

# Inferring phenotypic causal structures among meat quality traits and the application of a structural equation model in Japanese Black cattle<sup>1</sup>

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**ABSTRACT:** Meat quality is one of the most important traits determining carcass price in the Japanese beef market. Optimized breeding goals and management practices for the improvement of meat quality traits requires knowledge regarding any potential functional relationships between them. In this context, the objective of this research was to infer phenotypic causal networks involving beef marbling score (BMS), beef color score (BCL), firmness of beef (FIR), texture of beef (TEX), beef fat color score (BFS), and the ratio of MUFA to SFA (MUS) from 11,855 Japanese Black cattle. The inductive causation (IC) algorithm was implemented to search for causal links among these traits and was conditionally applied to their joint distribution on genetic effects. This information was obtained from the posterior distribution of the residual (co)variance matrix of a standard Bayesian multiple trait model (MTM). Apart from BFS, the IC algorithm implemented with 95% highest posterior density (HPD) intervals detected only undirected links among the traits. However, as a result of the application of 80% HPD intervals, more links were recovered and the undirected links were changed into directed ones, except between FIR and TEX. Therefore, 2 competing

causal networks resulting from the IC algorithm, with either the arrow  $FIR \rightarrow TEX$  or the arrow  $FIR \leftarrow TEX$ , were fitted using a structural equation model () to infer causal structure coefficients between the selected traits. Results indicated similar genetic and residual variances as well as genetic correlation estimates from both structural equation models. The genetic variances in BMS, FIR, and TEX from the structural equation models were smaller than those obtained from the MTM. In contrast, the variances in BCL, BFS, and MUS, which were not conditioned on any of the other traits in the causal structures, had no significant differences between the structural equation model and MTM. The structural coefficient for the path from MUS (BCL) to BMS showed that a 1-unit improvement in MUS (BCL) resulted in an increase of 0.85 or 1.45 (an decrease of 0.52 or 0.54) in BMS in the causal structures. The analysis revealed some interesting functional relationships, direct genetic effects, and the magnitude of the causal effects between these traits, for example, indicating that BMS would be affected by interventions on MUS and BCL. In addition, if interventions existed in this scenario, a breeding strategy based only on the MTM would lead to a mistaken selection for BMS.

**Key words:** genetic parameter, inductive causation, Japanese Black, meat quality, phenotypic causal link, structural equation model

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## INTRODUCTION

Traits related to meat quality are important in determining carcass price in the Japanese beef market. In particular, beef marbling and a high ratio of unsaturated fatty acids are of great interest for Wagyu beef. In any

breeding or production system based on multiple traits, it is important to study potential causal relationships among the traits (Rosa et al., 2011). The inductive causation (IC) algorithm (Verma and Pearl, 1990; Pearl, 2000) allows searching for how variables are causally related. Valente et al. (2010) adapted the IC algorithm to a mixed models context and showed that applying this method to the posterior distribution of the residual (co)variance matrix of a standard multiple trait model (MTM) recovered the expected network in simulated data.

The causal structure inferred using the IC algorithm can then be applied to define a structural equation model (Wright, 1921; Haavelmo, 1943). Gianola and Sorensen (2004) were the first to propose adaptations of the structural equation model to the context of quantitative genetics and mixed effect models. Fitting structural equation models allows for inferences of direct genetic effects and the magnitude of causal effects between traits. A breeding strategy based only on MTM would cause a delay in achieving the breeding goal if interventions, which would block indirect genetic effects, exist among the traits (Valente et al., 2013).

A number of studies have applied mixed effects structural equation models in the animal breeding context. However, these studies assumed that causal structures were known a priori. More recently, some studies fitted structural equation models based on a data-driven causal structure search, namely applications to European quail (Valente et al., 2011) and to bovine milk fatty acids (Bouwman et al., 2014). The objective of this research was to search for causal structures among meat quality traits in Japanese Black cattle. In addition, a structural equation model was fitted using the selected links based on the results of the IC algorithm to quantify the relationships between these traits.

## MATERIALS AND METHODS

### Data

Data used in this study were obtained from carcass records (i.e., Animal Care and Use Committee approval was not necessary) of 11,855 Japanese Black steers and heifers under concentrate feeding (fattening) in Yamagata prefecture, Japan. Pedigree information comprised data from 57,523 animals. The data were the same as in our previous study (Inoue et al., 2016). Graded meat quality traits included beef marbling score (BMS) ranging from 1 (poor) to 12 (abundant), beef color score (BCL) ranging from 1 (light) to 7 (dark), firmness of beef (FIR) and texture of beef (TEX) ranging from 1 (poor) to 5 (excellent), and beef fat color score (BFS) ranging from 1 (white) to 7 (yellow). These measurements were evaluated between the sixth and

**Table 1.** Descriptive statistics of meat quality traits

Trait <sup>1</sup>	Mean	SD	Minimum	Maximum
BMS	6.9	2.25	2	12
BCL	3.7	0.62	2	6
FIR	4.2	0.87	2	5
TEX	4.3	0.77	2	5
BFS	3.0	0.22	2	5
MUS	1.9	0.24	1.1	2.9

<sup>1</sup>BMS = beef marbling score; BCL = beef color score; FIR = firmness of beef; TEX = texture of beef; BFS = beef fat color score; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

seventh rib in accordance with the Japan Meat Grading Association (1988). The meat quality trait related to the fatty acid composition was the ratio of MUFA to SFA (MUS) as an index of the level of unsaturation of i.m. fat and was calculated as follows:

$$\text{MUS} = (\text{C14:1} + \text{C16:1} + \text{C18:1}) / (\text{C14:0} + \text{C16:0} + \text{C18:0})$$

where C14:0 (myristic acid), C16:0 (palmitic acid), and C18:0 (stearic acid) were SFA with high fat melting points and C14:1 (myristoleic acid), C16:1 (palmi-toleic acid), and C18:1 (oleic acid) were unsaturated fatty acids with low melting points. These fatty acids were measured using GLC on i.m. adipose tissue samples from approximately 2 mm of sliced trapezius from between the sixth and seventh rib as described by Inoue et al. (2011).

