



## Review Article

# Influence of sex and rearing method on performance and flock uniformity in broilers—implications for research settings

Ashley England, Kosar Gharib-Naseri, Sarbast K. Kheravii, Shu-Biao Wu\*

School of Environmental and Rural Science, University of New England, Armidale, NSW, 2351, Australia

## ARTICLE INFO

## Article history:

Received 21 June 2022

Received in revised form

2 November 2022

Accepted 2 December 2022

Available online 7 December 2022

## Keywords:

Sex effect

Chicken

Gut microbiota

Nutrient transporter gene expression

Sexing method

## ABSTRACT

Male and female broiler chickens differ in their growth performance, carcass part weights and nutrient requirements. The potential reasons for these differences have been explored by looking at differences in nutrient digestibility, nutrient transporter gene expression as well as gut microbiota populations between male and female birds. Studies have shown that male broilers have higher crude protein requirements compared to female broilers. The expression of monosaccharide and amino acid transporters show conflicting results as expression depends on the interactions between sex and bird age and breed as well as which tissue is sampled. Differences in microbiota populations between the genders were reported which may contribute towards performance differences, however research in this area is limited. The differences observed between the sexes contribute to increased variation in nutrition trials, and the potential to rear birds as equally mixed-sex becomes an option to reduce the variation introduced by the sex effect. Difference in rearing options obviously would only be feasible provided a quick, practical and cost-effective method of sexing birds is available, a topic that is also discussed in this review.

© 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Growth performance and carcass characteristics of broiler chickens can be affected by many factors such as breed, age and sex as well as environmental factors including nutrition, stocking density and housing, and the interactions between different factors (Young et al., 2001; Mehaffey et al., 2006; Abdullah et al., 2010; Lopez et al., 2011). Conducting meaningful research requires any unwanted sources of variation within the experimental model to be minimized or accounted for. A source of variation that is often not considered are the growth performance differences between male and female broilers when mixed-sex broilers are used in

experiments. A plethora of research has been published that clearly demonstrates a difference between male and female broilers when it comes to body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). A recent challenge faced by researchers is that due to the changes in genetics, feather sexing day-old chickens in the most common broiler breeds used in the commercial industry is no longer possible in certain areas. Ross 308 still has feather sexable birds depending on where the birds are being sourced from; however, Ross 708 is more likely to be feather sexable. The change in broiler genetics occurred only recently and this change has started to affect the research experimental design and choice of sex options at least in Australia (Wu et al., 2021). It has therefore become important to investigate alternate sexing methods that can be used. This review aims to investigate the effect of rearing broilers either as single or mixed-sex on flock uniformity and bird performance as well as to explore some of the underlying reasons for performance differences between male and female chickens by looking at the effect of sex on nutrient transporter gene expression and gut microbiota.

\* Corresponding author.

E-mail address: [shubiao.wu@une.edu.au](mailto:shubiao.wu@une.edu.au) (S.-B. Wu).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



## 2. Use of single or mixed-sex broilers in research

Research in the past has been performed mainly on single-sex broilers due to their easy access for use in experiments and the reduced variation they provide compared to mixed-sex broilers (Gous, 2017). However, due to feather sexing not always being possible many researchers need to make use of mixed-sex birds in experiments or use single sex birds from parental lines. Currently, there is little clarity as to if rearing broilers as single-sex or mixed-sex will result in better bird performance for males and/or females. The separate sex rearing of broilers may be justified if it results in a better growth rate, more efficient utilisation of feed that has been designed to more accurately meet the different requirements of each sex or increased flock uniformity. Early studies have shown no beneficial effect of separate sex rearing on bird performance (Smith et al., 1954; Hess et al., 1960; Lang et al., 1960; Lamoreux and Proudfoot, 1969) while later reports show it is beneficial to rear single-sex birds (Deaton et al., 1973; Gehle et al., 1974; Laseinde and Oluayemi, 1994; de Albuquerque et al., 2006). With the advancement in broiler genetics, there is an immense difference between the broiler breeds currently being used in commercial production systems compared to the birds used in the older studies. The use of less selected broiler breeds in these studies meant that the BW used were recorded at much later ages. Hess et al. (1960) recorded the BW at 9 weeks of age to determine the effect of rearing system on performance. Gehle et al. (1974) did not correct BW for mortality and this will have an effect on the results from the study. Other factors that differed between these experiments were stocking density as well as environmental conditions such as exposure to heat stress which can play a role in the differences in results between different studies. Therefore, it becomes crucial to reassess the rearing system and find the optimal method to be used for modern commercial broiler strains.

