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Running head: Serum predictors for RFI in chickens

Assessing serum metabolite profiles as predictors for feed efficiency in broiler chickens reared at geographically distant locations

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Abstract

1. Various physiological mechanisms contribute to feed efficiency (FE) in chickens. Blood metabolite profiles may correlate to the animal's FE, but have rarely been evaluated in chickens. The objective of this study was to investigate differences in growth performance, serum intermediary metabolites, acute-phase-proteins and white blood cells in low, medium and high residual feed intake (RFI) chickens. It was also assessed if the environment affects the FE and FE-related performance and serum profiles of chickens.

2. Individual BW and feed intake (FI) were recorded from d 7 of life. At 5 weeks of age, female and male chickens (Cobb 500) were selected according to their RFI (L1: Austria; L2: UK; n = 9/RFI group, sex and location) and blood was collected.

3. Chickens at L1 had similar FI but a 15%-higher ($P < 0.001$) BW gain compared to chickens at L2. The RFI values of female chickens were -231, 8 and 215 g and those of male chickens -197, 0 and 267 g for low, medium and high RFI, respectively ($P < 0.001$).

4. Location affected serum glucose, urea, cholesterol, NEFA and ovotransferrin in females, and serum glucose and triglycerides in male chickens ($P < 0.05$). Serum uric acid and NEFA linearly increased from low to high RFI in females, whereas in males cholesterol showed the same linear response from low to high RFI ($P < 0.05$). Serum alpha-1-acid glycoprotein and blood heterophil-to-lymphocyte ratio linearly increased by 35 and 68%, respectively, from low to high RFI but only in male chickens at L1 ($P < 0.05$).

5. Regression analysis showed positive relationships between RFI and serum uric acid ($R^2 = 0.49$; $P < 0.001$) and cholesterol ($R^2 = 0.13$; $P < 0.001$).

6. We conclude that RFI-related variation in serum metabolites of chickens was largely similar for the two environments and that serum metabolite patterns could be used to predict RFI in chickens.

Key words: acute phase response, broilers, feed-efficiency predictor, residual feed intake, serum metabolites

Introduction

Improving feed efficiency (FE) is a continuing goal since feed is the major cost in chicken production. Improved FE is often associated with reduced feed intake (FI) (Bottje and Carstens, 2009). As it is a heritable trait and is independent of production traits, the residual feed intake (RFI) has become the metric of choice for studying physiological mechanisms underlying variation in FE of chickens and other livestock species (Herd and Arthur, 2009; Berry and Crowley, 2012). Generally, a chicken population from a commercial breed shows considerable variation in RFI (van Eerden *et al.*, 2004). As knowledge about RFI related physiological mechanisms in poultry and other livestock species advances, the biological basis of inter-animal variations associated with FE becomes clearer (Bottje and Carstens, 2009; Aggrey *et al.,* 2014; Lee *et al.* 2015; Mignon-Grasteau *et al.,* 2015; Zhuo *et al.,* 2015). In beef cattle and pigs some plasma metabolites and hormones correlated with animal's RFI and have been discussed as RFI predictors (Kelly *et al.*, 2010; Le Naou *et al.*, 2012; Montagne et al., 2014). Some evidence for RFI-associated differences in serum intermediary metabolites also exists for cockerels (Gabbarou *et al.*, 1997; Swennen *et al.*, 2007); however, due to the short production cycle, these have not been satisfactorily studied in meat-type chickens. In general, peripheral blood is more easily accessible than other body tissues and can provide useful information to identify the main biological processes which are modulated by genetic selection or by feeding strategies (Jegou *et al*., 2016).

The question whether meat-type chickens of diverging RFI respond differently to stressors which may affect growth performance (Zulkifli *et al.*, 2014) has also not been completely answered. In pullets, for instance, differences in RFI-related stress responses are small (van Eerden *et al.*, 2004). Overall, concentrations of blood corticosterone, acute-phase-proteins (APP) and heterophil-to-lymphocyte ratio (H-to-L) correlate in poultry (Gross and Siegel, 1983). Hence, APPs and the H-to-L ratio are commonly used as indices of stress in chickens

(Zulkifli *et al.*, 2000, 2014) and may help understanding RFI-related stress responses in broiler chickens.

In most studies, RFI was derived from one contemporary population of chickens (Bottje and Carstens, 2009), whereas information regarding the impact of the rearing environment on RFI-related variation is scarce. In considering that substantial batch-to-batch variation has been reported for the chicken gut microbiota under controlled conditions at one experimental setting (Stanley *et al.*, 2013, 2016; Ludvigsen *et al.*, 2016), it is feasible that the environment may modify RFI-related physiological responses. This is an important issue since any predictors or biomarkers of FE must be applicable across multiple environments and the result will influence the approaches used to measure and manipulate the underlying physiological mechanisms to improve FE gain.

We therefore hypothesized that, despite being raised in different environments, chickens of equal RFI would be characterized by similar RFI-related profiles for performance and serum parameters. The first objective of this study were to investigate differences in growth performance, FE, serum intermediary metabolites, acute-phase-proteins and white blood cells in low, medium and high residual feed intake (RFI) chickens. The second objective was to assess if the environment in which chickens were raised affect chicken's FE and FE-related performance and serum profiles.

Materials and Methods

Experimental design and chickens

Two chicken experiments using common protocols comprising the experimental setup, diet formulation, data and sample collection were conducted at the Institute of Animal Nutrition and Functional Plant Compounds [University of Veterinary Medicine Vienna, Austria; location 1 (L1)] and at the Agriculture Branch of Agri-Food and Biosciences Institute [Hillsborough, Northern Ireland, United Kingdom; location 2 (L2)] using a completely randomized study design. At both locations three replicate batches were performed using day-old mixed-sex Cobb 500FF chicks, resulting in a total population of 78 females and 79 males at L1 and in a total population of 96 females and 96 males at L2. Within each replicate batch, equal numbers of females and males, except for batch 2 with one more male at L1, were used. Due to the geographic distance, chickens came from different commercial hatcheries. The three chicken batches at each location were run in parallel. All animal experimentation procedures were approved by the institutional ethics committee at the University of Veterinary Medicine Vienna and the Austrian national authority according to paragraph 26 of Law for Animal Experiments, Tierversuchsgesetz 2012 – TVG 2012 (GZ 68.205/0131—II/3b/2013). At Agri-Food and Biosciences Institute the animal procedures were conducted under the project licence number PPL 2781 obtained from the Department of Health, Social Services and Public Safety (DHSSPS) which adhere to the Animals (Scientific Procedures) Act 1986.

At hatch, chicks were sexed and transported to L1 and L2 within the first day of life, where chicks were weighed and group-housed. From d 7 of life, chickens were separated and individually housed in cages until the end of the experimental period. The cage floors were made of wire mesh (10 mm × 10 mm) and padded with rubber tubing. The chickens received a light-to-dark ratio of 23:1h on the day of arrival which was gradually decreased to 18:6h on d 6 of life and was maintained throughout the experimental period. The temperature was maintained at 33ºC for the first 5 days after which it was gradually decreased to a temperature of 21ºC on d 21 of life. Each cage was equipped with one manual feeder and one drinker and feed and demineralized water were freely available.

Diets and Data Collection

Chickens were fed starter, grower and finisher diets based on corn and soybean meal (Table 1) from d 1 to 10, d 11 to 21, and d 22 to 42 of life, respectively. Diets did not contain antimicrobial growth promoters or coccidiostats. Starter, grower and finisher diets were mixed according to the same diet formulation at each location. At each location, starter, grower and finisher diets for the replicate batches came from the same batch of commercially prepared crumbles (starter diet) and pellets (3 mm; grower and finisher diets) and were stored in cool (< 15°C) and dry conditions for a duration of no longer than 6 months. Feed intake (FI) was determined weekly. Feed leftovers and spills were collected before recording feed intake on d 14, 21, 28, 35, 36 and 38 of life. Once a week (upon arrival, d 7, 14, 21, 28 and 35) and on the selection day, BW of all chickens were recorded at both locations.