The descriptive statistics of the meat quality traits are shown in Table 1.

### The Multiple Trait Model Analysis

Among the traits, BCL, FIR, TEX, and BFS could be regarded as ordered categorical traits, because they were distributed in 4 or 5 levels in the data set (Table 1). However, in the present study, the traits were assumed to be linear traits, because of extremely poor mixing of the Gibbs sampling when using a multitrait linear-threshold model. In addition, differences of the estimated parameters between a multitrait linear-threshold model (the current study) and a bivariate linear-threshold model (Inoue et al., 2016) for the traits were not large except for the heritability of BFS; the differences of heritability estimates except for BFS were 0.02 to 0.05 and those of genetic correlations were 0.00 to 0.17. The posterior mean  $\pm$  posterior SD (PSD) of heritability for BFS from both models were  $0.10 \pm 0.022$  (Table 2) and  $0.30 \pm 0.067$  (a univariate threshold model; Inoue et al., 2016).

A standard Bayesian MTM was used for estimating genetic and residual (co)variance components. The following MTM was fitted:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e},$$

with the joint distribution of vectors  $\mathbf{u}$  and  $\mathbf{e}$  as

$$\begin{bmatrix} \mathbf{u} \\ \mathbf{e} \end{bmatrix} \sim N \left\{ \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{G}_0 \otimes \mathbf{A} & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_0 \otimes \mathbf{I} \end{bmatrix} \right\},$$

where  $\mathbf{y}$  is a vector of observations;  $\boldsymbol{\beta}$  is a vector of systematic effects, including sex of animals (2 levels), slaughter year (15 levels), slaughter month (12 levels), farm (302 levels), and linear and quadratic terms for age at slaughter (average 31.8 mo);  $\mathbf{u}$  is a vector of random additive genetic effects;  $\mathbf{e}$  is a vector of random residuals;  $\mathbf{X}$  and  $\mathbf{Z}$  are known incidence matrices;  $\mathbf{G}_0$  is the additive genetic (co)variance matrix;  $\mathbf{A}$  is the additive genetic relationship matrix;  $\mathbf{R}_0$  is the residual (co)variance matrix; and  $\mathbf{I}$  is an identity matrix with suitable dimensions. The program GIBBS1F90 (Misztal et al., 2002) was used to obtain posterior distributions of genetic and residual (co)variances. A Gibbs sampling chain with 200,000 samples was generated, with the initial 100,000 samples discarded as burn-in based on visual inspection of trace plots and posterior distributions. The results of Geweke's diagnostic (Geweke, 1992) and effective sample size from the program POSTGIBBSF90 (Misztal et al., 2002) were also used to assess convergence and were less than 1.0 and over 10, respectively (their recommended criteria), for all variance and covariance parameters. Effective sample sizes were around 80 to 90 except for those related to covariances of BFS (around 20). The posterior distributions of the variance and covariance components were approximated based on the remaining 100,000 samples.

### The Inductive Causation Algorithm

The IC algorithm was applied to the residual (co)variances obtained from the MTM analysis. The residual (co)covariances were considered information from the joint distribution of the phenotypic traits conditional on genetic effects, such that they correct the confounding issues caused by such effects when the traits are genetically correlated (Valente et al., 2010). The IC algorithm performs a series of statistical decisions based on partial correlations between traits and consists of the following 3 steps (Pearl, 2000):

Step 1: If partial correlations of 2 traits that are conditional on every possible set of the remaining traits are different from 0, the 2 traits are connected with an undirected link (e.g.,  $y_1 - y_2$ ).

Step 2: Based on the undirected graph obtained in Step 1, if partial correlations of a pair of traits that are nonadjacent but share a common adjacent trait (e.g.,  $y_1$  and  $y_3$  in  $y_1 - y_2 - y_3$ ) are conditionally non-null on all

**Table 2.** Posterior means of heritability (diagonal) and genetic (above diagonal) and residual (below diagonal) correlations (with their posterior SD) obtained from the multiple trait model