A recent study by Da Costa et al. (2017b) showed that the BW of males reared as separate sex was negatively impacted from 17 to 32 days, whereas sex separate female birds had higher BW from day 25 to 41 compared to females reared as mixed-sex. Da Costa et al. (2017b) concluded that rearing females in a mixed-sex environment negatively impacted their performance, whereas males benefited from mixed-sex rearing. These differences may be related to feeder space and competition. When the females are reared as mixed-sex, the competition for feeder space from males may be higher and could result in the females having a lower feed intake and lighter BW under mixed-sex conditions. Conversely, males reared under mixed-sex conditions can easily exclude smaller birds, such as females, from the feeders allowing them to increase feed intake and grow faster. However, with male-only birds, the competition for feeder space will be higher amongst males and result in a slower growth rate in comparison with males reared under mixed-sex conditions. This study was performed using birds of an older genetic line where growth rates were not as fast resulting in birds that had lighter final BW. It was therefore observed by Da Costa et al. (2017b) that the birds were a lot more active and more inclined to interact with each other. However, the growth rate of broilers today is much faster and it would make sense that as birds become heavier the less they would be inclined to move around the pen. This means that even in mixed-sex pens there would be plenty of opportunities for all the birds to reach the feeders and males would not be dominant over the females. It is also important to note that according to the present guidelines for better animal welfare, the birds are reared in much larger spaces with more sufficient feeder space compared to the settings in the past. Therefore, it is now unlikely that the competition for feed between birds is an issue and the females no longer face being pushed away from the feeders by the males.

Uniformity within a flock is an important measure of performance and is influenced by a variation in genotype, nutrition and environmental conditions (Gous, 2017). High uniformity can facilitate optimisation of feed and feeding programmes as well as help reduce between-pen variation in experiments to more accurately determine the effect of the treatments on performance parameters. Da Costa et al. (2017a) reported a lower BW variability and thus a small coefficient of variation within separate sex flocks compared to birds reared as mixed-sex showing the beneficial effects of separate sex rearing of broilers. Alternatively, if as-hatched birds are used and sexing can take place prior to placement then it may be appropriate to use equal numbers of males and females within each pen. Previous studies have shown that the uniformity of single-sex flocks is still better than that of equally-mixed sex (de Albuquerque et al., 2006; Deaton et al., 1973; Gehle et al., 1974). However, again it would be beneficial to investigate this using more modern broiler strains.

An additional advantage of separate sex rearing is being able to feed each sex according to its optimum nutrient requirements and providing a more suitable environment, such as greater floor space for the same number of male birds (Gous, 2017), in order to enhance the performance of each sex. Overall, in a research setting, separate sex rearing of broilers can improve pen uniformity, thereby reducing the coefficient of variation of nutrition studies.

## 3. Common methods for sexing broiler chickens for use in research

Several methods of variable practicality exist to determine the sex of newly hatched chicks. These methods include feather and vent sexing which are more commonly used compared to molecular and in ovo sexing methods which have recently been developed. The most commonly used sexing methods have been summarised in Table 1. Other methods include differentiation of the sex of growing embryos on the basis of specific female hormone concentrations has been reported by Phelps et al. (2003). Oestrogen radio-immune assays conducted on allantoic fluids of embryos allow for the sex discrimination from 15 to 17 days of incubation. Weissmann et al. (2013) established a method for in ovo sex identification on day 9 of incubation by measuring oestrone sulphate in the allantoic fluid. It was observed that male embryos had significantly lower oestrogen hormone levels compared to females. The results of oestrogen measurements are available within a few hours, and mass screening of thousands of eggs per hour is possible (Phelps et al., 2003). Another in ovo sexing method includes genetic engineering modification. Doran et al. (2018) focused on the production of genetically engineered hens, and described the marking of the Z chromosome of breeding hens with green fluorescent protein. This method was successfully used for sex determination in layers, with the males being identified from the germinal disc fluorescence in non-incubated eggs while female eggs do not show such fluorescence and can be incubated to produce female chicks (Bruijnijis et al., 2015).

There are methods for determining the sex of both as hatched chickens as well as chicken embryos. However, many of these methods are difficult to apply practically and on a large scale leaving researchers and producers with few options available if sexed chickens are to be sourced for research trials. Therefore, more work needs to be done to find a sexing method that is both cost-effective, practical and will satisfy the needs of poultry researchers and producers. The potential to adapt molecular sexing methods using real-time PCR and high resolution melting curve analysis to shorten the time it takes to receive results can certainly be pursued.

