Genetic basis of *Campylobacter* colonisation in the broiler chicken and its impact on intestinal health following natural field exposure.

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Short title: Genetic basis of *Campylobacter* colonisation

Abstract

Campylobacter is the leading bacterial cause of food-borne diarrhoeal illness in humans and source attribution studies unequivocally identify handling or consumption of poultry meat as a key risk factor. *Campylobacter* colonises the avian intestines in high numbers and rapidly spreads within flocks. A need therefore exists to devise strategies to reduce *Campylobacter* populations in poultry flocks. There has been a great deal of research aiming to understand the epidemiology and transmission characteristics of *Campylobacter* in poultry as a means to reduce carriage rates in poultry and reduce infection in humans. One potential strategy for control is the

genetic selection of poultry for increased resistance to colonisation by *Campylobacter*. The potential for genetic control of colonisation has been demonstrated in inbred populations following experimental challenge with *Campylobacter* where quantitative trait loci associated with resistance have been identified. Currently in the literature there is no information of the genetic basis of *Campylobacter* colonisation in commercial broiler lines and it is unknown whether these QTL are found in commercial broiler lines. The aim of this study was to estimate genetic parameters associated with *Campylobacter* load and genetic correlations with gut health and production traits following natural exposure of broiler chickens to *Campylobacter*. The results from the analysis show a low but significant heritability estimate (0.095 ± 0.037) for C*ampylobacter* load which indicates that non-genetic factors have a greater influence on the level of *Campylobacter* found in the broiler chicken.

Furthermore, through examination of macroscopic intestinal health and absorptive capacity, our study indicated that *Campylobacter* has no detrimental effects on intestinal health and bird growth following natural exposure in the broiler line under study. These data indicate that whilst there is a genetic component to *Campylobacter* colonisation worthy of further investigation, there is a large proportion of phenotypic variance under the influence of non-genetic effects. As such the control of *Campylobacter* will require understanding and manipulation of non-genetic host and environmental factors.

Background

Campylobacter is the leading bacterial cause of human foodborne illness worldwide. It was estimated by the World Health Organisation to cause approximately 96 million illnesses, 21 thousand deaths and loss of 2.1 million

disability-adjusted life years in 2010 (Havelaar et al. 2015). Human campylobacteriosis is typically a self-limiting disease characterised by acute watery diarrhoea which is sometimes bloody and accompanied by abdominal cramp, fever and nausea. Symptoms typically persist for up to 10 days, however c. 10% of cases require hospitalisation and in rare cases severe sequelae can develop including reactive arthritis and inflammatory neuropathies such as Guillain-Barré Syndrome, sepsis and even death (Mishu and Blaser 1993). It has been suggested that the actual number of cases of campylobacteriosis in the UK community is nine times greater than that captured by national surveillance (Tam et al. 2012).

Sources of *Campylobacter* include the environment and a range of wild and domesticated animals (Penner 1988, Blaser 1997). It is widely accepted that farmed poultry are a key reservoir of human infections with studies into the epidemiology of *Campylobacter* outbreaks repeatedly identifying the consumption and handling of undercooked and raw chicken as a major risk factor (Mullner et al. 2009, Sheppard et al. 2009, Kaakoush et al. 2015). A survey in 2015-2016 by the UK Food Standards Agency (FSA) demonstrated that 61.3% of fresh chicken at retail sale was positive for *Campylobacter* above the minimum detection limit of 10 colony-forming units (CFU)/g (Jorgensen et al. 2016). *Campylobacter* levels in the intestinal tract of poultry can be in excess of 10^8 CFU/g of caecal contents and this can contaminate chicken meat in the event of leakage of gut contents during the slaughter process (Beery et al. 1988, Boyd et al. 2005).

Quantitative risk assessments have estimated that a 30 fold reduction of poultry-associated *Campylobacter* human infections is achievable through a $2\log_{10}$ reduction in the level of *Campylobacter* in broiler carcases (Rosenquist et al. 2003). The UK poultry industry initiated a large scale effort to find effective methods to reduce the

incidence and level of *Campylobacter* throughout the poultry supply chain. These interventions have included reviews of farm biosecurity and subsequent optimisation, processing technologies designed to kill bacteria such as steam treatment and blast chilling, and the introduction of leak proof packaging and guidance to consumers. One key focus for intervention is reducing the level of *Campylobacter* in poultry during production and this requires a better understanding of the contribution of avian and bacterial factors to colonisation. *Campylobacter* readily colonises the avian intestinal tract, typically in the absence of overt pathology, and for many years has been considered a commensal member of the normal chicken gut microbiota (Hermans et al. 2011). In recent years it has been suggested that *Campylobacter* is not merely a commensal and in some instances can be pathogenic (Humphrey et al. 2014). This shift in opinion is the product of published data describing innate immune responses to experimental *Campylobacter* inoculation coupled with evidence of inflammation and an increased influx of immune cells in some commercial broiler lines (Smith et al. 2008, Meade et al. 2009, Humphrey et al. 2014). Moreover, some have reported that *Campylobacter* colonisation impairs weight gain and alters gut morphology (Awad et al. 2014, 2015). In contrast, other published data show no evidence of gross or histopathological lesions following experimental inoculation of poultry (Beery et al. 1988, Dhillon et al. 2006, Pielsticker et al. 2012). Conflicting data describing the response of the chicken to *Campylobacter* inoculation is not wholly unexpected as the balance between inert commensal and opportunistic pathogen can be swayed depending on the strain of the bacterium, host genotype and immune status, diet and co-infection (Wigley 2015).

Differences in *Campylobacter* levels have been described in commercial broiler populations, with some data suggesting that slower growing broiler breeds harbour

less *Campylobacter* than standard commercial broiler breeds (Bull et al. 2008, Williams et al. 2013). Conversely, Gormley *et al.*(2014) demonstrated that there were no differences in *Campylobacter* levels in multiple commercial and slower growing broiler breeds when reared in the same environment under commercial conditions with natural exposure to field relevant populations of *Campylobacter*. Experimental inoculation of inbred chicken lines with *C. jejuni* revealed heritable differences in resistance or susceptibility to intestinal colonisation that were consistently observed with multiple strains (Boyd et al., 2005; Psifidi et al. 2016). Through the use of resistant and susceptible inbred chicken lines it has also been possible to demonstrate variation in immune response through gene expression analyses following experimental *C. jejuni* inoculation (Li et al. 2010, 2012, Connell et al. 2012). Attempts have been made to identify loci which may explain variation in resistance to *Campylobacter* with some candidate genes being identified via genome-wide association studies using the progeny of crosses of lines of varying resistance (Connell et al. 2013, Psifidi et al. 2016). Taken together, these findings indicate that *Campylobacter* colonisation in the gut is partly under genetic control and potentially provides a route by which *Campylobacter* could be controlled at the individual bird level (Lin 2009). However, research on avian heritable resistance to *C. jejuni* has mostly relied on inbred birds derived from layer lines, and the extent to which findings apply in commercial broilers is unclear.

