55	1.93	2.03	2.04	1.37	NS
Serosa O_2 consumption (μmol O_2/(min·g))	0.80^{ab}	0.73^{b}	0.88^{a}	0.91^{a}	0.04	0.82	0.85	0.03	NS
Ouabain sensitive proportion (%)	12.1	13.8	17.0	18.2	1.94	15.2	15.4	1.40	NS
Mucosa O_2 consumption (μmol O_2/(min·g))	2.29	2.06	2.21	2.19	0.11	2.10	2.27	0.08	NS
Ouabain sensitive proportion (%)	22.8	20.5	5.2	17.0	6.65	13.0	19.7	4.71	NS

[A, B] Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).
[a, b] Means in the same row without the same superscripts are significantly different between the lines ($p<0.05$).
[M, N] Means in the same row without the same superscripts are very significantly different between the treatments ($p<0.01$).
SEM = Standard error of mean. L×T: Interaction between line and treatment; NS indicates $p>0.05$.

Table 6. Jejunal glucose transport and its apparent energetic efficiency in 9-wk-old mice randomly selected (ICR) or selected for rapid growth (M16) and their reciprocal crosses provided drinking water containing monensin

Item	Line (L)					Treatment (T)			L×T
	ICR	M16	ICR×M16	M16×ICR	SEM	Control	Monensin	SEM	
Jejunal tissue glucose transport ηmol glucose/(min·g jejunum)									
Total	219^{A}	165^{B}	197^{AB}	208^{A}	8.5	189	206	6.01	NS
Active	143^{A}	91^{B}	133^{A}	145^{A}	10.3	118	138	7.28	NS
Passive	76	74	64	63	4.3	71	68	3.0	NS
Entire jejunum glucose transport ηmol glucose/min									
Total	292^{B}	394^{A}	293^{B}	321^{B}	16.8	313	336	12	NS
Active	193	214	198	222	16.6	192	222	12	NS
Passive	99^{B}	179^{A}	95^{B}	99^{B}	8.8	121	115	6.2	NS
Adjusted entire jejunum glucose transport ηmol glucose/(min·g FBW)									
Total	8.83^{A}	6.46^{C}	7.17^{BC}	7.96^{AB}	0.33	7.34	7.86	0.24	NS
Active	5.80^{A}	3.53^{B}	4.82^{AB}	5.50^{A}	0.38	4.56	5.26	0.27	NS
Passive	3.03	2.93	2.35	2.46	0.19	2.79	2.60	0.13	NS

[A, B, C] Means in the same row without the same superscripts are very significantly different between the lines ($p<0.01$).
[a, b, c] Means in the same row without the same superscripts are significantly different between the lines ($p<0.05$).
SEM = Standard error of mean. L×T: Interaction between line and treatment; NS indicates $p>0.05$.
FBW = Body weight after feed-deprived for 18 h.

M16 mice had denser (79.1 mg intestine/cm intestinal length) jejunums ($p<0.01$; Table 2) that were 31% shorter ($p<0.01$) than were observed for the randomly selected controls. Small intestinal length (49.6-52.7 cm/mouse) and FBW (40.3-40.7 g/mouse) of reciprocal crosses were intermediate between the ICR and M16 lines. The genetic lines of the mice had no effect on dry matter contents of jejunal intact, serosal or mucosal tissues. Neither effect of genetic lines on protein and DNA concentrations and protein/DNA ratio in jejunal mucosa was observed (Table 3).

M16 mice had jejunal villi that were 24% wider ($p<0.05$) than the controls (Table 3). Villus width for the reciprocal crosses was not different ($p>0.05$) from that of the controls. Jejunal villus height/crypt depth decreased 18% ($p<0.05$) in the M16 compared to that observed for the ICR controls (Table 3). Both reciprocal crosses tended to have similar decreases (12.2%-16.5%) in jejunal villus height/crypt depth in comparison to the controls (Table 3). Enterocyte height at mid jejunal villus in the M16 line was greater (28.8 vs. 24.4-25.1 μm; $p<0.01$) than those in the controls and in the reciprocal crosses.