**Table 1**  
Summary of the most common methods used for sexing broiler chickens.

Sexing method	Description	Advantages	Disadvantages
Feather sexing	Based on the judgement of the length of the long wing feathers compared to the covert feathers. Female chickens are rapid feathering and male chickens are slow feathering (Kaleta and Redmann, 2008).	Inexpensive Does not require extensive training	Due to the changes in the genetics of certain breeds, feather sexing is no longer possible in breeds from Aviagen (Ross breeds) and Cobb-Vantress (Cobb breeds) in certain areas.
Vent sexing	Performed looking at the presence or absence of a rudimentary male sex organ after everting the vent area of the chick.	Fast Accurate	Requires well trained and experiences personal (Otsuka et al., 2016). Stressful for the chicks. Can cause up to 1% increase in early chick mortality due to chick damage during handling (Phelps et al., 2003). The movement of vent sexers between hatcheries creates a biosecurity risk.
Molecular sexing	Makes use of the fact that in birds females are heterogametic, and males are homogametic (Smith and Sinclair, 2004). The sex of the birds can, therefore, be distinguished based on the presence or absence of the W chromosome.	Highly sensitive Accurate	The time it takes from taking samples to obtaining results does not make this method conducive to large scale nutrition trials, where the sex of chicks needs to be determined within a few days after hatch before experimental treatments are applied.
In-ovo sexing: Fluorescence and Ramen spectroscopy	Based on differences in the composition of embryonic blood between males and females (Galli et al., 2016).	High precision Short analysis time Allows for analysis during early embryonic stages. Less stress on the chicks. More economical for sexing large amounts of eggs.	Accuracy depends on egg size, age and storage conditions. Perforation of the egg shell is required which can reduce hatching rate and lead to the impairment of embryonic structures which can result in developmental disorders or embryonic death (Krautwald-Junghanns et al., 2017).

#### 4. Influence of sex on different parameters in broiler chickens

##### 4.1. Performance and carcass characteristics

It is well known that male and female broilers differ in their growth performance and this has been supported by many studies. Lopez et al. (2011) conducted a trial to evaluate the sex effect on final BW in broilers and found male and female broilers slaughtered at 42 days of age were significantly different with respect to live BW with males being heavier than the females. This is in agreement with other reports that males are heavier at the same age (Young et al., 2001; Kidd et al., 2005; Shafey et al., 2013; Benyi et al., 2015; Da Costa et al., 2017b; Madilindi et al., 2018). Although heavier, male broilers have a greater coefficient of variation (CV) as observed by Goo et al. (2019) and Peak et al. (2000). Da Costa et al. (2017b) reported that the CV of BW for both males and females decreased with age although overall females tended to be more uniform than males. An increase in BW variability can result in increased between-pen variation which is not favoured in experiments where the aim is to keep any unwanted variation to a minimum. In addition, male broilers also have a higher feed intake compared to female broilers (Shafey et al., 2013; Benyi et al., 2015; Da Costa et al., 2017b; Madilindi et al., 2018; Goo et al., 2019). Siaga et al. (2017) and Madilindi et al. (2018) noted that there was an insignificant effect ( $P > 0.05$ ) of sex on FCR during all stages of growth and that both males and females utilised the feed with the same degree of efficiency at the same ages. On the contrary, Benyi et al. (2015) found that sex had an influence on FCR with male birds having better FCR means. These results are conflicting and it may illustrate why FCR is not a good criterion for comparison purposes. If it is reported that both sexes had a similar FCR, it does not mean they would utilize feed with the same degree of efficiency due to the different BW of males and females at the same age. It would be more accurate to correct FCR for BW and make comparisons based on this number. Indeed, FCR that is not corrected for BW is used as a

reference parameter and is not an exact measure, however it is used by producers as a way to measure the economic impact of rearing birds with different FCR values.

According to Zerehdaran et al. (2005), differences between males and females for a specific trait cannot be attributed to a single factor. Factors such as broiler breed, competition for feed, increased aggressive behaviour in males, social dominance, differences in nutritional requirements and different hormone levels between sexes all have an effect on the differences observed between male and female birds. Benyi et al. (2015) reported that Ross males were lighter than Cobb males at 49 days, but at the same age Ross females were heavier than Cobb females. Shim et al. (2012) and Udeh et al. (2015) also found significant genotype  $\times$  sex effects on BW, BW gain, feed intake and FCR.