Selection procedure and calculation of FE

Due to the fact that chickens at L1 grew faster than chickens at L2, selection of chickens at L1 took place two days earlier on d 36 of life in order to achieve approximately similar BW at sacrifice and hence to minimize the effect of BW and body composition on parameters of interest. Chickens at L2 were weighed and ranked according to their RFI value on d 38 of life. The RFI was calculated for each chicken for the test interval between d 7 and d 36 of life at L1 and between d 7 and d 38 of life at L2, respectively. Data for net total FI (TFI), metabolic mid-test metabolic weight and total BW gain (TBWG) were used to estimate RFI and residual BW gain (RBG) values as the residuals over the test interval with a nonlinear mixed model in SAS (SAS Stat Inc., version 9.2; Cary; NC) as described in Metzler-Zebeli *et al.* (2016):

The MMW was calculated as:

$$MMW = [(BW \text{ at d 7 of life (g)} + BW \text{ at d 35 of life (g)}) / 2]^{0.75}.$$

The RFI and RBG were calculated as:

$$RFI\ (g) = TFI - (a_1 + b_1 \times MMW + b_2 \times TBWG),$$

where a_1 is the intercept and b_1 and b_2 are partial regression coefficients of MMW and TBWG on TFI, respectively. In addition, RBG, residual intake over gain (RIG) and feed conversion ratio (FCR) for the test interval were calculated for the selected chickens:

RBG (g) = TBWG - (a_2 + b_3 × MMW + b_4 × TFI),

where a_2 is the intercept and b_3 and b_4 are partial regression coefficients of MMW and TFI on TBWG, respectively.

The RIG was calculated as:

RIG (g) = RBG (g) – RFI (g).

The FCR was calculated as:

FCR (g/g) = TFI (g) / TBWG (g).

In each replicate, batch and location it was aimed to select the three chickens with the lowest RFI (Low RFI), the three chickens with the highest RFI (High RFI), and the three chickens with the medium RFI (Medium RFI; a RFI value close to zero), separately for female and male chickens. Finally, at location 1, each RFI group was represented by 9 females and 9 males. At location 2, 6 low RFI, 11 medium RFI and 6 high RFI female chickens and 10 low RFI, 9 medium RFI and 9 high RFI male chickens were selected. Only the data of the selected chickens at both locations were used for the comparison of FI, growth performance and FE. Moreover, blood samples were only collected from the selected chickens. The remaining chickens were removed from the experiment. TFI and TBWG were compared for the test interval from d 7 to 36 of life across locations.

Blood sampling

Body weight of selected chickens was recorded before chickens were humanely killed for blood sampling from d 37 to 42 of life. At L1, selected chickens were euthanized with an overdose of sodium pentobarbital (450 mg/kg, Release, WTD-Wirtschaftsgenossenschaft Deutscher Tierärzte, Garbsen, Germany) by i.v. injection into the caudal tibial vein from d 37 of life with three to six chickens per day, whereas at L2 selected chickens were sacrificed on d 41 and 42 of life. Immediately thereafter, blood from the vena jugularis at L1 and the heart at L2 was collected into serum collection tubes (Sarstedt, Nürnbrecht, Germany) and placed on ice until centrifugation (1 811 × g for 10 min and 1 500 × g at 4°C for 10 min at L1 and L2, respectively; Eppendorf Centrifuge 5810 R, Eppendorf, Hamburg, Germany), and stored at -20°C until analysis. At L1, 1 mL blood was additionally collected in tubes containing EDTA as anticoagulant (Sarstedt, Nürnbrecht, Germany) from which blood smears were prepared on glass slides (*n* = 4/chicken) to count white blood cells. The intestinal mucosa was checked for *Eimeria*-related lesions at the necropsy which could not be detected.

Chemical analysis and calculations

Proximate nutrient analysis of diet samples was performed according to standard protocols (Naumann and Basler, 2012). Dry matter was determined after oven-drying for 4 h at 103°C (method 3.1), crude ash by overnight incineration at 550ºC (method 8.1), and CP (nitrogen × 6.25) by the Kjeldahl method (method 4.1.1; Naumann and Basler, 2012). Diet samples were further analyzed for EE (method 5.1.1B), CF (method 6.1.1), total starch (method 7.2.1), sugar (method 7.1.1), calcium (method 10.3.2) and phosphorus (method 10.6.1; Naumann and Basler, 2012).

Blood leukocyte counts, serum metabolites, and acute-phase proteins

Blood smears were stained using the May-Grünwald-Giemsa stain (Hemacolor Rapid staining of blood smear kit; Merck KGaA, Darmstadt, Germany). A total of 100 leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes), were counted per slide using light microscopy (Leitz Orthoplan, Leitz, Wetzlar, Germany) at 100-times magnification, and the H-to-L ratio was calculated (Gross and Siegel, 1983). Serum glucose, uric acid, triglycerides, cholesterol and NEFA were determined by standard enzymatic colorimetric analysis using an autoanalyzer for clinical chemistry (Cobas 6000/c501; Roche Diagnostics GmbH, Vienna, Austria). Chicken specific commercial ELISA kits were used to determine the APPs ovotransferrin (OVT; Cusabio, Wuhan, China) and alpha-1-acid glycoprotein (AGP; Genway Biotech Inc., San Diego, CA, US) in serum according to the manufacturers' instructions. Samples were diluted 2 to 5-fold for both assays depending on the individual sample concentration. The intra- and interassay variability for the OVT and AGP kits were less than 10%, respectively, and the detection limit was 0.039 ng/ml and 3.125 ng/ml. All serum parameters were analyzed together at L1.

Statistical analysis

Feed efficiency, FI, growth performance and serum parameters from the selected low, medium and high RFI chickens (location 1: *n* = 9 low, medium and high RFI female and male chickens; location 2: *n* = 6 low RFI, *n* = 11 medium RFI and *n* = 8 high RFI females, and *n* = 10 low RFI, *n* = 9 medium RFI and *n* = 9 high RFI males) were first analysed for normality using Shapiro-Wilk test with the PROC UNIVARIATE in SAS. The Cook's distance (Cook's D) test was used to determine any influential observation on the model. Parameters of individual RFI, performance, and serum metabolites, APPs and white blood cells were analysed by ANOVA using the PROC MIXED in SAS. Two models were run. The first accounted for the fixed effects of sex, batch, location and RFI. Because chickens were sacrificed at different days of life and in order to consider that chickens were consecutively sampled, the first model included the random effect of chicken nested within day of life and chicken order at sacrifice. The effects of bird age and BW at 7 days of life were also separately tested as covariates in the model. As both covariate effects showed no significant influence on any response variable evaluated, these covariates were removed from the final model and not accounted for in the further analyses. However, sex and batch as fixed effects were found to be significant for many parameters. Therefore, data of female and male chickens were analysed separately using a second model which was fitted to take into account the fixed effects of RFI and location and their two-way-interaction. The random effect considered the chicken nested within batch, day of life and chicken order at sacrifice. Since white blood cell counts were only determined at L1, only the fixed effect of RFI was considered. Moreover, in the second model, orthogonal polynomial contrast statement was used to evaluate linear relationships. Degrees of freedom were approximated by the method of Kenward-Roger. Least squares means were computed and significance declared at $P \leq 0.05$. A trend was considered at $0.05 < P \leq 0.10$.

In order to investigate whether sex-independent relationships between chicken's individual RFI and serum metabolites existed linear discriminate analysis (LDA) and regression analysis were applied. The LDA was performed using JMP10 software (SAS Stat Inc.) with serum metabolites (glucose, urea, cholesterol, triglycerides and NEFA) as covariates and RFI group as the categorical variable. The LDA results were visualised using the first 2 principal components of the scores plot to identify characteristic trends or grouping among chickens of diverging RFI. Moreover, regression analysis (PROC REG of SAS) was used to establish and quantify the relationships between individual serum metabolites, serum APPs and blood H-to-L ratio and chickens' individual RFI values, irrespective of sex and location. For this, mixed modelling (PROC MIXED of SAS) of each serum metabolite was performed including the fixed effects RFI, sex and location. The slope and intercept by RFI, sex and location were included as random effects and the variance component structure was used as variance-covariance matrix. Significant relationships ($P < 0.05$) are shown in Fig. 1.