Genotype had no effect on mouse activity either before or after treating with monensin for 2 weeks (Table 4). Whole-body oxygen consumption adjusted for body weight was 28% less (1.68 vs. 2.34 μmol O_2/min·g FBW; $p<0.01$) in the M16 line than that in the randomly selected controls

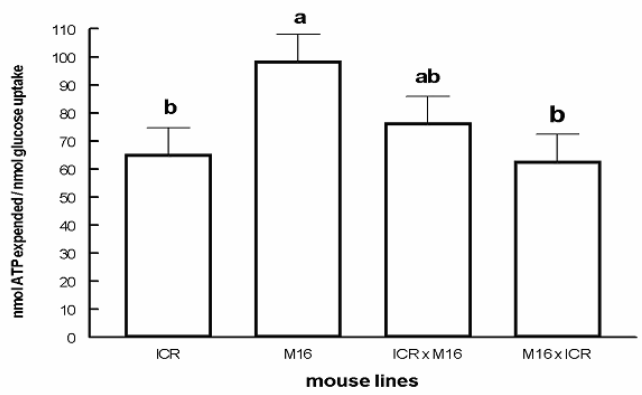

Figure 1. Effect of selection for growth in mice on the apparent energetic efficiency of active jejunal glucose absorption (APEE) as defined by Bird et al. (1994) and Croom et al. (1998). Histograms without common superscripts differ significantly ($p<0.05$).

before initiation of monensin treatment at 7 weeks of age. No differences were noted among the ICR and the reciprocal crosses (Table 4). The M16 line consumed more ($p<0.01$) oxygen than ICR line and the reciprocal crosses after treating with monensin for 2 weeks. However, the difference no more existed when whole-mouse oxygen consumption was expressed after adjusted by body weight.

Respiration of intact jejunum, including respiration of ouabain sensitive proportion, did not differ ($p>0.05$) between lines (Table 5). Alcohol, but not monensin, decreased jejunal respiration in M16 line in comparison with the lines of ICR and reciprocal crosses. Jejunal serosal respiration decreased as much as 20% (0.73 vs. 0.88-0.91 μmol O_2/min·g; $p<0.05$) in the M16 line in comparison with the reciprocal crosses. No differences were noted amongst mucosal respiration of the different lines.

Selection for increased growth (M16 line) decreased the rate of active, consequently total, jejunal tissue glucose transport by 25% ($p<0.01$; Table 6). Selection for increased growth increased estimated entire jejunum glucose transport (total: active plus passive) due to more mass of the small intestine. Selection for increased growth decreased total and active entire jejunal glucose transport adjusted for body weight by 27% and 39% ($p<0.01$), respectively, in comparison with that observed for the control line. The apparent energetic efficiency of active glucose transport decreased by 51% ($p<0.05$) when compared with the controls and the M16×ICR lines (Figure 1).

DISCUSSION

The growth and performance of the parent lines of mice (ICR and M16) were consistent with that reported in previous studies involving this well described mouse growth model (Eisen et al., 1975; Eisen and Leatherwood, 1978a, b; Fan et al., 1996). A re-analysis of the M16 line has verified the stability of this outbred stock in its value as a model for polygenic growth and obesity (Allan et al., 2004). The M16 line grew faster than the ICR line and with greater efficiency (Table 1). Changes in growth and performance of the reciprocal crosses in relation to the parent lines were inconsistent, as reported in a previous study (Fan et al., 1996). The inconsistency in growth and performance of the reciprocal crosses may be attributable to maternal effects influencing uterine function, lactation and/or behavior which could cause an indirect effect other than the direct effect of genetic background per se on the progenies from mothers of different lines.

Effect of genotype on response to monensin

Monensin did not have any significant effects on growth or feed efficiency across lines (Table 1). In the present study, monensin did significantly decrease water consumption in the ICR and reciprocal cross lines in comparison to what was observed for the M16 ($p<0.01$; Table 1). This has not been previously reported. Mouse is able to differentiate hazardous substances presenting in food or drinking water. Basing on the evidence of less intake of drinking water that contained monensin, the mice of ICR, a randomly bred one, and its reciprocal cross having half of ICR blood are believed still conserving the ability of differentiation. The differentiating ability is apparently genetic associating. In contrast, M16 mice may have lost their ability to differentiate the presence of hazardous chemicals such as monensin during the genetic selection for rapid growth.

The present study supports previous findings from this laboratory (Fan et al., 2003) that monensin has minimal effect on whole body energetics as well as on jejunal morphology, energetics and absorptive function (Tables 2, 4, 5 and 6), despite minor effects on villus architecture (Table 3).

Monensin did significantly decrease intestinal mucosal protein/DNA, across lines by 10% (Table 3). Additionally, significant line x treatment effects for mucosal protein and mucosal protein/DNA indicate that these parameters were affected differently amongst the different lines (Table 3). Monensin also increased villus width (Table 3). Monensin had no other effects on whole-body or intestinal function including jejunal glucose transport. No such effects were reported in 7-week-old Swiss-Webster mice by Fan et al. (2003). It should be noted that two major differences existed in the design of the previously reported study by Fan et al. (2003) and the present one that may account for these minor differences. First, Fan et al. (2003) used 7-week-old mice in contrast to the 9-week-old mice in the present report. Second, the earlier study administered 16.4 mM monensin in the drinking water as opposed to the 20 mM employed in the present study. Physiologically, a more

mature mouse of 9-week-old one should be more tolerable to the effect of monensin on the anatomical structure of the small intestine than the younger one such as 7-week-old mouse. Similarly, a higher dosage of 20 mM monensin is more intolerable comparing to the lower one of 16.4 mM. Taking the two issues together, it shows that the effect of monensin dosage is dominating that of maturity in mouse. It is true at least in these two experiments.