Trait <sup>1</sup>	BMS	BCL	FIR	TEX	BFS	MUS
BMS	<b>0.74</b> ( <b>0.049</b> )	-0.70 <sup>2</sup> (0.053)	0.99 <sup>2</sup> (0.000)	0.99 <sup>2</sup> (0.002)	-0.04 (0.132)	-0.13 <sup>2</sup> (0.065)
BCL	-0.15 (0.072)	<b>0.38</b> ( <b>0.048</b> )	-0.72 <sup>2</sup> (0.051)	-0.72 <sup>2</sup> (0.053)	0.04 (0.136)	0.08 (0.081)
FIR	0.57 <sup>2</sup> (0.062)	-0.16 <sup>2</sup> (0.060)	<b>0.66</b> ( <b>0.046</b> )	0.99 <sup>2</sup> (0.002)	-0.05 (0.130)	-0.13 <sup>2</sup> (0.065)
TEX	0.52 <sup>2</sup> (0.061)	-0.16 <sup>2</sup> (0.058)	0.71 <sup>2</sup> (0.032)	<b>0.62</b> ( <b>0.046</b> )	-0.06 (0.135)	-0.13 (0.067)
BFS	-0.04 (0.053)	0.03 (0.026)	-0.05 (0.043)	-0.04 (0.041)	<b>0.10</b> ( <b>0.022</b> )	0.51 <sup>2</sup> (0.102)
MUS	0.20 <sup>2</sup> (0.109)	0.05 (0.058)	0.15 (0.089)	0.13 (0.083)	0.03 (0.037)	<b>0.63</b> ( <b>0.045</b> )

<sup>1</sup>BMS = beef marbling score; BCL = beef color score; FIR = firmness of beef; TEX = texture of beef; BFS = beef fat color score; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

<sup>2</sup>95% highest posterior density region does not include 0.

possible subsets of the remaining variables that contain the adjacent trait ( $y_2$  in the given example), arrowheads pointing to the common adjacent trait are added (i.e.,  $y_1 \rightarrow y_2 \leftarrow y_3$ ), forming a so-called "unshielded collider."

Step 3: With the partially oriented graph obtained in Step 2, as many undirected links as possible are oriented without creating a new unshielded collider or cycle.

Statistical decisions regarding whether to declare partial correlations as null or not were based on highest posterior density (HPD) intervals. If the interval contained the value 0, the correlation was declared null. Outputs may differ according to the probability content used for the decision. We applied different HPD intervals (80, 85, 90, and 95%) to evaluate the structure sensitivity. The analysis was performed using the program written in R (R Development Core Team, 2009) by Valente and Rosa (2013).

### The Structural Equation Model Analysis

The structural equation model was fitted to the causal network inferred by the IC algorithm application. The structural equation model can be described as

$$\mathbf{y} = (\boldsymbol{\gamma} \otimes \mathbf{I})\mathbf{y} + \mathbf{X}^* \boldsymbol{\beta}^* + \mathbf{Z}\mathbf{u}^* + \mathbf{e}^*,$$

with the joint distribution of vectors  $\mathbf{u}^*$  and  $\mathbf{e}^*$  as

$$\begin{bmatrix} \mathbf{u}^* \\ \mathbf{e}^* \end{bmatrix} \sim N \left\{ \begin{bmatrix} \mathbf{0} \\ \mathbf{0} \end{bmatrix}, \begin{bmatrix} \mathbf{G}_0^* \otimes \mathbf{A} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\psi}_0 \otimes \mathbf{I} \end{bmatrix} \right\},$$

where  $\mathbf{y}$ ,  $\boldsymbol{\beta}^*$ ,  $\mathbf{u}^*$ ,  $\mathbf{e}^*$ ,  $\mathbf{X}$ ,  $\mathbf{Z}$ ,  $\mathbf{A}$ , and  $\mathbf{I}$  have meanings similar to those described above for the MTM, but these

vectors represent systematic and random effects directly affecting each trait, that is, effects that are not mediated by other phenotypic traits (Gianola and Sorensen, 2004; Rosa et al., 2011; Valente et al., 2013). Additionally,  $\Lambda$  is a  $t \times t$  matrix (where  $t$  is the number of traits) with 0 on the diagonal and with structural coefficients (linear effects between pairs of traits) or 0 on the off-diagonals,  $\mathbf{G}_0^*$  is the structural equation model additive genetic (co)variance matrix (i.e., it describes variances and covariances of direct genetic effects), and  $\Psi_0$  is a diagonal matrix with the structural equation model residual variances. The residual covariances between the traits in the structural equation model were assumed to be 0. This is equivalent to assuming that all variables that simultaneously affect 2 or more phenotypic traits in the system have already been accounted for by the model. Such parametric restriction confers identifiability to the structural coefficients in the likelihood function.

The program used for the standard MTM (GIBBS1F90; Misztal et al., 2002) was also used for obtaining posterior distributions of the structural equation model parameters. Model effects and initial values for parameters were set similarly to the MTM analysis. For the structural equation model, however, causal parents (e.g.,  $y_1$  is the causal parent of  $y_2$  in  $y_1 \rightarrow y_2$ ) of a given trait were included as covariates in the equations assigned to that trait and a diagonal residual covariance matrix was imposed. A Gibbs sampling chain with 1,000,000 samples was generated, with the initial 500,000 samples discarded as burn-in as decided by visual inspection and a thinning interval of 10 iterations. Larger sample size and thinning were required to fulfill the convergence criteria, which were less than 1.0 for the results of Geweke's diagnostic (Geweke, 1992) and over 10 for effective sample size from the program POSTGIBBSF90 (Misztal et al., 2002) for all variance and covariance parameters. Therefore, the posterior probabilities of the structural equation model unknowns were characterized based on the remaining 50,000 samples.