Results

Chicken performance and feed efficiency

Sex did not affect BW on d 7 of life, whereas male chickens weighed approximately 300 g more on d 36 of life than females ($P < 0.001$; Table 1 and 2). Similarly, TFI and TBWG were higher ($P < 0.001$) in males compared to females. Location affected BW on d 7 and 36 of life. While female and male chickens weighed about 10 g more on d 7 of life at L2 compared to L1, they gained about 350 to 400 g less by d 36 at L2 compared to L1 ($P < 0.001$). In contrast, TFI between d 7 and 36 of life was not influenced by location. Likewise, location did not affect the FE metrics RFI, RBG and RIG; providing similar values for female and male chickens of the same RFI group, whereas FCR was about 12 % lower (P < 0.001) in chickens of L1 compared to chickens of L2.

The RFI ranged on average from -231 to 215 g in females and from -197 to 267 g in males representing a difference of 330 and 500 g TFI between most and least efficient female and male chickens (*P* < 0.001; Table 1 and 2). Body weight at d 36 and TBWG were similar among chickens of diverging RFI. Likewise, the RBG of the selected chickens was similar among the three RFI groups, whereas the RIG linearly decreased in the same range observed for the increase in RFI from low to high RFI chickens, irrespective of sex. The FCR linearly increased from low to high RFI by on average 13% (*P* < 0.001). There was a sex effect and location effect for FCR showing a 0.06 g/g-lower FCR in males compared to females as well as a 0.19 g/g lower FCR in chickens at L1 compared to chickens at L2 (*P* < 0.001).

At sacrifice, male chickens at both locations had similar BW across locations (3.03 and 3.02 ± 0.062 kg at L1 and L2, respectively; *P* = 0.859) and RFI groups (3.04, 2.96 and 3.08 ± 0.076 kg for low, medium and high RFI, respectively; *P* = 0.535). By contrast, BW in female chickens at sacrifice differed across locations with females at L1 weighing about 270 g more than females at L2 (2.85 versus 2.58 ± 0.063 kg at L1 versus L2, respectively; *P* = 0.001), but their BW was not different among RFI groups (2.72, 2.71 and 2.73 ± 0.065 for low, medium and high RFI, respectively; *P* = 0.974).

Serum metabolite profiles and acute phase proteins

Results for serum metabolite profiles and acute-phase-proteins examined for female and male chickens are presented in Table 3 and 4, respectively. There was a location effect for serum OVT in females showing that chickens at L1 had a 2-fold higher serum OVT concentration than that of chickens at L2 (*P* < 0.05). Moreover, we observed a linear increase (*P* < 0.05) in serum AGP from low to high RFI in male chickens at L1 but not at L2. Sex affected (*P* < 0.05) serum NEFA concentrations which were higher in males. Female chickens at L1 had a lower serum glucose and NEFA and higher serum urea and cholesterol than females at L2 (*P* < 0.05). In males, serum glucose and triglycerides were lower at L1 compared to L2 (*P* < 0.01). Despite differences in actual serum concentrations, FE-effects for glucose, uric acid and cholesterol among RFI groups were similar at both locations in females. There was a linear increase in serum uric acid (*P* < 0.05), and a tendency for a linear increase in serum cholesterol and triglycerides (*P* < 0.1) from low to high RFI in female chickens. Serum NEFA showed a FE × location effect (P < 0.01) by increasing by 57% from low to high RFI at L2 but not in females at L1. Similar to the females, serum cholesterol linearly increased (P < 0.05) and triglycerides tended (*P* < 0.1) to increase by about 17 and 31% from low to high RFI in male chickens, respectively.

White blood cell counts

White blood cell counts were only determined at L1 (Table 5). Females and males differed in their white blood cell counts with females having more lymphocytes but less monocytes and heterophils than males (*P* < 0.05). In females, FE tended to affect only monocyte counts with chickens of low RFI having less monocytes than chickens of medium and high RFI. In males, lymphocyte counts linearly decreased (*P* = 0.012) from low to high RFI, whereas heterophils linearly increased from low to high RFI (*P* = 0.031). Because of this, there was a linear (*P* = 0.027) increase in the H-to-L ratio of 68% from low to high RFI in males.

Multivariate and regression analysis

The LDA plot of RFI groups and serum metabolites showed separate clustering for serum metabolites for low and high RFI, whereas the 95% confidence intervals of medium RFI overlapped with those of low and high RFI (Figure 1A). Serum glucose discriminated best for low RFI, whereas serum triglycerides, uric acid and cholesterol correlated with high RFI. When comparing locations (Figure 1B), the LDA showed clear clustering of serum metabolites between L1 and L2, whereby serum NEFA correlated to L2 and urea to L1. Due to the separate clustering in the LDA together with trends for linear relationships between some serum metabolites and RFI groups, relationships between serum parameters and the individual RFI values of chickens from both sexes and locations were regressed. Regression analysis showed positive relationships between serum cholesterol and RFI ($R^2 = 0.13$; $P < 0.001$; Figure 2A) and serum uric acid and RFI ($R^2 = 0.49$; $P < 0.001$; Figure 2B). There was also a weak positive relationship between the H-to-L ratio and RFI values for chickens at L1 ($R^2 = 0.15$; $P = 0.003$; Figure 2C).

Discussion

Our understanding of the physiological mechanisms underlying the FE of chicken's is steadily advancing (e.g., Aggrey *et al.*, 2014; Lee *et al.*, 2015; Zhou *et al.*, 2015). However, the contribution of the rearing environment has not yet been sufficiently elucidated. In the current study, chickens from one hybrid line were raised using similar management protocols at two distinct geographic locations to investigate if RFI-related performance traits and serum profiles are affected by the rearing environment. Similar to Stanley *et al.* (2016), the present chicken populations met or exceeded the expected average growth rate, and the range in TFI, growth, and FE data recorded was consistent with previous studies in chickens selected for RFI (e.g., Zhuo *et al.*, 2015). Although the TFI from d 7 to 36 of life was similar across locations, results indicated a marked location effect on TBWG of chickens between locations which was apparent throughout all replicate batches and for both sexes. Furthermore, we could distinguish RFI-related profiles for certain serum intermediary metabolites, but not acute-phase-proteins, in the current chicken populations, whereby RFI-effects were different in males and females. The regression models implemented established linear relationships between RFI and serum uric acid and cholesterol, suggesting them as predictors for RFI in the current chicken populations irrespective of sex and location. Despite these relationships and clear clustering between low and high RFI in the LDA plots, the actual concentrations of serum metabolites were location-specific which may render it difficult to predict universal serum threshold values for low, medium and high RFI chickens. Moreover, as present relationships between RFI and serum cholesterol and uric acid were weak to moderate, it may be advisable to use serum metabolite patterns rather than individual metabolites to predict the RFI in chickens.

Chicken RFI values were similar across locations, but it should be considered that chicken's RFI values were determined two-days apart. The RFI is phenotypically independent of BW and level of production (e.g., ADG; Bottje and Carstens, 2009), and may have therefore remained similar across locations in the current study despite differences in TBWG and ADG. Similar observations were made for RBG and the combined metric RIG of the selected chickens. Inconsistent findings exist in the literature for RFI-related differences in BW and BW gain in low and high RFI chickens (van Eerden *et al.*, 2004; Zhuo *et al.*, 2015). Irrespective of location, chickens of diverging RFI could not be distinguished based on their BW or TBWG. In contrast to some studies with short measurement periods of only one week (e.g., Zhuo *et al.*, 2015), we determined the FE over a period of 29 and 31 days at L1 and L2, respectively. It is highly likely that this improved the accuracy of RFI prediction in the present study as we observed slight differences in the FE and grouping of chickens according to their RFI when assessed only on a weekly basis. Differences in TFI between low and high RFI chickens were considerable and were already present at 21 days of life (Supplemental Table 1). Notably, irrespective of the two-day difference in selection for RFI, location effects were distinguishable when using the ratio metric FCR. This leads to the assumption that the FCR may more accurately predict FE-related differences in growth performance among chicken flocks, whereas the RFI may be the FE metric of choice to equally rank chickens independent from the environment.