Here, for the first time, we aimed to estimate the genetic basis of *Campylobacter* colonisation within an outbred pure-bred commercial broiler line reared under commercial conditions with natural exposure to *Campylobacter*. To further examine the influence of *Campylobacter* on the intestinal health of the chicken, the gut tissues of all birds were examined using a *post mortem* gut health scoring system developed

by Aviagen®. This technique uses a severity scale to macroscopically characterise enteritis and intestinal imbalance based on the appearance of the intestinal tissues and contents. By analysing these phenotypes along with body weight, we aimed to provide more information on the impact of *Campylobacter* on bird performance along with the health and function of the intestinal tract of commercial broiler chickens under relevant farming conditions with natural exposure to *Campylobacter*.

Materials and methods

Birds, Housing and Management

The data for this study originate from the ongoing recording of health and performance traits within the Aviagen (Newbridge, UK) breeding program. The birds were housed within a non-bio-secure environment referred to as sib-test environment aimed to resemble broader commercial conditions and where full-sibs and half-sibs of selection candidates are placed (Kapell et al. 2012). A detailed description of environmental parameters can be found in Table 1. Birds were fed a standard feed ration (maize-based to provide the carotenoid source) in the form of a starter, grower and finisher diet in line with industry practice. All birds throughout the study received the same vaccinations as per commercial regimen and were reared under the same management practices. Phenotypic data were collected from 3,000 individual birds and genetic parameters were estimated using five generations of pedigree. To ensure the birds from each flock were exposed to *Campylobacter* during the study, the farm environment was tested for the presence of *Campylobacter spp.* prior to sampling using the "boot sock" method as described by Gormley *et al.* (2014).

Recording of traits

All birds in this study were hatched in the same hatchery, fully pedigreed and uniquely tagged with a barcode wingband. Sampling was performed at 35 days of age with sampling occurring every two weeks in batches of 100 birds (50 males and 50 females) giving a total of 3,000 birds over a period of 16 months. Birds were weighed and euthanised humanely by cervical dislocation by trained personnel. After euthanasia, a millilitre of blood was collected from the heart for assessment of blood carotenoid levels. Furthermore the intestinal tract of each bird was assessed after euthanasia and scored to characterise any gross intestinal abnormalities which could indicate enteritis or enteropathy. During this process the two intact caeca were aseptically removed for *Campylobacter* enumeration.

Microbiology

To enumerate *Campylobacter* in intestinal contents, seven serial ten-fold dilutions of caecal content were prepared in phosphate-buffered saline and 100 µl plated to modified charcoal deoxycholate (mCCDA) agar supplemented with cefoperazone (32 mg/L) and amphotericin B (10 mg/L; Oxoid), followed by incubation for 48 h under microaerophilic conditions (5% O_2, 5% CO_2, and 90% N_2) at 41°C. Dilutions were plated in duplicate and colonies with morphology typical of *Campylobacter* enumerated. The number of colony-forming units (CFU)/g of caecal content was then calculated and the theoretical limit of detection by the method used was 100 CFU/g of content. In instances where no colonies were observed after direct plating, a *Campylobacter* load equal to the theoretical limit of detection was assumed, as enrichment to confirm the absence of *Campylobacte*r in the caecal content was not performed.

Gut health assessment

The whole intestinal tracts of the birds were examined immediately *post mortem* and intestinal health was evaluated based on a gut health index developed by Aviagen®. The underlying principle of this gut health index is to examine each section of the small intestine and assess the muscular tone of the gut wall, signs of inflammation on the gut surface, the consistency of the gut contents and presence of mucus. In addition the quality of the caecal contents and any evidence of infectious agents is recorded. The scoring of muscular tone, inflammation and consistency is based on a scale of 0 (normal), 1 (mildly abnormal) and 2 (severely abnormal); for the presence of mucus it is scored as 0 (absent) or 1 (present). Gut health index scoring was performed on each region of the small intestine (duodenum, jejunum and ileum) and the caeca. The gut health index score for each individual bird was calculated as the sum of all the scores across gut sections. The maximum available score is 23 which would indicate a severely affected intestinal tract; the scoring criteria for each aspect of the gut health index are outlined in Table 2.

Serum optical density

The absorptive capacity of the gut can be assessed by measuring the level of carotenoid levels in the blood Blood was allowed to clot at room temperature and 200µl of serum was removed with a pipette and placed into a flat-bottomed 96 well plate. Carotenoid levels were measured via spectrophotometry using a Tecan Sunrise microplate reader at 450nm to obtain the optical density (OD_{450}) of the sera. Due to the fragility of avian erythrocytes, haemolysis can sometimes occur and cause discolouration of the sera. This discolouration interferes with the measurement of carotenoids and samples found to be haemolysed were not included in the analysis. In this data set 148 samples were found to be haemolysed and treated as missing values in subsequent analyses. The

analyses were performed both with and without the birds with the missing values and no significant differences were seen in the resultant parameters.

Statistical Analyses of Genetic Parameters

The phenotypic traits of 35 days body weight (BW), gut health index score (GS), serum carotenoid level (via optical density at 450nm) (OD) and *Campylobacter* load (CP) were analysed in the following multivariate animal model to estimate genetic parameters:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e},$$

Where: **y** is the vector of observations of the traits, **b** the vector of the fixed effect accounting for the interaction between the sex, hatch-week, pen and contributing mating group. To account for the potential impact of seasonal variation on *Campylobacter* load within poultry flocks the model includes the week of hatch of sampled birds as a fixed effect. The vector of additive genetic effects is denoted by **a**, the vector of permanent environmental effects of the dam is denoted by **c,** and **e** represents the vector of residuals. **X**, **Z** and **W** represent incidence matrices relating the vectors **b**, **a**, and **c** to **y**. The assumed (co)variance structure was:

$$V\begin{bmatrix}\mathbf{a}\\ \mathbf{c}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\otimes\mathbf{G} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\otimes\mathbf{C} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{I}\otimes\mathbf{R}\end{bmatrix},$$

Where: **A** and **I** are the additive genetic relationship matrix and identity matrix, respectively. **G**, **C** and **R** represent the variance and covariance matrices of additive genetic effects, permanent environmental effects of the dam and residual effects,

respectively. All variance component analyses were performed using ASReml (v3.0) software (Gilmour et al. 2009).