## RESULTS AND DISCUSSION

### *The Multiple Trait Model Analysis*

The results of the MTM analysis, in terms of posterior means of heritability and genetic and residual correlations of meat quality traits, are shown in Table 2 with their respective PSD. The posterior means and PSD of the variance components are presented in Table 3. Heritability estimates and  $\pm$ PSD of meat quality traits were moderate ( $0.38 \pm 0.048$  for BCL) to high ( $0.74 \pm 0.049$  for BMS) except for BFS ( $0.10 \pm 0.022$ ). These estimates are comparable to the results reported by Yokota et al. (2011) for a Japanese Black breed from

a different herd. These authors reported heritability estimates of 0.50, 0.24, 0.49, 0.34, 0.02, and 0.60 for BMS, BCL, FIR, TEX, BFS, and MUFA, respectively. In addition, the heritability estimates for BCL, FIR, and TEX as linear traits in the present study were similar to those that Inoue et al. (2016) estimated using a univariate threshold model as categorical traits, although only the estimates for BFS were different between the studies. The heritability ( $\pm$ PSD) for BFS in the present study was lower ( $0.10 \pm 0.022$ ) than that ( $0.30 \pm 0.067$ ) from Inoue et al. (2016). The difference between the 2 heritability estimates for BFS could be due to differences in the fitted models (i.e., a linear vs. a threshold model), because heritability estimates obtained from a threshold model are generally larger than those obtained from a linear model (Matos et al., 1997; Varona et al., 1999; Kizilkaya et al., 2002). The decrease in heritability by fitting a linear model was larger in BFS than in the other traits. Small phenotypic variation for BFS can also be one cause of lower heritability for BFS than for the other traits from a linear model.

Genetic correlations among BMS, FIR, and TEX were almost 1.00 and those of BCL with BMS, FIR, and TEX were highly negative (around  $-0.70$ ). By contrast, genetic correlations between BFS and the remaining traits were approximately 0.00, with the 95% HPD region including 0.00, except between BFS and MUS ( $0.51 \pm 0.102$ ). Similarly, weak genetic correlations ( $-0.13 \pm 0.065$  to  $0.08 \pm 0.081$ ) were found between MUS and the remaining traits except for BFS. Generally, these results were similar to those of the previous studies of Wagyu (Shirai et al., 1996; Yokota et al., 2011; Sato et al., 2013; Inoue et al., 2016).

Significantly, strong positive residual correlations were found among BMS, FIR, and TEX ( $0.52 \pm 0.061$  to  $0.71 \pm 0.032$ ). Residual correlations of BCL with BMS, FIR, and TEX were weakly negative ( $-0.16 \pm 0.058$  and  $0.060$  to  $-0.15 \pm 0.072$ ). Those between MUS and meat quality traits were weak and not significant ( $0.03 \pm 0.037$  to  $0.15 \pm 0.089$ ), except between MUS and BMS ( $0.20 \pm 0.109$ ). Despite our concern for a fitting a linear model for BFS, estimates of residual correlations between BFS and the other graded meat quality traits in this study were similar to those presented by Inoue et al. (2016).

### *The Inductive Causation Algorithm*

Using different HPD intervals for the decisions of the IC algorithm resulted in the 3 alternative graphs depicted in Fig. 1. Using 95% HPD intervals, links among the meat quality traits, except for BFS, were detected, although their directions were not resolved (Fig. 1a). When 90 and 85% HPD intervals were applied, an additional link between BCL and FIR (Fig. 1b) was detected. When

**Table 3.** Posterior means and posterior SD (PSD) of the variance components for the multiple trait model (MTM) and the structural equation model