The present environmental effects clearly suggest that physiological differences between low and high RFI chickens may largely vary between farms due to environment-specific factors. Parents' own FE essentially determines development and FE of the chicks post-hatch (Bottje and Carstens, 2009; Romero *et al.*, 2011). This may have been of less relevance in the present study as chickens used in the present trials were not related within or between locations (see Relationship analysis in Supplemental Material). The main environment-specific factors were likely the diet, even though it was of the same formulation, the housing environment including environmental microbes at the hatcheries and rearing location as well as the personnel handling the chickens. The immediate colonization of chicken's intestine post-hatch with microbes from the egg shell and environment is critical because it has a long lasting effect on chicken's performance by influencing the further microbial colonization, intestinal development and priming of the immune system (Brisbin *et al.*, 2008; Schokker *et al.*, 2015). The intestinal microbiota interacts with the host via several routes including microbial metabolites and receptor-recognition pathways (Blaut, 2015). As a result, different bacterial colonization patterns may have caused a more pronounced stimulation of the immune system throughout the growing phase at one location which may have decreased the energy available for growth. Also, different bacterial colonization across locations may have led to diverging profiles of intestinally produced short-chain fatty acids which, after being absorbed, may have affected lipogenesis of the host and present serum profiles. Especially acetate serves as substrate for *de novo* lipogenesis in the liver, whereas propionate is used for hepatic gluconeogenesis (Blaut, 2015). In general, due to the hygienic standards in modern hatcheries, microbial colonization of the gastrointestinal tract of newly hatched chicks is more influenced by microbes encountered in their wider environment (e.g., personnel, housing, water and diet) than by the normal chicken gut microbiota (Stanley *et al.*, 2013; Ludvigsen *et* *al.*, 2016). Because current chickens came from different hatcheries, the early microbial colonization may have been one of the most influential factors for the variation between both locations. This would be supported by different RFI-associated bacterial microbiome profiles in chickens between the two locations at 6 weeks of life (Siegerstetter *et al.*, 2016). Moreover, although the dietary formulations were the same and concentrations of most nutrients were equal, natural differences in the raw materials, i.e. corn and soybean meal, between locations (e.g. dietary fiber composition; Rodehutscord *et al.*, 2016) may have altered digestive, absorptive and fermentative processes. This probably affected the present results for growth performance and serum metabolite profiles across locations.

The BW at sacrifice and thus body composition may have also contributed to the variation in serum parameters in female chickens across locations and were likely depicted in chickens' serum metabolite and APP concentrations. Accordingly, serum profiles suggested that chickens at L2 had either an increased intestinal glucose release or altered systemic glucose metabolism than those at L1, irrespective of sex. Moreover, differences in BW and thus adipose tissue accretion likely led to the variation in serum lipids across locations. Moreover, the increased OVT response in females at L1 compared to L2 may indicate an increased abundance of microbial stressors at L1. As an iron binding protein OVT provides antimicrobial properties by sequestering iron and modulates heterophil and macrophage function in chickens (Murata *et al.*, 2004). In spite of the observed location effects, the fact that location × FE interactions were almost absent in our study allows assuming that RFI-related differences in performance traits and serum profiles were similar across locations.

Although influenced by prandial activity, blood metabolites and hormones associated with feed intake, growth, nutrient repartitioning and utilization may serve as potential physiological markers for FE in various livestock species (Richardson *et al.*, 2004; Kelly *et al.*, 2010; Montagne *et al.*, 2014; Jegou *et al.*, 2016). Likewise, serum intermediary metabolites suggest RFI-related differences in systemic lipid and protein metabolism in the chicken populations of the present study. Controversial results were previously reported for serum triglycerides, NEFA and uric acid in cockerel lines selected for low and high RFI (Gabbarou *et al.,* 1997; Swennen *et al.*, 2007), whereas, to our awareness, little information exists for broiler chickens of diverging RFI. Although the selection strategy and age of the chickens differed, Gabbarou *et al.* (1997) found a comparable increase in plasma triglycerides and plasma glucose and uric acid concentrations in cockerels which corresponded to our results in male chickens. According to the present linear FE-effects and regression analysis, serum concentrations of uric acid and serum cholesterol might be considered as predictors for RFI in chickens. The higher FI in high RFI chickens should have increased the intestinal glucose uptake and postprandial insulin level as well as peak duration. Accordingly, equal serum glucose concentrations may indicate improved energy saving capacity or lower glucose uptake and metabolism of peripheral organs in low versus high RFI chickens (Bottje and Carstens, 2009). Some authors (Richardson *et al.*, 2004; Kelly *et al.*, 2010) have proposed a decrease in insulin sensitivity in muscle tissue in energetically inefficient animals. Concurrently, higher basal insulin concentrations in high-RFI animals may be linked to greater fat deposition because insulin reduces lipolysis and stimulates lipogenesis in adipose tissue (Kelly *et al.*, 2010; Le Naou *et al.*, 2012; Montagne *et al.*, 2014; Zhuo *et al.*, 2015). Accordingly, Zhuo *et al.* (2015) showed that abdominal adipose tissue of high RFI chickens had a greater expression of lipid synthesis genes and decreased expression of triglyceride hydrolysis and cholesterol transport genes. Moreover, in their study, low RFI chickens had a potentially more active glucose-to-lipid conversion and different insulin signaling in adipose tissue at transcriptome level compared to high RFI chickens (Zhuo *et al.*, 2015). The latter may explain the elevated postprandial serum triglycerides and cholesterol observed for high RFI males and females compared to their low RFI counterparts in the present study. Varying RFI-related serum profiles in males and females indicated that differences were more pronounced in females than males. Despite not having measured serum insulin levels, elevated serum uric acid and NEFA in high RFI females may confirm our assumption of reduced insulin sensitivity since both metabolites are typically raised during insulin resistance due to increased lipolysis and deamination of amino acids for energy provision (e.g., Yuan *et al.*, 2008; Ji *et al.*, 2012). In addition, raised serum uric acid in high RFI animals may also suggest less efficient nitrogen recycling as recently shown for a different chicken line (Aggrey *et al.*, 2014).

Inconclusive results exist on whether diverging RFI is accompanied by a change in the stress response of meat-type chickens. As part of the physiological stress response via the hypothalamic-pituitary-adrenal axis and sympathetic system, increased systemic levels of corticosterone induces a general acute-phase response including OVT and AGP in chickens (O'Reilly and Eckersall, 2014; Zulkifli *et al.*, 2014). Moreover, increased corticosterone levels were associated with modified insulin sensitivity, reduced muscle protein accretion and raised plasma lipids and uric acid in chickens (Dong *et al.*, 2007; Yuan *et al.*, 2008) which may have contributed to RFI-related metabolic alterations and serum metabolite profiles. Present results for RFI-related differences in serum APPs were not, however, conclusive and only indicated a linear relationship between AGP and RFI in males at L1. Similar to AGP, the H-to-L ratio showed the same RFI-related pattern in males at L1 only. AGP has an immunoregulatory function by influencing T-cell function and thus white blood cell production (Murata *et al.*, 2004). Since males and females were evenly distributed across the experimental room for all three batches at L1, a greater immune response due to infectious disease agents may be excluded as an explanation for the gender difference seen here. The question then arises as to whether the high RFI males at L1 showed a greater excitability or aggressiveness compared to the female chickens. Despite the weak linear relationship between RFI and serum H-to-L, its reliability to predict chicken's RFI should be evaluated in further experiments since only data from L1 were available for regression analysis in the present study.