Results

Phenotypic averages and descriptive statistics

Table 3 summarises the least square means with standard errors for all the traits by sex. The results show that male birds had a significantly higher *Campylobacter* load (7.145 $\log_{10}$ CFU/g ±0.040) compared to the female birds (6.888 $\log_{10}$ CFU/g ±0.040). The difference in *Campylobacter* load between the sexes, albeit significant, is small and may not represent biologically relevant variation. The mean caecal *Campylobacter* loads demonstrated in this study are comparable to the loads reported in Gormley *et al.* (2014) where *Campylobacter* colonisation was via natural exposure as per this study. There were no significant differences seen in the gut scores between males (2.197 ±0.048) and females (2.210 ±0.048), and considering the total possible cumulative score of 23 both these scores are very low and indicating good intestinal health overall in both sexes. Serum carotenoid levels as shown by serum OD_{450nm} were significantly higher ($p=0.005$) in males (0.526 ±0.006) compared to females (0.509 ±0.006) indicating that the males, despite higher level of *Campylobacter,* have a better absorptive capacity of pigments (and by inference lipids).

Impact of *Campylobacter* on bird performance

The relationship of *Campylobacter* load with body weight, gut pathology score and serum carotenoid level is shown as scatter (XY) plots in Figures 1, 2 and 3 respectively. These data indicate that following natural exposure of the commercial broiler line studied to *Campylobacter* colonisation, the caecal *Campylobacter* load has no statistically significant impact on bird performance (in agreement with Gormley *et al.*

*(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)*2014), macroscopic gut health or ability to absorb carotenoid pigments (and thus lipids).

Genetic parameters

The genetic and phenotypic correlations between BWT, GS, OD and CP are presented in Table 4. The phenotypic correlations (below the diagonal shown in bold text in Table 4) of *Campylobacter* load with body weight, gut score and serum carotenoid levels were low. There was a positive correlation between body weight and serum carotenoid level indicating that those birds which have increased ability to absorb carotenoid (thus lipids) grow better.

The heritabilities for all the traits are displayed in Table 4. The heritability for body weight is moderate and in line with previously published data (Kapell et al. 2012, Bailey et al. 2015). The heritabilities for gut score, carotenoid level and *Campylobacter* were low with estimates of 0.074, 0.136 and 0.095 respectively.

Table 5 shows the proportion of phenotypic variance accounted for by environmental and maternal environment effects. For all the traits analysed, the permanent maternal environment accounted for 1.5-3.4% of the phenotypic variance which is similar to the range reported by Kapell et al (2012) for body weight and dermatitis in the same environment. The residual variance is shown to be responsible for the majority of the phenotypic variance for all traits analysed in this study accounting for 57.7% of the phenotypic variance of body weight and between 84.2-90.6% of the phenotypic variance of gut score, *Campylobacter* load and carotenoid level (as shown by serum OD_{450}). The genetic correlations (Table 4, above the diagonal) of *Campylobacter* load with body weight and gut score were low ($\leq$0.062), and moderate with serum carotenoid

level (0.301), however these were not statistically significant. The relationship of body weight with intestinal health parameters indicated a low genetic correlation with gut score (0.024) and moderate positive correlation with carotenoid level (0.244). The low correlation of body weight and gut score may reflect the fact that gut health was generally good across all birds leading to low phenotypic variance in the population. A positive genetic correlation was seen between gut pathology score and serum carotenoid level (0.482) however, since this correlation was not statistically different from zero robust conclusions cannot be drawn.

Discussion

Strategies are urgently required to reduce the burden of *Campylobacter* in poultry to limit the incidence of human infection. The poultry industry has already been successful at reducing the presence of *Campylobacter* in chicken found in retail outlets. Reports from the Food Standards Agency show 6.5% of chickens testing positive for the highest level of contamination (carrying more than 1,000 cfu/g) compared to 9.3% for the same period in the previous year (FSA 2017). Here we sought to evaluate if genetic selection could be an additional tool to reduce *Campylobacter* levels in commercial poultry. As observations of avian resistance to *C. jejuni* to date have relied on inbred layer lines of questionable relevance to commercial broilers (Boyd et al. 2005; Connell et al. 2013; Psifidi et al. 2016), we estimated the genetic basis of *Campylobacter* colonisation in commercial broilers following natural exposure under relevant rearing conditions. We also estimated the genetic correlations of *Campylobacter* load with body weight and intestinal health traits in order to ascertain if selecting for *Campylobacter* resistance may have adverse effects on bird performance and vice-versa. The data presented shows a low but significant heritability estimate for

Campylobacter colonisation in the test population. These data indicate that whilst there is a genetic component to *Campylobacter* colonisation worthy of further investigation, there is a large proportion of phenotypic variance under the influence of non-genetic effects. As such the control of *Campylobacter* will require understanding and manipulation of non-genetic host and environmental factors.

The relationship between *Campylobacter* and its poultry host following exposure is not fully understood. In some cases *Campylobacter* elicits a negative effect on broiler performance and intestinal health (Smith et al. 2008, Meade et al. 2009, Humphrey et al. 2014), whereas in other cases *Campylobacter* has no significant impact on bird weight, intestinal health or immune status (Beery et al. 1988, Dhillon et al. 2006, Pielsticker et al. 2012). In the current study we showed no correlation between caecal *Campylobacter* load and body weight at the phenotypic or genetic level in the broiler line under study. These findings are in agreement with the data from Gormley *et al.* (2014) where no correlation between *Campylobacter* load and body weight was reported. In this study we measured intestinal health and function using macroscopic gut scoring and serum carotenoid levels as a means to investigate whether or not *Campylobacter* was impacting upon the gut of the birds in this study. Typically, during an intestinal challenge, the gut contents have a greater liquid fraction due to secretion of immune cells into the gut lumen, reduced absorption and an increase in water intake by the affected bird (Manning et al. 2007). Additionally it is common for an inflammatory response to be seen on the gut surface particularly in the gut-associated lymphoid tissue (Chen et al. 2015) along with thinning and loss of muscle tone in the intestinal wall (Teirlynck et al. 2011). When the intestinal tract is compromised malabsorption can occur resulting in the caecal microbiota becoming imbalanced leading to a change from the normal dark brown pasty caecal contents to paler coloured,

watery and gassy contents (Wilson et al. 2005, Teirlynck et al. 2011, Sergeant et al. 2014). The absorptive capacity of the gut can be assessed using the level of carotenoids in the blood. These naturally occurring pigments, found in many plants such as maize, influence the yellow pigmentation found in the skin and legs of poultry (Rajput et al. 2013). Carotenoids are fat soluble and thus absorbed with lipids during digestion where they enter the blood stream and can be laid down in the body tissues (Ullrey 1972, Yonekura and Nagao 2007, Nagao 2011). In the event of enteric disease there is a reduction in fat absorption which in turn leads to a reduction in carotenoid absorption resulting in poor pigmentation; this is seen in coccidiosis, mycotoxicosis and malabsorption syndromes (Tung and Hamilton 1973, Tyczkowski et al. 1991a, 1991b, Zhao et al. 2006). The data presented demonstrates that there is no correlation between *Campylobacter* load and intestinal health as examined by macroscopic gut scoring of the intestinal tract and the ability to absorb carotenoids (through serum optical density) as an indicator of intestinal function. Assuming that caecal *Campylobacter* load is representative of colonisation in other parts of the intestinal tract, this result indicates that in this study *Campylobacter* colonisation does not have a negative impact upon intestinal health of the birds.