Variance component <sup>1</sup>	MTM		Structural equation model (FIR → TEX)		Structural equation model (TEX → FIR)	
	Mean	PSD	Mean	PSD	Mean	PSD
$\sigma_e^2$ BMS	1.367	0.227	1.576	0.167	1.563	0.163
$\sigma_e^2$ BCL	0.210	0.013	0.212	0.012	0.211	0.012
$\sigma_e^2$ FIR	0.265	0.031	0.179	0.002	0.117	0.002
$\sigma_e^2$ TEX	0.228	0.024	0.107	0.002	0.160	0.003
$\sigma_e^2$ BFS	0.041	0.001	0.041	0.001	0.041	0.001
$\sigma_e^2$ MUS	0.019	0.002	0.018	0.002	0.018	0.002
$r_e$ BMS, BCL	-0.148	0.072	–	–	–	–
$r_e$ BMS, FIR	0.568 <sup>2</sup>	0.062	–	–	–	–
$r_e$ BMS, TEX	0.524 <sup>2</sup>	0.061	–	–	–	–
$r_e$ BMS, BFS	-0.040	0.053	–	–	–	–
$r_e$ BMS, MUS	0.202 <sup>2</sup>	0.109	–	–	–	–
$r_e$ BCL, FIR	-0.158 <sup>2</sup>	0.060	–	–	–	–
$r_e$ BCL, TEX	-0.158 <sup>2</sup>	0.058	–	–	–	–
$r_e$ BCL, BFS	0.028	0.026	–	–	–	–
$r_e$ BCL, MUS	0.054	0.058	–	–	–	–
$r_e$ FIR, TEX	0.706 <sup>2</sup>	0.032	–	–	–	–
$r_e$ FIR, BFS	-0.050	0.043	–	–	–	–
$r_e$ FIR, MUS	0.154	0.089	–	–	–	–
$r_e$ TEX, BFS	-0.042	0.041	–	–	–	–
$r_e$ TEX, MUS	0.127	0.083	–	–	–	–
$r_e$ BFS, MUS	0.026	0.037	–	–	–	–
$\sigma_g^2$ BMS	3.851	0.338	3.132	0.334	3.090	0.316
$\sigma_g^2$ BCL	0.129	0.019	0.126	0.017	0.127	0.017
$\sigma_g^2$ FIR	0.525	0.046	0.024	0.003	0.012	0.002
$\sigma_g^2$ TEX	0.376	0.035	0.009	0.004	0.030	0.009
$\sigma_g^2$ BFS	0.005	0.001	0.004	0.001	0.004	0.001
$\sigma_g^2$ MUS	0.032	0.003	0.033	0.003	0.033	0.003
$r_{g_e}$ BMS, BCL	-0.701 <sup>2</sup>	0.053	-0.623 <sup>2</sup>	0.073	-0.630 <sup>2</sup>	0.073
$r_{g_e}$ BMS, FIR	0.999 <sup>2</sup>	0.000	0.936 <sup>2</sup>	0.013	0.928 <sup>2</sup>	0.034
$r_{g_e}$ BMS, TEX	0.996 <sup>2</sup>	0.002	0.606 <sup>2</sup>	0.217	0.870 <sup>2</sup>	0.051
$r_{g_e}$ BMS, BFS	-0.035	0.132	-0.231 <sup>2</sup>	0.056	-0.204 <sup>2</sup>	0.060
$r_{g_e}$ BMS, MUS	-0.132 <sup>2</sup>	0.065	-0.250 <sup>2</sup>	0.050	-0.144 <sup>2</sup>	0.043
$r_{g_e}$ BCL, FIR	-0.716 <sup>2</sup>	0.051	-0.697 <sup>2</sup>	0.061	-0.678 <sup>2</sup>	0.072
$r_{g_e}$ BCL, TEX	-0.720 <sup>2</sup>	0.053	-0.472 <sup>2</sup>	0.213	-0.631 <sup>2</sup>	0.104
$r_{g_e}$ BCL, BFS	0.039	0.136	0.208 <sup>2</sup>	0.063	0.222 <sup>2</sup>	0.068
$r_{g_e}$ BCL, MUS	0.075	0.081	0.145 <sup>2</sup>	0.025	0.143 <sup>2</sup>	0.025
$r_{g_e}$ FIR, TEX	0.996 <sup>2</sup>	0.002	0.641 <sup>2</sup>	0.214	0.759 <sup>2</sup>	0.104
$r_{g_e}$ FIR, BFS	-0.049	0.130	-0.339 <sup>2</sup>	0.049	-0.262 <sup>2</sup>	0.077
$r_{g_e}$ FIR, MUS	-0.129 <sup>2</sup>	0.065	-0.064	0.040	-0.033	0.046
$r_{g_e}$ TEX, BFS	-0.063	0.135	-0.280 <sup>2</sup>	0.129	-0.355 <sup>2</sup>	0.080
$r_{g_e}$ TEX, MUS	-0.128	0.067	-0.080	0.057	-0.070	0.038
$r_{g_e}$ BFS, MUS	0.514 <sup>2</sup>	0.102	0.601 <sup>2</sup>	0.060	0.624 <sup>2</sup>	0.072

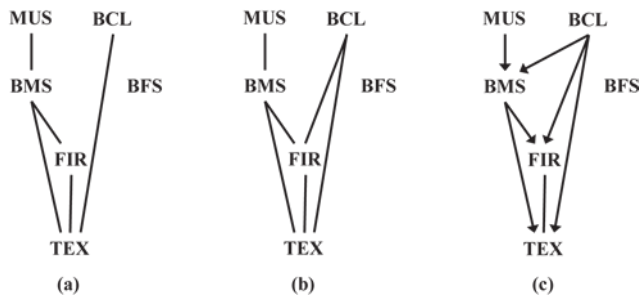
<sup>1</sup> $\sigma_e^2$  = residual variance;  $r_e$  = residual correlation;  $\sigma_g^2$  = genetic variance;  $r_g$  = genetic correlation; BMS = beef marbling score; BCL = beef color score; FIR = firmness of beef; TEX = texture of beef; BFS = beef fat color score; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

<sup>2</sup>95% highest posterior density region does not include 0.

80% HPD intervals were used, an extra link between BCL with BMS was recovered and most links were directed, except for the one between FIR and TEX (Fig. 1c). As expected, applying narrower HPD intervals resulted in more links being recovered. Furthermore, it led to detecting unshielded colliders in Step 2.

### The Structural Equation Model Analysis

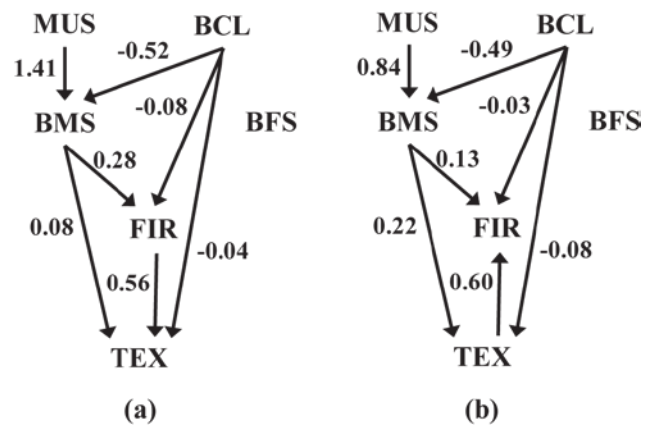
The causal network shown in Fig. 1c was chosen for fitting the structural equation model, which produced an almost fully directed graph. The only exception was the link between FIR and TEX, which remained undirected.