In conclusion, the results of the present study demonstrate that chickens reared at two geographically distinct locations showed similar RFI-related variation in serum intermediary metabolites. Regression analysis confirmed the usefulness of serum metabolite patterns as RFI predictors for the current chicken populations. Due to the environment-specific differences observed here, further research is warranted to validate the reliability of serum metabolites, such as uric acid and cholesterol, as RFI predictors in chickens.

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Disclosure statement

The authors state no conflict of interest.

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Table 1. Feed intake, growth performance and feed efficiency metrics in female broiler chickens raised at two different locations.

		Residual feed intake (RFI)[1,2]				*P*-value[3,4]		
Item	Location	Low	Medium	High	SEM	FE	location	FE × location
BW, d 7 of life (g)	L1+2	145	145	147	2.6	0.805	0.001	0.802
	L1	141	138^{y}	141^{y}	3.6			
	L2	149	151^{x}	153^{x}	2.6			
BW, d 36 of life (g)	L1+2	2253	2187	2215	50.4	0.654	<0.001	0.670
	L1	2392^{x}	2359^{x}	2420^{x}	68.9			
	L2	2115^{y}	2015^{y}	2009^{y}	73.2			
Total feed intake, d 7-36 of life (g)	L1+2	3447^{b}	3485^{ab}	3774^{a}	91.1	0.027*	0.479	0.566
	L1	3334^{b}	3510^{ab}	3751^{a}	123.2			
	L2	3559	3461^{B}	3797^{A}	131.0			
Total body weight gain, d 7-36 of life (g)	L1+2	2108	2042	2068	49.5	0.647	<0.001	0.643
	L1	2251^{x}	2220^{x}	2279^{x}	67.7			
	L2	1966^{y}	1865^{y}	1856^{y}	72.0			
RFI (g)	L1+2	-231	8	215	20.1	<0.001***	0.412	0.201
	L1	-195	18	197	27.5			
	L2	-267	-3	232	29.2			
RBG (g)	L1+2	-0.9	1.0	1.7	4.13	0.901	0.775	0.993
	L1	-2.1	0.5	1.3	5.65			
	L2	0.2	1.5	2.1	6.01			
RIG (g)	L1+2	230	-7	-213	20.2	<0.001***	0.380	0.195
	L1	193	-18	-196	27.6			
	L2	267	4	-231	29.3			

FCR (g/g)	L1+2	1.55	1.63	1.76	0.019	<0.001***	<0.001	0.108
	L1	1.46[y]	1.55[y]	1.62[y]	0.026			
	L2	1.65[x]	1.71[x]	1.89[x]	0.028			

FE, feed efficiency; FCR, feed conversion ratio; RBG, residual BW gain; RIG, residual intake over gain; L1, University of Veterinary Medicine Vienna (Vienna, Austria); L2, Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ female chickens at location 1; $n = 6$ low RFI, $n = 11$ medium RFI and $n = 8$ high RFI females at location 2.

[3]P: probability level.

[4]Linear polynominal contrast: *$P \leq 0.05$, and ***$P \leq 0.001$.

[a-c]Least squares means within a row without a common lowercase superscript differ among RFI groups ($P < 0.05$).

[A,B]Least squares means within a row without a common uppercase superscript tend to differ among RFI groups ($P < 0.1$).

[x,y]Least squares means within a column without a common lowercase superscript differ between locations ($P < 0.05$).

Table 2. Feed intake, growth performance and feed efficiency metrics in male broiler chickens raised at two different locations.

		Residual feed intake (RFI)[1,2]				*P*-value [3,4]		
Item	Location	Low	Medium	High	SEM	FE	location	FE × location
BW, d 7 of life (g)	L1+2	145	145	148	2.2	0.704	<0.001	0.919
	L1	139^{x}	140^{x}	141^{x}	3.0			
	L2	152^{y}	150^{y}	154^{y}	3.0			
BW, d 36 of life (g)	L1+2	2562	2483	2546	55.4	0.577	<0.001	0.560
	L1	2712^{x}	2733^{x}	2756^{x}	79.0			
	L2	2380^{y}	2233^{y}	2367^{y}	79.4			
Total feed intake, d 7-36 of life (g)	L1+2	3753^{b}	3879^{b}	4253^{a}	70.1	<0.001***	0.340	0.573
	L1	3682^{b}	3901^{b}	4185^{a}	99.9			
	L2	3823^{b}	3857^{b}	4321^{a}	98.2			
Total body weight gain, d 7-36 of life (g)	L1+2	2401	2338	2414	54.5	0.582	<0.001	0.560
	L1	2573	2593^{x}	2615^{x}	77.7			
	L2	2228	2083^{y}	2214^{y}	76.4			
RFI	L1+2	-197	0	267	21.8	<0.001***	0.149	0.610
	L1	-183	6	303	31.1			
	L2	-211	-6	231	30.6			
RBG	L1+2	5.5	-1.1	3.8	4.40	0.550	0.166	0.687
	L1	6.8	1.8	10.4	6.27			
	L2	4.2	-3.9	-2.7	6.16			
RIG	L1+2	202	-1.	-263	22.0	<0.001***	0.247	0.699
	L1	190	-4	-292	31.3			
	L2	215	2	-234	30.8			

FCR	L1+2	1.50	1.58	1.70	0.019	<0.001***	<0.001	0.774
	L1	1.41^{y}	1.48^{y}	1.61^{y}	0.028			
	L2	1.58^{x}	1.69^{x}	1.79^{x}	0.027			

FE, feed efficiency; RBG, residual BW gain; RIG, residual intake over gain; L1, University of Veterinary Medicine Vienna (Vienna, Austria); L2, Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ male chickens at location 1; $n = 10$ low RFI, $n = 9$ medium RFI and $n = 9$ high RFI males at location 2.

[3]P: probability level.

[4]Linear polynominal contrast: ***$P \leq 0.001$.

[a-c]Least squares means within a row without a common lowercase superscript differ among RFI groups ($P < 0.05$).

[x,y]Least squares means within a column without a common lowercase superscript differ between locations ($P < 0.05$).

Table 3. Serum metabolites and acute-phase-proteins in female broiler chickens raised at two different locations.

		Residual feed intake[1,2]				*P*-value[3,4]		
Parameter	Location	Low	Medium	High	SEM	FE	location	FE × location
Glucose (mg/dl)	L1+2	304	283	310	16.8	0.450	0.002	0.920
	L1	268^{X}	256^{X}	276^{x}	23.0			
	L2	340^{Y}	310^{Y}	344^{y}	24.4			
Urea (mg/dl)	L1+2	2.27^{b}	2.42^{ab}	2.83^{a}	0.182	0.101*	0.005	0.701
	L1	2.46^{b}	2.76^{abx}	3.25^{ax}	0.248			
	L2	2.08	2.08^{y}	2.41^{y}	0.264			
Cholesterol (mg/dl)	L1+2	132	138	145	5.1	0.244†	0.002	0.628
	L1	139	152^{X}	154	7.0			
	L2	125	125^{Y}	135	7.4			
Triglycerides (mg/dl)	L1+2	93^{B}	101	126^{A}	11.8	0.135†	0.802	0.882
	L1	86^{B}	103	126^{A}	16.2			
	L2	99	99	127	17.2			
NEFA (µmol/l)	L1+2	204	241	269	11.6	0.002***	<0.001	0.008
	L1	199	214^{y}	208^{y}	15.8			
	L2	210^{c}	269^{bx}	330^{ax}	16.8			
Ovotransferrin (µg/ml)	L1+2	13.2	10.8	14.1	0.34	0.761	0.031	0.226
	L1	17.8	11.1^{B}	22.1^{Ax}	4.59			
	L2	8.5	10.6	6.0^{y}	4.83			
Alpha-1-acid glycoprotein (µg/ml)	L1+2	221.1	204.7	209.6	13.13	0.686	0.139	0.342
	L1	240.7	223.8	205.5	18.04			
	L2	201.5	185.7	213.8	18.99			

FE, feed efficiency; L1 University of Veterinary Medicine Vienna (Vienna, Austria); L2, Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ female chickens at location 1; $n = 6$ low RFI, $n = 11$ medium RFI and $n = 8$ high RFI females at location 2.