The differences seen in host response between experimental infection and natural exposure may be linked, in part, to the way by which the bacterium is introduced to the birds. Experimental infection of birds with *Campylobacter* is usually with a high concentration of a single strain at one time point whereas natural exposure occurs gradually with one or multiple strains initially at lower doses (Beery et al. 1988, Newell and Fearnley 2003, Boyd et al. 2005, Psifidi et al. 2016). It is possible that in the case of experimental inoculation, the introduction of a large dose of a single bacterium has the potential to upset the balance of the resident microbiota resulting in dysbacteriosis

leading to a disruption in intestinal health and function. Furthermore, the procedure of handling and dosing a bird during experimental inoculation may cause stress to the bird which may have the potential to influence the physiology of the bird and the activity of the bacterium once it enters the gastrointestinal tract. This could aid the proliferation of *Campylobacter*, especially if there are host related factors favouring *Campylobacter* colonisation such as in the case of susceptible inbred lines. At the farm level, a key risk factor for increasing levels of *Campylobacter* in a broiler flock is through the process of partial depopulation (also called 'thinning') where a proportion of the flock are removed at a certain body weight and the remaining birds are kept on the farm to allow them to grow for a longer period of time (Cloak et al. 2002). Opportunities for breaks in biosecurity and increasing bird age may be responsible for these increases in *Campylobacter* levels (Smith et al. 2016), as well as the stress associated with the process of partial depopulation (Robyn et al. 2015). Catecholamines released during stress, such as adrenaline and noradrenaline, can impact negatively upon the intestinal environment (Siegel 1971, 1980, Virden and Kidd 2009) and promote motility and growth of *Campylobacter* (Cogan et al. 2007, Xu et al. 2015). The manner and extent by which a particular strain of *Campylobacter* responds to noradrenaline has been shown to be highly variable (Aroori et al. 2014) and thus the outcome of an *Campylobacter* challenge may be dependent on which strain is introduced to the intestinal tract of the bird. The impact of *Campylobacter* on its poultry host is highly variable and understanding the factors which can result in colonisation or a negative interaction may inform strategies for controlling the bacterium.

The caecal microbiota has long been recognised influencing susceptibility to disease and colonisation by zoonotic pathogens (Stanley et al., 2014). Certain bacterial species are known to affect the growth of *Campylobacter* (Nishiyama et al. 2014, Mañes-

Lázaro et al. 2017) and there have been reports of differences in intestinal microbiota composition in birds positive for *Campylobacter* (Sofka et al. 2015, Indikova et al. 2015). Transfer of microbiota between inbred mice differing in susceptibility to the enteric pathogen *Citrobacter rodentium* resulted in a reciprocal transfer of susceptibility and resistance (Willing et al. 2011). Thus, while a host genetic component to resistance can exist, this may be exerted in part though differences in the microbiota. Studies are therefore required to associate *Campylobacter* burden with the composition of indigenous microbial communities to explore the extent to which this may explain variation in *C. jejuni* colonisation phenotypes.

Whilst the present study provided evidence of a genetic component affecting *Campylobacter* colonisation, the estimate of heritability for *Campylobacter* load in the caeca is low and would mean that any progress through selection is likely to be slow and very modest in impact due to a low accuracy of predicting breeding values. Importantly, the lack of genetic correlation between *Campylobacter* load with body weight and gut health traits indicates that any selection for *Campylobacter* would not be detrimental for bird performance. Selection for disease resistance or resilience is a common goal in many livestock breeding programs however success is heavily reliant on two important things; firstly the animals from within the study population need to be inoculated with the target organism and secondly a reliable phenotype is needed to measure the presence or impact of the organism on the host (Bishop 2012). A breeding strategy for reducing *Campylobacter* colonisation would need to be based on natural exposure to *Campylobacter*, as experimental bacterial challenge as part of a routine program has ethical and safety implications. When using natural exposure, inoculation with the target organism is dependent on the seasonality of the organism and studies have shown that the presence of *Campylobacter* in poultry environments is seasonal

(Chowdhury et al. 2012). The consequence of seasonality is that exposure will vary from flock to flock thus the accuracy of the estimation of variance components is compromised (Bishop and Woolliams 2014). Our results should be interpreted in the context of the limitations and advantages of field studies (Bishop and Woolliams 2010, Bishop et al. 2012). Compared to controlled challenge experiments, unknown and uncontrolled exposure to infections, may reduce the power of a field study but does not constitute a fatal flaw in demonstrating host genetic differences in resistance (Bishop and Woolliams 2010). In addition, the natural infections that characterise field studies offer a more realistic picture of the genetic variation and yield results that are more relevant to practical genetic improvement programmes.

When dealing with complex traits where heritabilities are low and a reliable phenotype cannot be established, molecular genomic methods may be required to achieve resistance (Bishop and Woolliams 2014). The use of genome-wide association studies for the identification of single nucleotide polymorphisms or QTL conferring resistance to disease has been successful in a number of animal species in selecting for disease resistance (Houston et al. 2008, Bermingham et al. 2014). The low heritability estimate for campylobacter colonisation indicates that there doesn't seem to be any QTL of large effect for resistance or any QTL present are already at a high frequency in the population under study. Our ongoing research seeks to define the genomic architecture of the *Campylobacter* resistance in commercial broiler chickens.

In conclusion this study indicates that *Campylobacter* colonisation in the broiler intestinal tract following natural exposure is partially under genetic control with the majority of phenotypic variance being under the influence of environmental factors. Understanding the environmental factors that influence *Campylobacter* prevalence at the farm level will be required to devise strategies for control of *Campylobacter* in

broilers and genetic selection may be only a minor part of an integrated solution to the problem. Additionally by examining body weight along with macroscopic intestinal health and absorptive capacity it was shown that, following natural exposure, *Campylobacter* has no detrimental impact upon bird health.

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References

Aroori, S. V., T. A. Cogan, and T. J. Humphrey. 2014. Effect of noradrenaline on the virulence properties of campylobacter species. International Journal of Microbiology 2014:279075.

Awad, W. A., J. R. Aschenbach, K. Ghareeb, B. Khayal, C. Hess, and M. Hess. 2014. Campylobacter jejuni influences the expression of nutrient transporter genes in the intestine of chickens. Veterinary microbiology 172:195–201.

Awad, W. a, A. Molnár, J. R. Aschenbach, K. Ghareeb, B. Khayal, C. Hess, D. Liebhart, K. Dublecz, and M. Hess. 2015. Campylobacter infection in chickens modulates the intestinal epithelial barrier function. Innate immunity 21:151–160.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poultry Science 94:2870–2879.

Beery, J. T., M. B. Hugdahl, and M. P. Doyle. 1988. Colonization of gastrointestinal tracts of chicks by Campylobacter jejuni. Appl. Envir. Microbiol. 54:2365–2370.