In terms of the difference in carcass part weights between male and female broilers, it is important to make the comparison based on relative BW when evaluating differences based on the same age as obviously with males having a higher BW at the same age the weight of their different carcass parts will also be heavier. For example, Olawumi and Fagbuaro (2011) and Lopez et al. (2011) reported significant differences between males and females in the weights of various carcass parts; however, the weights were not expressed relative to BW, making these results not very useful when making comparisons. Benyi et al. (2015) reported a significant effect of sex on relative back, wing and leg weights with higher means for males than females but no significant effect of sex on relative breast weight. Shim et al. (2012) also reported no significant effect of sex on relative breast weight. It has been shown that there is a breed  $\times$  sex interaction on carcass part weights such as breast, back, wing, and leg weights (Olawumi and Fagbuaro, 2011; Benyi et al., 2015). Therefore, when making comparisons between studies it is important to take note of which breed of bird is being used. For example Castellini et al. (2006) reported no effect of gender on any of the relative carcass part weights; however, in this experiment slow growing chicken genotypes were used and so it

would not be accurate to compare this experiment with those that make use of modern fast growing broiler strains. Another important factor to highlight is that in the commercial broiler industry, birds are marketed at a similar BW rather than age. Da Costa et al. (2017a) reported that females were shown to have a significantly larger increase in breast meat yield at the same BW as males.

Fat deposition occurs earlier on in female broilers compared to males, Le Bihan-Duval et al. (1998) suggested this could be due to the difference in hormone production between males and females and their influence on fat deposition. A higher proportion of abdominal fat was reported in females compared to male Cobb broilers at day 42 (Madilindi et al., 2018). Benyi et al. (2015) also found Ross females had significantly more abdominal fat compared to Ross males. This is contrary to the results reported by Abdullah et al. (2010), who noted that although Lohman and Hubbard females had a higher abdominal fat percentage compared to males at day 43, this difference was not significant Fanatico et al. (2007) amongst others reported that slow growing broiler strains have a lower fat deposition compared to fast growing strains meaning that the differences between the sexes may not be as big in these slow growing breeds. Gous et al. (1999) collected data from both males and females of 2 different broiler strains and used it to describe the potential growth and lipid content curves (Figs. 1 and 2). It is shown in these figures that lipid deposition is affected by both bird strain and sex. At a younger age, when there is a smaller difference in BW between the sexes the lipid deposition differences between males and females are also very small. However, as the birds age and their weights increase, the differences in lipid deposition between the sexes increases with females having a much greater deposition compared to males.

Another variable to consider when assessing the difference in fat deposition between the sexes is the crude protein (CP) content of the feed. Chrystal et al. (2020) and Musigwa et al. (2020) reported that broilers fed diets low in CP deposited more fat. It has been shown that female broilers have lower CP requirements compared to males (Hernandez et al., 2012). Therefore, feeding diets low in CP relative to the specific breed guidelines should not result in a significant increase in fat deposition in females whereas males would have a higher fat deposition when fed a diet low in CP. This is also a reason why CP requirements should be based on lean BW and not total BW.

In summary, differences between males and females for a specific trait are influenced by broiler breed, competition for feed, increased aggressive behaviour in males, social dominance, hormone levels and differences in nutritional requirements. From the

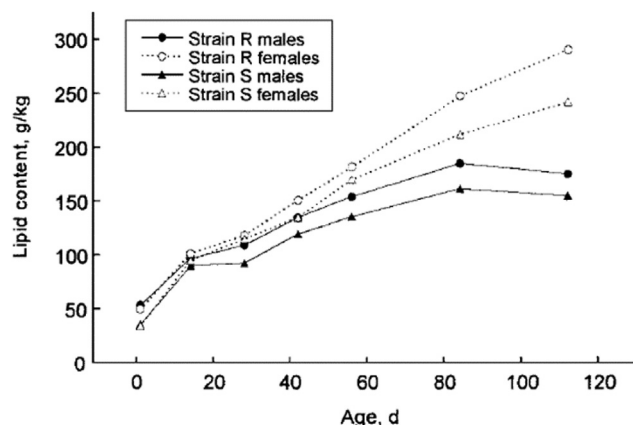


Fig. 2. The development of body lipid content with age in the males and females of 2 strain-crosses of broiler chicken. R = Ross male × Arbor Acres female; S = Steggles male × Arbor Acres female (Gous et al., 1999).

data gathered, there is enough evidence to suggest a difference between male and female birds in terms of BW and feed intake with male birds having a heavier final BW and higher feed intake compared to females. When it comes to determining the effect of sex on carcass part weights, it is important to make comparisons based on relative BW and also to take note of the broiler breed being used. In research, these differences may increase experimental variation when using mixed-sex birds for nutrition studies and bird sex will need to be taken into account for variation to be minimised.