[3]P: probability level.

[4]Linear polynominal contrast: *$P \leq 0.05$, ***$P \leq 0.001$, and †$P \leq 0.10$.

[a-c]Least squares means within a row without a common lowercase superscript differ among RFI groups ($P < 0.05$).

[A,B]Least squares means within a row without a common uppercase superscript tend to differ among RFI groups ($P < 0.1$).

[x,y]Least squares means within a column without a common lowercase superscript differ between locations ($P < 0.05$).

[X,Y]Least squares means within a column without a common uppercase superscript tend to differ between locations ($P < 0.1$).

Table 4. Serum metabolites and acute-phase-proteins in male broiler chickens raised at two different locations.

Parameter	Location	Residual feed intake[1,2]			SEM	*P*-value[3,4]		
		Low	Medium	High		FE	location	FE × location
Glucose (mg/dl)	L1+2	295	312	317	15.9	0.585	<0.001	0.377
	L1	270	257^{x}	272^{x}	23.1			
	L2	320	368^{y}	362^{y}	21.8			
Urea (mg/dl)	L1+2	2.30	2.38	2.66	0.194	0.406	0.126	0.665
	L1	2.34	2.61	2.93	0.283			
	L2	2.27	2.16	2.40	0.267			
Cholesterol (mg/dl)	L1+2	134^{A}	142^{A}	157^{B}	5.2	0.010**	0.133	0.453
	L1	143	142	162	7.5			
	L2	125^{a}	142^{ab}	153^{b}	7.2			
Triglycerides (mg/dl)	L1+2	91^{B}	102	119^{A}	11.9	0.248†	0.001	0.226
	L1	84	71^{x}	86^{x}	17.3			
	L2	98	133^{y}	153^{y}	16.3			
NEFA (µmol/l)	L1+2	253	295	293	25.9	0.429	0.354	0.126
	L1	244	318	238^{x}	37.7			
	L2	262^{B}	273	348^{yA}	35.6			
Ovotransferrin (µg/ml)	L1+2	7.61	11.86	13.24	3.06	0.394	0.743	0.904
	L1	7.79	11.71	14.96	4.22			
	L2	7.43	12.01	11.52	4.33			
Alpha-1-acid glycoprotein (µg/ml)	L1+2	202.1	227.1	235.0	16.59	0.338	0.164	0.246
	L1	195.3^{b}	241.7^{ab}	267.9^{aX}	24.46			
	L2	208.8	212.5	202.1^{Y}	23.50			

FE, feed efficiency; L1, University of Veterinary Medicine Vienna (Vienna, Austria); L2, Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ male chickens at location 1; $n = 10$ low RFI, $n = 9$ medium RFI, and $n = 9$ high RFI males at location 2.

[3]P: probability level.

[4]Linear polynominal contrast contrast: **$P \leq 0.01$, and †$P \leq 0.10$.

[a-c]Least squares means within a row without a common lowercase superscript differ among RFI groups ($P < 0.05$).

[A,B]Least squares means within a row without a common uppercase superscript tend to differ among RFI groups ($P < 0.1$).

[x,y]Least squares means within a column without a common lowercase superscript differ between locations ($P < 0.05$).

[X,Y]Least squares means within a column without a common uppercase superscript tend to differ between locations ($P < 0.1$).

Table 5. White blood cells in female and male broiler chickens raised at location 1.

Parameter	Residual feed intake[1,2] Low	Medium	High	SEM	FE, *P*-value[3,4]
Females					
Lymphocytes (%)	86.3	83.8	84.9	1.36	0.465
Heterophils (%)	12.1	13.4	13.2	1.25	0.730
Basophils (%)	0.07	0.17	0.03	0.05	0.160
Monocytes (%)	1.57	2.52	1.92	0.27	0.064
H-to-L proportion (%)	14.2	16.3	15.8	1.76	0.680
Males					
Lymphocytes (%)	83.6	82.6	75.3	2.14	0.023*
Heterophils (%)	13.9	15.0	20.6	2.06	0.067*
Basophils (%)	0.14	0.00	0.23	0.08	0.121
Monocytes (%)	2.37	2.32	2.81	0.39	0.629
H-to-L proportion (%)	17.0	18.7	28.5	3.44	0.057*

FE, feed efficiency; location 1, University of Veterinary Medicine Vienna (Vienna, Austria).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ chickens females and males.

[3]*P*: probability level.

[4]Linear polynominal contrast: $*P \leq 0.05$; $**P \leq 0.01$, $***P \leq 0.001$, and $\dagger P \leq 0.10$.

[5]Nitrogen × 6.25.

Figure captions

Figure 1. a) Linear discriminant analysis of RFI groups and serum metabolites: low RFI group (○), medium RFI group (◇), and high RFI group (●). b) Linear discriminant analysis of location and serum metabolites: location 1 (Austria (●)), and location 2 (UK (○)). Circles indicate 95% confidence intervals.

Figure 2. Quantification of relationships between RFI values and serum metabolites in male and female chickens from both locations (A-C). Relation between chicken's RFI value (x) and serum concentration (y) of cholesterol (A) and serum uric acid (B): linear regression, A) $y = 140.72 + 0.039 \times x$, RMSE = 20.652, $R^2 = 0.13$, $P < 0.001$ and B) $y = 2.34 + 0.00070 \times x$, root mean square error (RMSE) = 0.143, $R^2 = 0.49$, $P < 0.001$. Relation between RFI value (x) and blood heterophil-to-lymphocyte proportion in chickens at location 1 (C): linear regression, $y = 17.98 + 0.018 \times x$, RMSE = 8.358, $R^2 = 0.15$, $P = 0.003$.

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Supplemental Table 1. Ingredients and chemical composition of diets.

Item	Starter[1]	Grower[2]	Finisher[3]
Ingredient (g/kg as-fed)			
Corn	612	660	679
Soybean meal	331	282	260
Soybean oil	17.5	20.6	27.7
Limestone flour	11.0	9.8	7.0
Salt	2.0	2.0	2.3
Dicalcium phosphate	16.1	15.0	13.4
Vitamin/mineral-premix	11.0	11.0	10.0
Analyzed chemical composition (g/kg DM) at L1			
Dry matter	926	923	914
Crude protein	243	223	216
Ether extracts	50	52	59
Crude fiber	31	27	28
Crude ash	69	62	55
Starch	462	506	514
Sugar	40	46	49
Calcium	11.9	10.7	8.9
Phosphorus	8.2	7.8	6.9
Analyzed chemical composition (g/kg DM) at L2			
Dry matter	908	902	902
Crude protein	221	219	209
Crude ash	94	81	72
Metabolizable energy[4] (MJ/kg)	13.7	14.3	14.6

[1]Premix provided per kilogram of starter diet: vitamin A as retinyl acetate, 13,000 IU; vitamin D_3 as cholecalciferol, 5,000 IU; vitamin E as alpha-tocopherol-acetate, 80 IU; vitamin K, 3 mg; thiamin, 3 mg; riboflavin, 9 mg; pyridoxine, 4 mg; vitamin B_{12}, 20 µg; biotin, 0.15 mg; calcium pantothenate, 15 mg; nicotinic acid, 60 mg; folic acid, 2 mg; 500 mg choline chloride; methionine, 3,405 mg; threonine, 745 mg; lysine, 2,812 mg; I, 1 mg as calcium iodate; Se, 0.35 mg as sodium selenite; Fe, 40 mg as ferrous sulphate; Mo, 0.5 mg as sodium molybdate; Mn, 100 mg as manganous oxide; Cu, 15 mg as copper sulfate; Zn, 100 mg as zinc oxide.