Bermingham, M. L., S. C. Bishop, J. A. Woolliams, R. Pong-Wong, A. R. Allen, S. H. McBride, J. J. Ryder, D. M. Wright, R. A. Skuce, S. W. McDowell, and E. J.

Glass. 2014. Genome-wide association study identifies novel loci associated with resistance to bovine tuberculosis. Heredity 112:543–551.

Bishop, S. C. 2012. A consideration of resistance and tolerance for ruminant nematode infections.

Bishop, S. C., A. B. Doeschl-Wilson, and J. A. Woolliams. 2012. Uses and implications of field disease data for livestock genomic and genetics studies. Frontiers in Genetics 3.

Bishop, S. C., and J. A. Woolliams. 2010. On the genetic interpretation of disease data. PLoS ONE 5.

Bishop, S. C., and J. A. Woolliams. 2014. Genomics and disease resistance studies in livestock. Livestock science 166:190–198.

Blaser, M. J. 1997. Epidemiologic and clinical features of Campylobacter jejuni infections. The Journal of infectious diseases 176 Suppl:S103–S105.

Boyd, Y., E. G. Herbert, K. L. Marston, M. A. Jones, and P. A. Barrow. 2005. Host genes affect intestinal colonisation of newly hatched chickens by Campylobacter jejuni. Immunogenetics 57:248–53.

Bull, S. A., A. Thomas, T. Humphrey, J. Ellis-Iversen, A. J. Cook, R. Lovell, and F. Jorgensen. 2008. Flock health indicators and Campylobacter spp. in commercial housed broilers reared in Great Britain. Applied and environmental microbiology 74:5408–13.

Chen, J., G. Tellez, J. D. Richards, and J. Escobar. 2015. Identification of Potential Biomarkers for Gut Barrier Failure in Broiler Chickens. Frontiers in Veterinary Science 2:1–10.

Chowdhury, S., M. Sandberg, G. E. Themudo, A. K. Ersboll, K. Jones, B. Borck, M. Madsen, M. Kuusi, J. Reiersen, I. Hansson, E. Olsson Engvall, M. Løfdahl, A. J.

Wagenaar, W. van Pelt, and M. Hofshagen. 2012. Risk factors for Campylobacter infection in Danish broiler chickens. Poultry Science 91:2701–2709.

Cloak, O. M., B. T. Solow, C. E. Briggs, C.-Y. Chen, and P. M. Fratamico. 2002. Quorum sensing and production of autoinducer-2 in Campylobacter spp., Escherichia coli O157:H7, and Salmonella enterica serovar Typhimurium in foods. Applied and environmental microbiology 68:4666–71.

Cogan, T. A., A. O. Thomas, L. E. N. Rees, A. H. Taylor, M. A. Jepson, P. H. Williams, J. Ketley, and T. J. Humphrey. 2007. Norepinephrine increases the pathogenic potential of Campylobacter jejuni. Gut 56:1060–1065.

Connell, S., K. G. Meade, B. Allan, A. T. Lloyd, T. Downing, C. O'Farrelly, and D. G. Bradley. 2013. Genome-wide association analysis of avian resistance to Campylobacter jejuni colonization identifies risk locus spanning the CDH13 gene. Genetics of Immunity 3:881–890.

Connell, S., K. G. Meade, B. Allan, A. T. Lloyd, E. Kenny, P. Cormican, D. W. Morris, D. G. Bradley, and C. O'Farrelly. 2012. Avian resistance to Campylobacter jejuni colonization is associated with an intestinal immunogene expression signature identified by mRNA sequencing. PloS one 7:e40409.

Dhillon, A. S., H. L. Shivaprasad, D. Schaberg, F. Wier, S. Weber, and D. Bandli. 2006. Campylobacter jejuni infection in broiler chickens. Avian diseases 50:55–8.

FSA. 2017. Campylobacter contamination in fresh whole chilled UK-produced chickens at retail: January-March 2017.

Gilmour, a R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml user guide release 3.0.

Gormley, F. J., R. A. Bailey, K. A. Watson, J. McAdam, S. Avendano, W. A. Stanley, and A. N. M. Koerhuis. 2014. Campylobacter Colonization and Proliferation in the Broiler Chicken upon Natural Field Challenge Is Not Affected by the Bird Growth Rate or Breed. Applied and Environmental Microbiology 80:6733–6738.

Havelaar, A. H., M. D. Kirk, P. R. Torgerson, H. J. Gibb, T. Hald, R. J. Lake, N. Praet, D. C. Bellinger, N. R. de Silva, N. Gargouri, N. Speybroeck, A. Cawthorne, C. Mathers, C. Stein, F. J. Angulo, B. Devleesschauwer, and World Health Organization Foodborne Disease Burden Epidemiology Reference Group. 2015. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. PLOS Medicine 12:e1001923.

Hermans, D., K. Van Deun, A. Martel, F. Van Immerseel, W. Messens, M. Heyndrickx, F. Haesebrouck, and F. Pasmans. 2011. Colonization factors of Campylobacter jejuni in the chicken gut. Veterinary Research 42:82.

Houston, R. D., C. S. Haley, A. Hamilton, D. R. Guy, A. E. Tinch, J. B. Taggart, B. J. McAndrew, and S. C. Bishop. 2008. Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (Salmo salar). Genetics 178:1109–1115.

Humphrey, S., G. Chaloner, K. Kemmett, N. Davidson, N. Williams, A. Kipar, T. Humphrey, and P. Wigley. 2014. Campylobacter jejuni is not merely a commensal in commercial broiler chickens and affects bird welfare. mBio 5:e01364-14.

Indikova, I., T. J. Humphrey, and F. Hilbert. 2015. Survival with a Helping Hand: Campylobacter and Microbiota. Frontiers in Microbiology 6:1266.

Jorgensen, F., A. Charlett, E. Arnold, C. Swift, B. Madden, and N. C. Elviss. 2016. FSA Project FS102121 Year 2 Report A microbiological survey of

Campylobacter contamination in fresh whole UK-produced chilled chickens at retail sale.

Kaakoush, N. O., N. Castaño-Rodríguez, H. M. Mitchell, and S. M. Man. 2015. Global Epidemiology of Campylobacter Infection. Clinical microbiology reviews 28:687–720.

Kapell, D. N. R. G., W. G. Hill, A. M. Neeteson, J. McAdam, A. N. M. Koerhuis, and S. Avendano. 2012. Genetic parameters of foot-pad dermatitis and body weight in purebred broiler lines in 2 contrasting environments. Poultry Science 91:565–574.

Li, X., C. L. Swaggerty, M. H. Kogut, H.-I. Chiang, Y. Wang, K. J. Genovese, H. He, F. M. McCarthy, S. C. Burgess, I. Y. Pevzner, and H. Zhou. 2012. Systemic response to Campylobacter jejuni infection by profiling gene transcription in the spleens of two genetic lines of chickens. Immunogenetics 64:59–69.