Alcalde et al. (1987) reported that amoxicillin inhibited galactose absorption from the jejunum of rats. They postulated that amoxicillin appears to inhibit galactose transport, in the SGLT1 Na-dependent glucose transporter, in a competitive fashion without affecting the oxygen consumption of the tissue. In contrast, Radja (1988) reported the ionophore antibiotic, valinomycin, increased glucose transport in the duodenum of mice. Ionophore antibiotics are known to insert themselves within the membranes of cells and act as artificial ion channels for different cations (Bergen and Bates, 1984). Monensin inhibits Na^+/K^+ ATPase located on eukaryotic cell membranes so that the enzyme is no longer able to maintain intracellular Na homeostasis. It would thus appear that the effects of antibiotics on the intestine are directly related to antibiotic structural and mechanistic characteristics. Based on the failure of monensin to alter ouabain sensitive respiration in the jejunum (Table 5), it would appear that monensin has no effect on enterocyte metabolism.

The lack of a generalized metabolic effect by monensin on body metabolism or intestinal function leads us to conclude that monensin has no effect on these physiological parameters in mice, regardless of genotype. When considering previous reports from this laboratory (Fan et al., 2003), monensin had no described effect on whole-body metabolism and intestinal function in five genetic lines or crosses of mice and in 2 different age groups. We feel this observation is important and indicates that extrapolation of these finding to domestic livestock and poultry is not unreasonable.

Effect of genotype on energetics and jejunal function

We have previously noted that whole-body O_2 consumption of 5-week-old M16 mice was 19% greater per gram of FBW than that of ICR mice (Fan et al., 1996). In the present study, before initiation of monensin treatment at 7 weeks of age, the M16 line consumed 28% less oxygen per unit of FBW than the ICR line. No differences were noted after monensin treatment at 9 weeks of age among the M16 line and the randomly selected controls or reciprocal crosses (Table 4).

Fan et al. (1996) reported no effects in selection for growth on jejunal tissue respiration (including serosal and mucosal components) as well as the rate of jejunal glucose absorption, total jejunal glucose absorption and the apparent energetic efficiency of active jejunal glucose absorption. A 36% decrease ($p<0.01$; Table 6) in active jejunal glucose transport rate, a 39% decrease ($p<0.01$) in estimated total jejunal glucose absorption adjusted for FBW and a 51% ($p<0.05$; Figure 1) decrease in the apparent energetic efficiency of active jejunal glucose transport compared to the ICR controls as evidenced by the increase in the relative amounts of ATP expanded per unit of glucose uptake.

It is important to reconcile difference on the effects of genotype on whole body energetics and jejunal function noted, herein, as compared to that previously reported from this laboratory (Fan et al., 1996), especially since the same lines of mice and experimental design were used in each study. Of special interest are the differences noted in whole-body O_2 consumption, jejunal O_2 consumption and jejunal glucose transport. We believe that these differences may be due to an age effect. Fan et al. (2003) used 5-week-old mice whereas herein monensin treatment was begun at 7 weeks of age and lasted until 9 weeks of age. Bird et al. (1994b) estimated that glucose absorption in mice did not peak in growing mice until approximately 8 weeks of age, at which time the mice are considered fully mature. The differences in whole-body oxygen consumption and the rate, total amount and apparent energetic efficiency of jejunal glucose absorption between the study of Fan et al. (2003) and the present one were likely due to ontogenetic differences in metabolism and intestinal function between a young 5 week-old mouse, a more mature 7 week-old mouse and a mature 9 week-old mouse.

The present data presented confirm previous conclusions from our laboratory that selection for enhanced production parameters may not be synchronized with beneficial changes in the rates or energetic efficiencies of intestinal absorption (Croom et al., 1999). This asynchrony might result in limitations on economically important phenotypic expression of a genotype selected for rapid growth (Croom et al., 1998).

CONCLUSIONS

The lack of effects of the ionophore antibiotic, monensin, on whole-body metabolism and jejunal function, as reported by Fan et al. (2003), is not altered by differences in genotype. Given the widespread use of this and similar compounds across a variety of genotypes, within a number of domestic livestock species, these studies provides evidence that the use of this class of feed supplement will not cause alterations in whole-body metabolism or intestinal function across different genetic lines. It is clear, however, that genetic selection for production traits does not result in concomitant improvements in intestinal function and nutrient absorption.

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