[2]Premix provided per kilogram of grower diet: vitamin A as retinyl acetate, 10,000 IU; vitamin D_3 as cholecalciferol, 5,000 IU; vitamin E as alpha-tocopherol-acetate, 50 IU; vitamin K, 3 mg; thiamin, 2 mg; riboflavin, 8 mg; pyridoxine, 3 mg; vitamin B_{12}, 15 µg; biotin, 0.12 mg; calcium pantothenate, 12 mg; nicotinic acid, 50 mg; folic acid, 2 mg; 400 mg choline chloride; methionine, 3,018 mg; threonine, 726 mg; lysine, 2,831 mg; I, 1 mg as calcium iodate; Se, 0.35 mg as sodium selenite; Fe, 40 mg as ferrous sulphate; Mo, 0.5 mg as sodium molybdate; Mn, 100 mg as manganous oxide; Cu, 15 mg as copper sulfate; Zn, 100 mg as zinc oxide.

[3]Premix provided per kilogram of finisher diet: vitamin A as retinyl acetate, 10,000 IU; vitamin D_3 as cholecalciferol, 5,000 IU; vitamin E as alpha-tocopherol-acetate, 50 IU; vitamin K, 3 mg; thiamin, 2 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 15 µg; biotin, 0.12 mg; calcium pantothenate, 10 mg; nicotinic acid, 50 mg; folic acid, 1 mg; 350 mg choline chloride; methionine, 2,514 mg; threonine, 361 mg; lysine, 1,779

mg; I, 1 mg as calcium iodate; Se, 0.35 mg as sodium selenite; Fe, 40 mg as ferrous sulphate; Mo, 0.5 mg as sodium molybdate; Mn, 100 mg as manganous oxide; Cu, 15 mg as copper sulfate; Zn, 100 mg as zinc oxide.

[4]Calculated according to NRC (1994).

Supplemental Table 2. Body weight, feed intake and growth performance between d 7 and 21 of life of female and male broiler chickens raised at two different locations.

		Residual feed intake[1,2]				*P*[3,4]		
Item	Location	Low	Medium	High	SEM	FE	location	FE × location
Females								
Body weight, d 7 of life (g)	L1+2	145	145	147	2.6	0.805	0.001	0.802
	L1	141	138[y]	141	3.6			
	L2	149	151[x]	153[x]	2.6			
Body weight, d 21 of life (g)	L1+2	906	848	893	21.56	0.133	<0.001	0.817
	L1	972[x]	895[x]	852[x]	29.48			
	L2	840[y]	801[y]	834[y]	31.35			
Total feed intake, d 7-21 of life (g)	L1+2	1009[b]	1023[abB]	1083[aA]	23.07	0.067*	0.339	0.391
	L1	1001[b]	1059[ab]	1094[a]	31.55			
	L2	1017	987[B]	1073[A]	33.55			
Total body weight gain, d 7-21 of life (g)	L1+2	761	703	746	21.02	0.131	<0.001	0.845
	L1	831[x]	757[x]	811[x]	28.75			
	L2	691[y]	650[y]	681[y]	30.57			
Males								
Body weight, d 7 of life (g)	L1+2	145	145	148	2.173	0.704	<0.001	0.919
	L1	139[x]	140[x]	141[x]	3.010			
	L2	152[y]	150[y]	154[y]	3.047			
Body weight, d 21 of life (g)	L1+2	920	928	933	19.09	0.895	<0.001	0.046

	L1	933	999	1005	27.23			
	L2	908	856	861	26.76			
Total feed intake, d 7-21 of life (g)	L1+2	1049^{b}	1088^{ab}	1155^{a}	20.15	0.002***	0.985	0.042
	L1	1008^{b}	1096^{ab}	1187^{a}	28.74			
	L2	1089^{ab}	1080^{b}	1123^{a}	28.25			
Total body weight gain, d 7-21 of life (g)	L1+2	775	782	785	17.77	0.914	<0.001	0.032
	L1	794^{B}	859^{x}	863^{xA}	25.34			
	L2	756	705^{y}	707^{y}	24.91			

FE, feed efficiency; RFI, residual feed intake; L1, University of Veterinary Medicine Vienna (Vienna, Austria); L2, Agri-Food and Biosciences Institute (Hillsborough, Northern Ireland, UK).

[1]Values are least squares means ± standard error of the mean (SEM).

[2]Each RFI group represents $n = 9$ female and male chickens at location 1; $n = 6$ low RFI, $n = 11$ medium RFI, and $n = 8$ high RFI females as well as $n = 10$ low RFI, $n = 9$ medium RFI, and $n = 9$ high RFI males at location 2.

[3]*P*: probability level.

[4]Linear polynominal contrast: $*P \leq 0.05$, and $***P \leq 0.001$.

[a-c]Least squares means within a row without a common lowercase superscript differ among RFI groups ($P < 0.05$).

[A,B]Least squares means within a row without a common uppercase superscript tend to differ among RFI groups ($P < 0.1$).

[x,y]Least squares means within a column without a common lowercase superscript differ between locations ($P < 0.05$).

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Relationship analysis

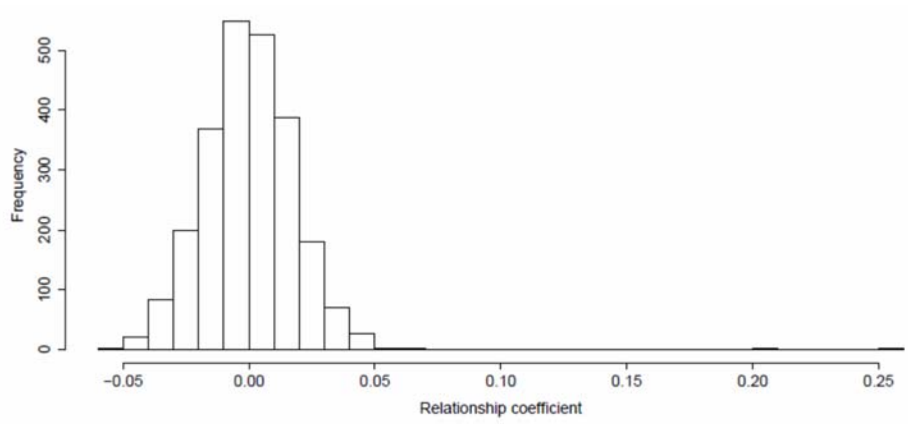
Single nucleotide polymorphism genotypes were used to examine the genetic relationship of all birds within and between each population received. In order to achieve the genetic relationship of each pair of samples supplied, a G-matrix was established using the PreGS program by Prof I. Misztal (Animal Breeding and Genetics group, University of Georgia, Athens, GA, USA). Supplemental Table 2 lists the relationship statistic per population.

These data indicate that there is very little genetic relationship between any two birds within replicate batch 1 and replicate batch 2 from the location 1. In replicate batch 3 at location 1, two birds appeared to be half-sibs (relationship of 0.25). Similarly, the replicate batch 1 from location 2 appeared to contain two birds that are half-sibs (relationship of 0.20). The overall relationships within and between populations has been plotted and is illustrated in Supplemental Figure 1.