Li, X., C. L. Swaggerty, M. H. Kogut, H.-I. Chiang, Y. Wang, K. J. Genovese, H. He, and H. Zhou. 2010. Gene expression profiling of the local cecal response of genetic chicken lines that differ in their susceptibility to Campylobacter jejuni colonization. PloS one 5:e11827.

Lin, J. 2009. Novel approaches for Campylobacter control in poultry. Foodborne pathogens and disease 6:755–65.

Mañes-Lázaro, R., P. M. Van Diemen, C. Pin, M. J. Mayer, M. P. Stevens, and A. Narbad. 2017. Administration of Lactobacillus johnsonii FI9785 to chickens affects colonisation by Campylobacter jejuni and the intestinal microbiota. British Poultry Science Online:1–9.

Manning, L., S. a Chadd, and R. N. Baines. 2007. Water consumption in broiler chicken: a welfare indicator. World's Poultry Science Journal 63:63–71.

Meade, K. G., F. Narciandi, S. Cahalane, C. Reiman, B. Allan, and C. O'Farrelly. 2009. Comparative in vivo infection models yield insights on early host immune response to Campylobacter in chickens. Immunogenetics 61:101–10.

Mishu, B., and M. J. Blaser. 1993. Role of Infection Due to Campylobacter jejuni in the Initiation of Guillain-Barre Syndrome. Clinical Infectious Diseases 17:104–108.

Mullner, P., S. E. F. Spencer, D. J. Wilson, G. Jones, A. D. Noble, A. C. Midwinter, J. M. Collins-Emerson, P. Carter, S. Hathaway, and N. P. French. 2009. Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach. Infection, Genetics and Evolution 9:1311–1319.

Nagao, A. 2011. Absorption and metabolism of dietary carotenoids. BioFactors 37:83–87.

Newell, D. G., and C. Fearnley. 2003. Sources of Campylobacter Colonization in Broiler Chickens. Applied and Environmental Microbiology 69:4343–4351.

Nishiyama, K., Y. Seto, K. Yoshioka, T. Kakuda, S. Takai, Y. Yamamoto, and T. Mukai. 2014. Lactobacillus gasseri sbt2055 reduces infection by and colonization of campylobacter jejuni. PLoS ONE 9.

Penner, J. L. 1988. The genus Campylobacter: A decade of progress. Clinical Microbiology Reviews 1:157–172.

Pielsticker, C., G. Glünder, and S. Rautenschlein. 2012. Colonization properties of Campylobacter jejuni in chickens. European journal of microbiology & immunology 2:61–5.

Psifidi, A., M. Fife, J. Howell, O. Matika, P. M. van Diemen, R. Kuo, J. Smith, P. M. Hocking, N. Salmon, M. A. Jones, D. A. Hume, G. Banos, M. P. Stevens, and P. Kaiser. 2016. The genomic architecture of resistance to Campylobacter jejuni

intestinal colonisation in chickens. BMC genomics 17:293.

Rajput, N., M. Naeem, S. Ali, J. F. Zhang, L. Zhang, and T. Wang. 2013. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. Poultry science 92:1177–85.

Robyn, J., G. Rasschaert, F. Pasmans, and M. Heyndrickx. 2015. Thermotolerant *Campylobacter* during Broiler Rearing: Risk Factors and Intervention. Comprehensive Reviews in Food Science and Food Safety 14:81–105.

Rosenquist, H., N. L. Nielsen, H. M. Sommer, B. Nørrung, and B. B. Christensen. 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic Campylobacter species in chickens. International Journal of Food Microbiology 83:87–103.

Sergeant, M. J., C. Constantinidou, T. A. Cogan, M. R. Bedford, C. W. Penn, and M. J. Pallen. 2014. Extensive Microbial and Functional Diversity within the Chicken Cecal Microbiome. PLoS ONE 9:e91941.

Sheppard, S. K., J. F. Dallas, N. J. C. Strachan, M. MacRae, N. D. McCarthy, D. J. Wilson, F. J. Gormley, D. Falush, I. D. Ogden, M. C. J. Maiden, and K. J. Forbes. 2009. Campylobacter genotyping to determine the source of human infection. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 48:1072–1078.

Siegel, H. S. 1971. Adrenals, Stress and the Environment. World's Poultry Science Journal 27:327–349.

Siegel, H. S. 1980. Physiological Stress in Birds. BioScience.

Smith, C. K., M. AbuOun, S. A. Cawthraw, T. J. Humphrey, L. Rothwell, P. Kaiser, P. A. Barrow, and M. A. Jones. 2008. Campylobacter colonization of the chicken induces a proinflammatory response in mucosal tissues. FEMS Immunology and

Medical Microbiology 54:114–121.

Smith, S., L. L. M. Messam, J. Meade, J. Gibbons, K. McGill, D. Bolton, and P. Whyte. 2016. The impact of biosecurity and partial depopulation on Campylobacter prevalence in Irish broiler flocks with differing levels of hygiene and economic performance. Infection ecology & epidemiology 6:31454.

Sofka, D., A. Pfeifer, B. Gleiss, P. Paulsen, and F. Hilbert. 2015. Changes within the intestinal flora of broilers by colonisation with Campylobacter jejuni. Berliner und Munchener tierarztliche Wochenschrift 128:104–10.

Tam, C. C., L. C. Rodrigues, L. Viviani, J. P. Dodds, M. R. Evans, P. R. Hunter, J. J. Gray, L. H. Letley, G. Rait, D. S. Tompkins, S. J. O'Brien, and IID2 Study Executive Committee. 2012. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 61:69–77.

Teirlynck, E., M. D. E. Gussem, J. Dewulf, F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2011. Morphometric evaluation of "dysbacteriosis" in broilers. Avian pathology : journal of the W.V.P.A 40:139–144.

Tung, H.-T., and P. B. Hamilton. 1973. Decreased Plasma Carotenoids During Aflatoxicosis. Poultry Science 52:80–83.

Tyczkowski, J. K., P. B. Hamilton, and M. D. Ruff. 1991a. Altered metabolism of carotenoids during pale-bird syndrome in chickens infected with Eimeria acervulina. Poultry science 70:2074–81.

Tyczkowski, J. K., J. L. Schaeffer, and P. B. Hamilton. 1991b. Measurement of malabsorption of carotenoids in chickens with pale-bird syndrome. Poultry science 70:2275–9.

Ullrey, D. E. 1972. Biological availability of fat-soluble vitamins: vitamin A and

carotene.

Virden, W. S., and M. T. Kidd. 2009. Physiological stress in broilers: Ramifications on nutrient digestibility and responses 1,2. J. Appl. Poult. Res 18:338–347.

Wigley, P. 2015. Blurred Lines: Pathogens, Commensals, and the Healthy Gut. Frontiers in veterinary science 2:40.