**Figure 1.** Links between traits detected by the inductive causation algorithm with 95% (a), 90 and 85% (b), and 80% (c) of highest posterior density intervals. Links without arrowheads represent associations between traits and links with arrowheads represent causal relationships toward arrowheads. BMS = beef marbling score; BCL = beef color score; FIR = firmness of beef; TEX = texture of beef; BFS = beef fat color score; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

Biological prior knowledge about the relationship between these traits could be applied to specify a direction, but no specific direction seemed more reasonable than the other in this case. It should be emphasized that this decision cannot be made on a statistical basis, as both models are statistically equivalent (Valente et al., 2010). Therefore, we applied the 2 types of structures based on Fig. 1c with a directed link for the undirected link between FIR and TEX, or  $\text{FIR} \rightarrow \text{TEX}$  ( $\lambda_{\text{TEX}, \text{FIR}}$ ) and  $\text{FIR} \leftarrow \text{TEX}$  ( $\lambda_{\text{FIR}, \text{TEX}}$ ). Here,  $\lambda_{i,j}$  denotes a structural coefficient from the  $j$ th trait to the  $i$ th trait.

The posterior means and PSD of the variance components for the structural equation models are shown in Table 3, together with those from the MTM. The posterior means, PSD, and 95% HPD intervals of the structural coefficients inferred using the structural equation models are also presented in Table 4, and the means and links between traits are depicted in Fig. 2. Similar posterior means of genetic and residual dispersion parameters (Table 3) were inferred by the structural equation model with either a directed link of  $\text{FIR} \rightarrow \text{TEX}$  (Fig. 2a) or a directed link of  $\text{FIR} \leftarrow \text{TEX}$  (Fig. 2b). As expected, posterior distributions of structural equation model variances assigned to BMS, FIR, and TEX were different from those from the MTM, because each of the 3 traits was conditioned on the other ones. In the structural equation model, genetic effects not only directly affect the phenotypes (direct effects) but also affect the phenotypes through the causal structure among traits (indirect effects). The structural equation model can identify these 2 effects, whereas the genetic effect derived by MTM includes both effects (Valente et al., 2013). Therefore, the genetic variance for the traits conditioned on the other traits can be different between the 2 models. The genetic variances for these traits from the structural equation model were smaller than the analogous parameters from the MTM. On the other hand, the posterior means of genetic and residual variances of BCL, BFS, and MUS of



**Figure 2.** Links and posterior means of structural coefficients between traits with directed link of firmness of beef (FIR) to texture of beef (TEX) (a) and TEX to FIR (b) based on results of the inductive causation algorithm with 80% of highest posterior density intervals. Links without arrowheads represent associations between traits and links with arrowheads represent causal relationships toward arrowheads. BMS = beef marbling score; BCL = beef color score; BFS = beef fat color score; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

structural equation models and the MTM were similar. This was expected as these 3 traits were not conditioned on any of the other traits in the directed acyclic graphs (DAG); therefore, their equations were similar in both models. Most of the posterior means of genetic correlations from the structural equation model were different from those of the MTM analysis, albeit the correlations from the 2 models were not directly comparable because of the relationship  $\mathbf{G}_0^* = (\mathbf{I} - \mathbf{\Lambda})^{-1} \mathbf{G}_0 (\mathbf{I} - \mathbf{\Lambda})^{-1}$

Valente et al. (2013) discussed that the genetic effects from MTM and structural equation models have different meanings: the former represent overall genetic effects that include all direct and indirect (i.e., mediated by other phenotypic traits) effects on each trait, whereas the latter represent only direct effects (i.e., not mediated by other traits in the causal network). For instance, when trait 1 has a causal effect on trait 2 ( $\lambda_{2,1}$ ) in the 2-trait model, the overall genetic effect on trait 2 ( $u_2^*$ ) in MTM can be represent as  $u_2^* = \lambda_{2,1}u_1 + u_2$ ; here,  $u_1$  and  $u_2$  are the direct genetic effects on traits 1 and 2, respectively, in the structural equation model. The magnitude of the posterior means of genetic variances of BMS, FIR, and TEX decreased in the structural equation models compared with the MTM parameters (Table 3). These traits were conditioned on the other traits, for example, BMS on BCL and MUS, FIR on BCL and BMS, and TEX on BCL, BMS, and FIR, according to Fig. 2a. These results suggest that the upstream traits (BCL and MUS in the current results) explained the genetic variability of the downstream traits (BMS, FIR, and TEX in the current results) in the MTM. Bouwman et al. (2014) observed that reductions of variances for upstream traits were smaller than those for downstream traits in bovine milk fatty acid. They also acknowledged the differences in the meaning of genetic

**Table 4.** Posterior means, posterior SD (PSD), and 95% highest posterior density (HPD) intervals of the structural coefficients resulting from the structural equation model