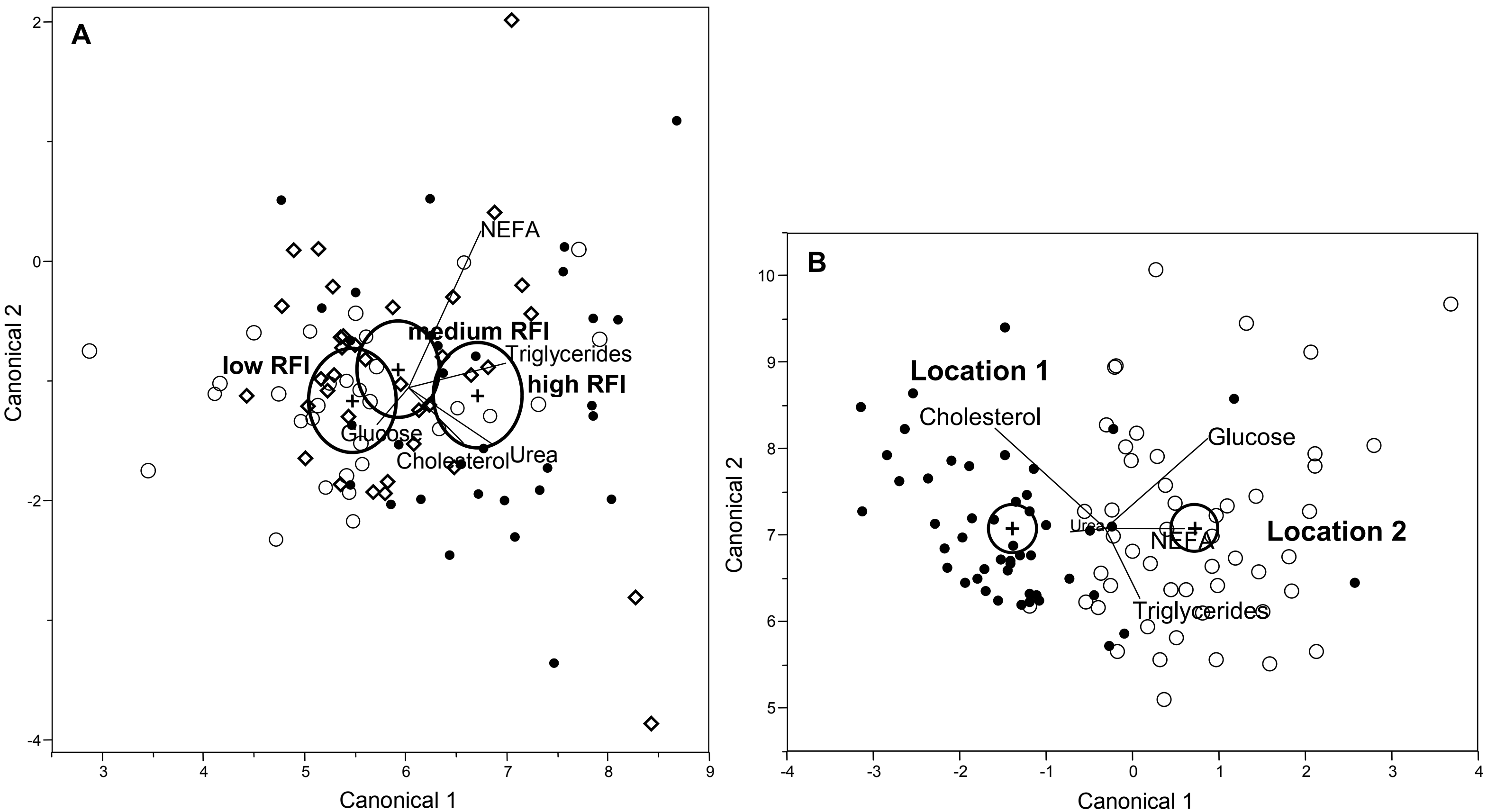
Supplemental Table 3. Genomic relationships among chickens.

	comparisons	genomic relationships among birds			
		mean	sd	min	max
Location 1 + 2	2415	0	0.02	-0.05	0.25

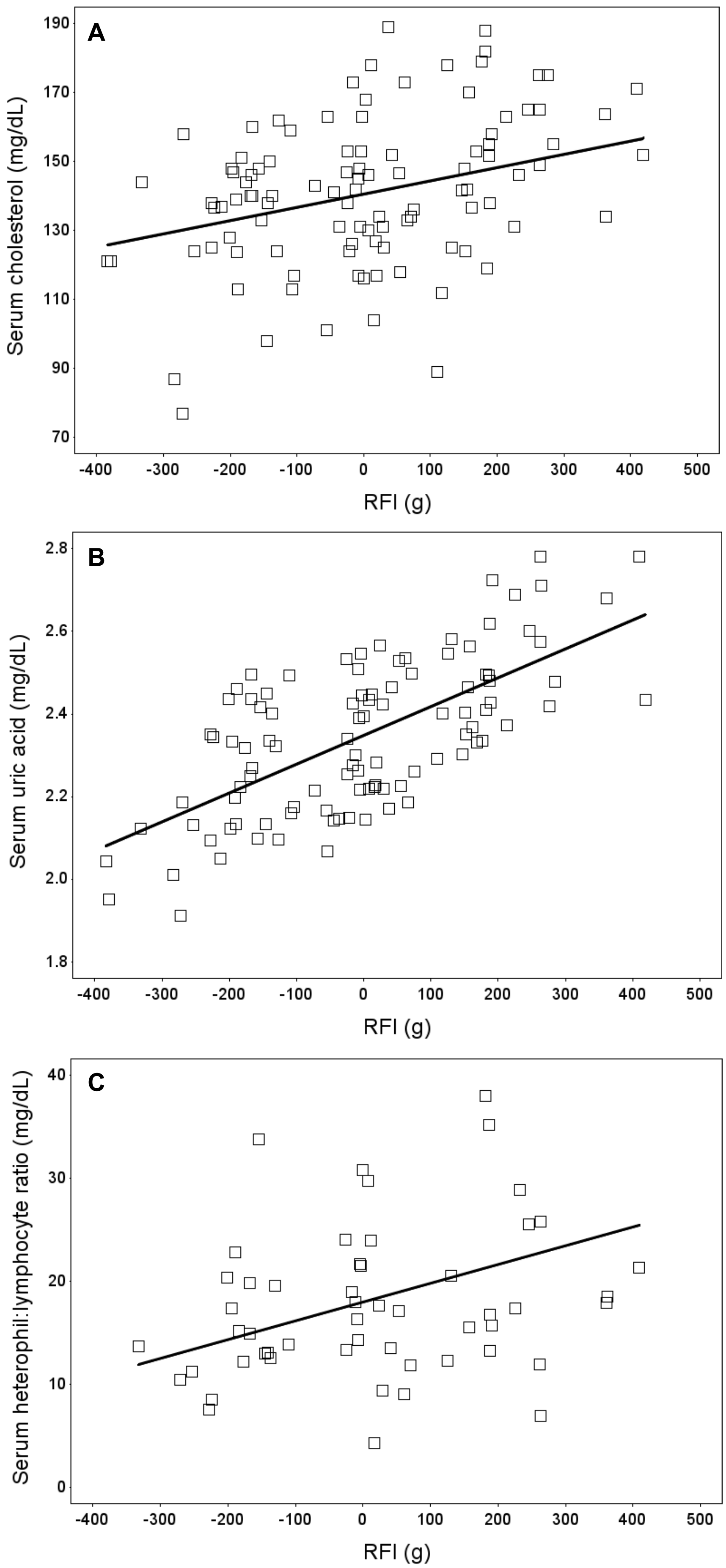
Supplemental Figure 1. G-relationships among chickens from both locations.



Assessing serum metabolite profiles as predictors for feed efficiency in broiler chickens reared at geographically distant locations



A
Canonical 2
Canonical 1
NEFA
medium RFI
low RFI
Triglycerides
high RFI
Glucose
Cholesterol
Urea
B
Location 1
Cholesterol
Glucose
Urea
NEFA
Location 2
Triglycerides


A
Serum cholesterol (mg/dL)
190
170
150
130
110
90
70
-400 -300 -200 -100 0 100 200 300 400 500
RFI (g)
B
Serum uric acid (mg/dL)
2.8
2.6
2.4
2.2
2.0
1.8
-400 -300 -200 -100 0 100 200 300 400 500
RFI (g)
C
Serum heterophil:lymphocyte ratio (mg/dL)
40
30
20
10
0
-400 -300 -200 -100 0 100 200 300 400 500
RFI (g)