Williams, L. K., L. C. Sait, E. K. Trantham, T. A. Cogan, and T. J. Humphrey. 2013. Campylobacter infection has different outcomes in fast- and slow-growing broiler chickens. Avian diseases 57:238–41.

Willing, B. P., A. Vacharaksa, M. Croxen, T. Thanachayanont, and B. B. Finlay. 2011. Altering host resistance to infections through microbial transplantation. PLoS ONE 6.

Wilson, J., G. Tice, M. L. Brash, S. St Hilaire, and S. S. Hilaire. 2005. Manifestations of Clostridium perfringens and related bacterial enteritides in broiler chickens. World Poultry Sci J 61:435–449.

Xu, F., C. Wu, F. Guo, G. Cui, X. Zeng, B. Yang, and J. Lin. 2015. Transcriptomic analysis of Campylobacter jejuni NCTC 11168 in response to epinephrine and norepinephrine. Frontiers in Microbiology 6:452.

Yonekura, L., and A. Nagao. 2007. Intestinal absorption of dietary carotenoids.

Zhao, J., Y. Guo, X. Suo, and J. Yuan. 2006. Effect of dietary zinc level on serum carotenoid levels, body and shank pigmentation of chickens after experimental infection with coccidia. Archives of animal nutrition 60:218–28.

Table 1. Environmental parameters for the farm where birds were housed in this study.

Environmental parameter	Target
Feed days: 0-10	Starter (195g CP/kg; 12.0 MJ ME/kg)

Feed days: 11-25	Grower (170g CP/kg; 12.7 MJ ME/kg)
Feed days: 25-final weighing	Finisher (170g CP/kg; 12.7 MJ ME/kg)
Stocking density	29 to 32 kg bird weight per m^2
Temperature	Gradually reduced from 35 to 24°C
Photoperiod day 0-7	23L:1D
Photoperiod day 8-final weighing	18L:6D
Light intensity day 0-7	40 lux
Light intensity day 8-final weighing	Gradually reduced from 20 to 10 lux

1

	Score		
	0	**1**	**2**
	Normal/Healthy	**Mildly abnormal**	**Severely abnormal**
(T) Tone of intestinal wall ***(Based on cutting into the intestinal wall longitudinally)***	When cutting into the gut wall the wall immediately folds back on itself	On cutting into the gut, the wall folds back but it does not occur immediately and there is a delay (more than 5 seconds) in the wall moving.	The gut wall fails to fold back on itself when cut
(C) Consistency of intestinal contents based on region of small intestine ***(Based on quality of intestinal contents when cutting into the intestinal tract to assess tone)***	*Duodenum:* Typically the contents resemble coarse porridge but must be of a uniform consistency. *Jejunum:* Contents here should contain less water than the duodenal contents and the colour should be darker. *Ileum:* Contents should be starting to form firm bolus and colour should be much darker	*Duodenum:* Contents not uniform with a distinguishable fluid and solid fraction. *Jejunum:* Contents not uniform with a distinguishable fluid and solid fraction but less water than the duodenal contents. *Ileum:* Bolus is forming but it is does not hold its shape but colour of contents is darker than the jejunal contents.	*Duodenum:* Distinguishable fluid and solid fraction however it is predominately fluid. *Jejunum:* Distinguishable water and solid fraction colour same as duodenal contents *Ileum:* No bolus formation with soft/wet contents. colour may be similar to contents in jejunum
(I) Mucosal inflammation	Mucosal surface light pink colour with no evidence of reddening on surface.	Localised inflammation around GALT or diffuse localised reddening of mucosa in small areas	Profuse inflammation and reddening covering extensive areas of the mucosa
(M) Mucus production **(*Based on presence or absence)***	No mucus seen	Obvious layer of opaque mucus lining region of intestinal tract	(Not applicable for this criteria)
(Ca) Caecal health	Dark brown/green contents, pasty in consistency and no gas present	Pale in colour, pasty consistency and small amount of gas bubbles present	Contents are pale in colour and have a fluid consistency. Contents leak out when caeca cut. Caeca more than 50% filled with gas.

Table 2. Outline of scoring criteria for Gut Health Index – must be performed within 15 minutes of euthanasia otherwise post mortem intestinal autolysis may interfere with the results. Scores added with a higher score indicating more severe intestinal imbalance/disturbance. Maximum

1 score is 23 which is obtained by assessing T+C+I+M (which has a maximum of 7) for each small intestinal region and the Ca scores which has a

2 maximum of 2 – these scores are then added together to give the final score (7+7+7+2=23)

Table 3. Descriptive statistics for the traits for each sex.

Trait	Male Mean	Female Mean	Standard Error	P value
Body weight dg (BW) *	156.9	143.9	0.600	0.001
***Campylobacter* load (Log cfu/g) (CP) ***	7.145	6.888	0.048	0.001
Gut Score (GS) *	2.197	2.210	0.048	0.088
Serum carotenoid level (OD)	0.526	0.509	0.006	0.001

Table 4. Estimates of heritabilities (bold, diagonal), genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for body weight (BW), gut score (GS), Serum carotenoid level (OD) and *Campylobacter* load (CP). Standard errors are displayed in parentheses.

BW	GS	OD	CP
0.389(0.063)	0.024(0.265)	0.244(0.170)	0.062(0.193)
-0.019	**0.074(0.048)**	0.482(0.358)	0.054(0.399)
0.136	-0.056	**0.136(0.043)**	0.301(0.259)
-0.023	-0.021	-0.067	**0.095(0.037)**

Table 5. Phenotypic (PHEN), Residual (RES) maternal permanent environmental (PEm) variances and proportions of Phenotypic variance accounted for by RES (Prop RES) and PEm (Prop PEm) for Body weight (BW), *Campylobacter* level (CP), Gut score (GS) and Carotenoid level (OD)

	Line A				
Trait	**PHEN**	**RES**	**PEM**	**Prop RES**	**Prop PEm**
BW	402.44	232.23	13.79	0.577	0.034
GS	1.273	1.153	0.026	0.906	0.020
CP	143.19	127.41	2.164	0.890	0.015
OD	149.49	125.88	3.342	0.842	0.022

Figure Legends:

Figure 1. Scatter (XY) plot of *Campylobacter* load (Log_{10} CFU/g) and bodyweight (dg) showing there is no relationship between *Campylobacter* burden and bird weight.
Figure 2. XY plot of *Campylobacter* load (Log_{10} CFU/g) and gut score showing there is no relationship between *Campylobacter* and gut pathology score.
Figure 3. Scatter (XY) plot of *Campylobacter* load (Log_{10} CFU/g) and carotenoid level (serum OD_{450}) showing there is no relationship between *Campylobacter* and carotenoid level, and by inference ability of the gut to absorb lipids.