Structural component <sup>1</sup>	Structural equation model (FIR → TEX)			Structural equation model (TEX → FIR)		
	Mean	PSD	95% HPD interval	Mean	PSD	95% HPD interval
$\lambda_{\text{BMS, MUS}}$	1.446	0.305	0.844, 2.037	0.851	0.239	0.378, 1.312
$\lambda_{\text{BMS, BCL}}$	-0.539	0.158	-0.861, -0.216	-0.519	0.156	-0.833, -0.192
$\lambda_{\text{FIR, BCL}}$	-0.077	0.014	-0.106, -0.049	-0.024	0.011	-0.047, -0.002
$\lambda_{\text{TEX, BCL}}$	-0.033	0.016	-0.066, 0.001	-0.078	0.020	-0.118, -0.039
$\lambda_{\text{FIR, BMS}}$	0.275	0.004	0.267, 0.284	0.127	0.005	0.117, 0.137
$\lambda_{\text{TEX, BMS}}$	0.074	0.008	0.059, 0.089	0.222	0.009	0.204, 0.239
$\lambda_{\text{TEX, FIR}}$	0.553	0.011	0.534, 0.572	-	-	-
$\lambda_{\text{FIR, TEX}}$	-	-	-	0.601	0.009	0.582, 0.620

<sup>1</sup> $\lambda$  = structural coefficient; BMS = beef marbling score; BCL = beef color score; FIR = firmness of beef; TEX = texture of beef; MUS = the ratio of MUFA to SFA [(C14:1 + C16:1 + C18:1)/(C14:0 + C16:0 + C18:0)].

variances of MTM and structural equation models. This explains the difference of magnitude of parameters from both models, which also applies to our results.

Signs of the posterior means of structural coefficients resulting from the structural equation model with a directed link of FIR → TEX (Fig. 2a) and with a directed link of FIR ← TEX (Fig. 2b) were the same between the 2 models (Table 4). The coefficients from MUS to BMS ( $\lambda_{\text{BMS, MUS}}$ ) were positive and those from BCL to BMS ( $\lambda_{\text{BMS, BCL}}$ ), FIR ( $\lambda_{\text{FIR, BCL}}$ ), and TEX ( $\lambda_{\text{TEX, BCL}}$ ) were negative. The signs of the 4 coefficients were the same as the signs of the MTM residual covariances among the traits, because MUS and BCL had no other traits as causal parents. At the same time, the signs of the coefficients from BMS to FIR ( $\lambda_{\text{FIR, BMS}}$ ) and TEX ( $\lambda_{\text{TEX, BMS}}$ ), from FIR to TEX ( $\lambda_{\text{TEX, FIR}}$ ), and from TEX to FIR ( $\lambda_{\text{FIR, TEX}}$ ) were the same as the signs of the residual covariances between them resulting from the MTM in this study, although the coefficients were inferred from a conditional association and the residual covariances were marginal associations.

Although the signs of the posterior means of structural coefficients were the same for the 2 structural equation models, the magnitude of most of them was significantly different between the 2 models (Table 4). However, the sum of  $\lambda_{\text{FIR, BMS}}$  and  $\lambda_{\text{TEX, BMS}}$  from the structural equation model with the directed link of FIR → TEX (Fig. 2a) and with the directed link of FIR ← TEX (Fig. 2b) was the same value (0.349). The sum of  $\lambda_{\text{FIR, BCL}}$  and  $\lambda_{\text{TEX, BCL}}$  in the structural equation models was also almost the same (-0.110 and -0.102 for the structural equation models with the directed link of FIR → TEX and with the directed link of FIR ← TEX, respectively). The direction of the link between FIR and TEX could cause the differences of causal effects between them. This is because these terms should explore different conditional as-

sociations depending on the direction, as this should change the remaining covariates involved in the vicinities of FIR and TEX. The estimate of  $\lambda_{\text{FIR, TEX}}$  (0.60) from the structural equation model with the directed link of FIR ← TEX was significantly larger than  $\lambda_{\text{TEX, FIR}}$  (0.55) from the structural equation model with the directed link of FIR → TEX. In addition, the deviance information criterion (Spiegelhalter et al., 2002) from the structural equation model with the directed link of FIR ← TEX (-34,543.0) was smaller than that with the directed link of FIR → TEX (-34,461.0). These suggest that the DAG with the directed link of FIR ← TEX was more feasible for this structure.

The present study indicated that BMS is affected by MUS and BCL. The effect of MUS on BMS ( $\lambda_{\text{BMS, MUS}}$ ) was inferred as 1.45 and 0.85 in the DAG with the directed link of FIR → TEX (Fig. 2a) and in the DAG with the directed link of FIR ← TEX (Fig. 2b), respectively. Also, the effect of BCL on BMS ( $\lambda_{\text{BMS, BCL}}$ ) was inferred as -0.54 (Fig. 2a) and -0.52 (Fig. 2b) under both structural equation models. Understanding the physiological and functional mechanisms behind BMS is relevant because it is the most influential trait in determining the price of a carcass in the Japanese market.

Biological reasons for the direct effect of MUS on BMS could be due to regulating adipocyte development during feeding of animals. Vernon (1977) reported that C18:0 was the most effective inhibitor of fatty acids in his study on the effect of fatty acids on lipogenesis in ovine adipose tissue in vitro. There is also a study that reported that high concentrate diets increased the amount of i.m. lipids and MUFA as well as the stearoyl-CoA desaturase (SCD) gene expression and activity (Smith et al., 2009). In addition, a high-oleate diet increased SCD activity in Simmental cattle (Chang et al., 1992). These studies suggest that feeding a high-SFA diet is one cause of depression of SCD activity, which

results in inhibiting unsaturation of lipids and increasing the proportion of C18:0. Consequently, this causes inhibition of lipogenesis and decreases the amount of lipids. Additionally, there are reports that aging changes SCD gene expression. Martin et al. (1999) showed that SCD gene expression increased between weaning to 12 mo of age in subcutaneous adipose tissue in Angus steers. Similarly, peak SCD mRNA levels in muscle tissues were observed at 12 mo of age in Hanwoo steers (Lee et al., 2005). Therefore, the positive coefficient between MUS and BMS ( $\lambda_{\text{BMS, MUS}}$ ) could reflect the degree of saturation or unsaturation source and feeding management of the diet at the age of high SCD activity.

The structural coefficient of  $\lambda_{\text{BMS, BCL}}$  was negative (Table 4), suggesting that darker beef color negatively affects the marbling score. One possible explanation for this might be related to serum vitamin A levels during the feeding period. There are reports that vitamin A inhibits lipogenesis (Matsui et al., 1996; Ohya et al., 1998) and feeding a diet with low levels of vitamin A results in improved BMS (Oka et al., 1998; Hayashi et al., 2003). Many feeders in Japan use a management technique based on this concept, in terms of vitamin A restriction during the feeding period to enhance BMS. On the other hand, it is known that vitamin A makes beef color darker (Hayashi et al., 2003; Itoh and Hirooka, 2003). These imply that the vitamin A level is a hidden common cause between BMS and BCL. In addition, negative phenotypic correlations between BMS and BCL have been reported in the Wagyu breed:  $-0.28$  for Japanese Black cattle (Osawa et al., 2004) and  $-0.31$  for Japanese Brown cattle (Shirai et al., 1996). These results also imply the causal effect of BCL on BMS. In contrast, Hodgson et al. (1992) reported the opposite relationship between lean color and marbling in cow carcasses in the United States. This difference could be partly due to the differences in age of the animals and the average degree of marbling between the U.S. cow meat and Japanese Wagyu meat.

Our study showed the same associations as those reported by Shirai et al. (1996) and Hodgson et al. (1992) regarding lean color with lean FIR and lean TEX, although the last one was only a phenotypic association. In our study, inferred values for  $\lambda_{\text{FIR, BCL}}$  and  $\lambda_{\text{TEX, BCL}}$  were negative but weak (Table 4). Although the magnitude of  $\lambda_{\text{FIR, BMS}}$  and  $\lambda_{\text{TEX, BMS}}$  was not too high, the influences due to these effects could be strong, given that the phenotypic CV of BMS is the largest among the meat quality traits in this study (Table 1). These causal effects suggest favorable conditions for the joint improvement and selection of the 3 traits. Higher BMS would lead to better FIR and TEX, and this could be due to positive relationships between BMS and the ratio of fat content in meat. It is well known that BMS is strongly related to

fat content and there are negative relationships between the ratio of fat content and moisture in meat (Savell et al., 1986; Ueda et al., 2007). Higher fat content in meat improves the FIR and TEX of the meat (Breidenstein et al., 1968). Favorability of conditions for joint selection should also ultimately consider the correlation between direct genetic effects and would be expressed by the reduced structural equation model or by the MTM (Valente et al., 2013). Regardless, structural equation model analysis provides insights and hypotheses about how causal relationships among traits contribute to this setting.

The important issue is an external intervention in the causal network among phenotypes, such as artificially defining the value of a trait, blocking causal associations, or modifying their magnitudes. Occasionally, hard interventions occur in a breeding scenario, for example, cross-fostering for litter size in pigs or cesarean sections for calving difficulty. If such interventions existed in a scenario where traits present causal effects among them, breeding strategies based only on MTM analysis could lead to wrong selection decisions. For the set of traits studied here, the most important issues are interventions that would affect selection for BMS, because of its economic relevance. For example, if hard interventions were made on MUS and BCL, such as controlling dietary unsaturation levels or vitamin A restrictions during the feeding period, only the direct genetic effects would influence BMS, as the interventions would block the indirect genetic effects through MUS or BCL (Valente et al., 2013). Inferences from structural equation models indicate that the posterior means of genetic variances of BMS were smaller (3.132 and 3.090 in the DAG presented in Fig. 2a and 2b, respectively) compared with the inferences based on the MTM (3.851). This suggests that genetic variance for BMS inferred using the MTM would be overestimated in a scenario under hard interventions made on MUS and BCL. This would result in a slower selection progress than expected, even if the ranking of individuals is the same for direct and overall breeding values. The same would apply for FIR and TEX, for which the posterior means of genetic variances from the structural equation models were dramatically reduced compared with the inferences using the MTM (Table 3). Therefore, careful considerations are required for setting an appropriate breeding strategy for FIR and TEX.

## Conclusion

Potential functional relationships were detected among the meat quality traits considered in this study, except for BFS. As expected, applying smaller HPD intervals detected more links. In addition, most of the undirected links were changed into directed ones, except for



the link between FIR and TEX. The final inferred DAG suggests that BMS would be affected by interventions on MUS and BCL. Fitting a structural equation model using the causal structure based on the IC algorithm output allowed inferences regarding direct genetic effects, as well as the magnitude of the causal effects between the traits, to be made. The decreased genetic variance of BMS from the structural equation model suggests that a breeding strategy based only on the MTM would cause a delay for achieving the breeding goal if interventions on MUS or BCL (such as controlling dietary unsaturation levels or vitamin A restrictions during the feeding period) were applied. Furthermore, the dramatically decreased genetic variances of FIR and TEX in the structural equation model should require careful considerations for designing an optimized breeding strategy for improvement of FIR and TEX.

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