Figure 1

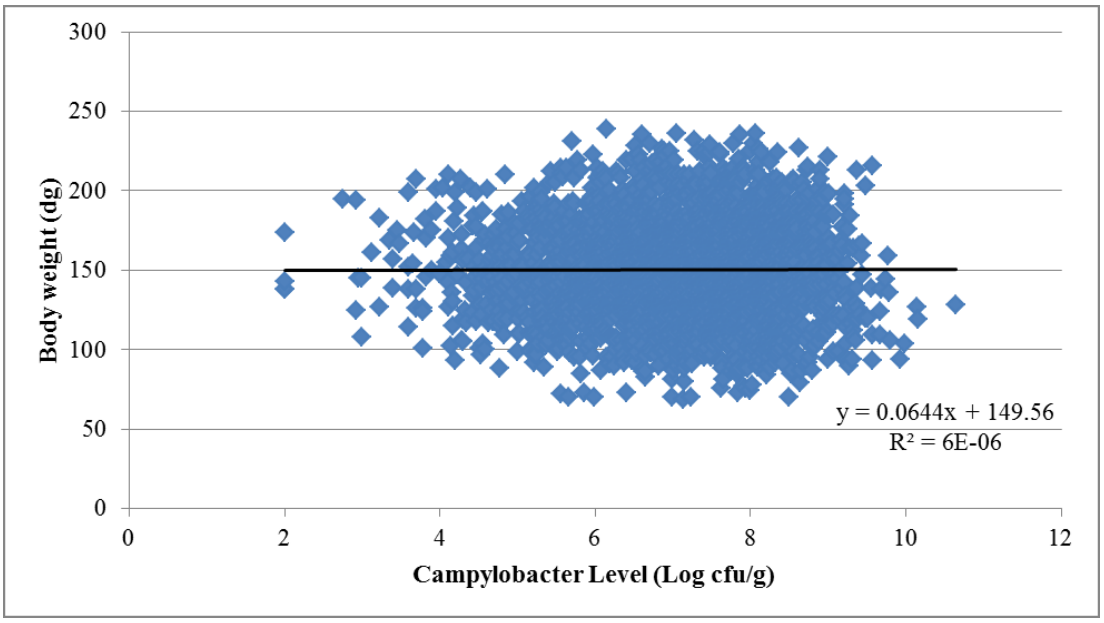


Figure 2

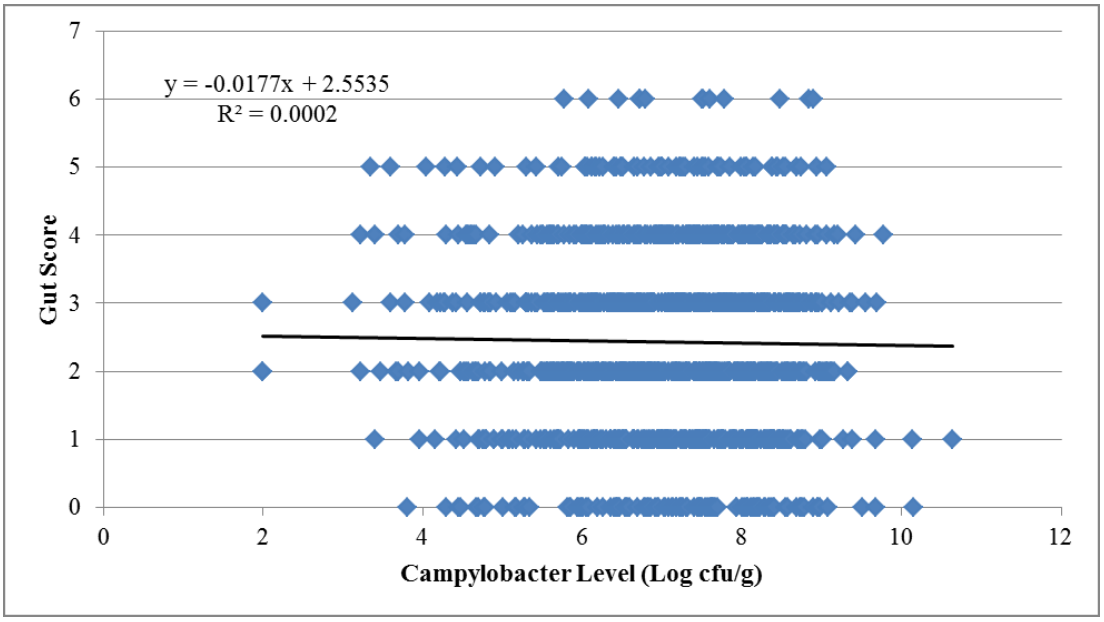
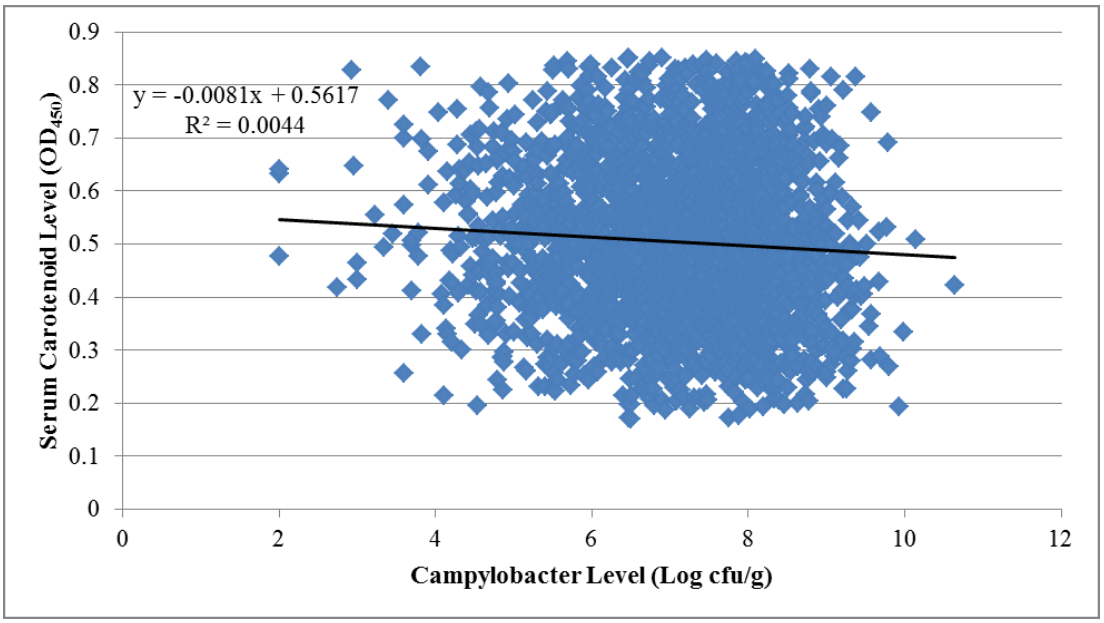


Figure 3

1


y = -0.0081x + 0.5617
R² = 0.0044
Serum Carotenoid Level (OD450)
Campylobacter Level (Log cfu/g)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0
2
4
6
8
10
12

2