#### 4.2. Differences in CP requirements and amino acid digestibility

Sex is known to have an effect on lean tissue deposition and amino acid requirements (Samadi and Liebert, 2006). Hernandez et al. (2012) conducted a trial in which male and female Ross 308 birds were fed a control diet containing a CP level of 24.5%, 23.0%, 21.5%, and 20.5%, respectively, for each phase, and medium and low CP treatments containing 1.5% and 3% less protein than the control diet, respectively, for each of the 4 phases. It was concluded that up to 3% reduction in CP did not affect female performance during the 4 phases. However, in males, a reduction in dietary CP levels negatively affected performance. These results identify a difference in protein requirements between sexes with female broilers potentially having a lower CP requirement compared to male birds. There may also be a possible benefit of separate sex rearing as it becomes easier to feed the 2 sexes more precisely, thereby optimising performance and at the same time reducing N excretion. Ravindran et al. (2004) conducted a study to determine nitrogen digestibility by measuring apparent ileal nitrogen digestibility (AIND). Results showed that AIND differed between males and females at day 42 with female broilers tending to have a higher ileal nitrogen digestibility compared to males (0.795 vs. 0.786,  $P < 0.1$ ). Similar results were reported by Doeschate et al. (1993), who found that female broilers had nitrogen digestibility coefficients that were 3% higher than those of male birds which provides an explanation why the performance of females is not negatively impacted on a low CP diet. When it comes to the digestibility of AA, Doeschate et al. (1993) reported that female broilers had higher AA digestibility coefficients compared to males. However, Zuprizal et al. (1992) reported higher true AA digestibility of rapeseed meal in male broilers at 6 weeks of age compared to females, but no sex differences were observed at 3 weeks of age. Other studies reported no significant effect of sex on AA digestibility (Awad et al., 2016;

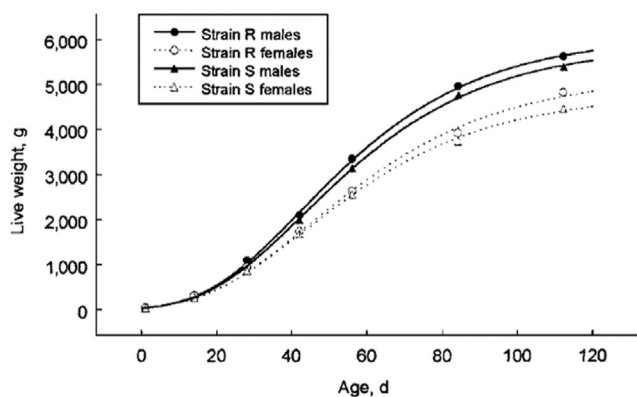


Fig. 1. The growth curves of males and females of 2 strain-crosses of broiler chicken. R = Ross male × Arbor Acres female; S = Steggles male × Arbor Acres female (Gous et al., 1999).



Gruhn and Zander, 1989; Kim and Corzo, 2012; Zelenka and Liska, 1986). These results are conflicting which may be because of the effect FI has on AA digestibility. Moter and Stein (2004) reported differences in AA digestibility based on FI for growing pigs and it was explained that the reason for this was that a decrease in FI causes a decrease in ileal endogenous AA flow and therefore higher apparent ileal AA digestibility. A higher feed consumption is also generally associated with a reduced retention time of digesta in the gastrointestinal tract, resulting in a reduced contact time between nutrients and digestive enzymes (Ravindran and Abdollahi, 2021). As previously mentioned, female broilers have a lower FI compared to males which in some circumstances would explain why some studies reported higher AA digestibility values in females. It is clear that CP requirements differ between male and female broilers which has implications for researchers when formulating diets for mixed-sex flocks. Being able to meet the nutrient requirements more accurately for each sex through separate-sex rearing will result in better performance and BW uniformity. On the other hand, it is difficult to evaluate the effect of sex on AA digestibility as the studies that have been done provide conflicting results as there are also other factors to consider such as differences in feed intake between the sexes.

#### 4.3. Gut microflora

Microorganisms in the gastrointestinal tract (GIT) of animals have an effect on health, nutrition utilisation, physiology and performance (Lan et al., 2005). Facultative and microaerophilic bacteria dominate the ileum of chickens while obligate anaerobes dominate the caeca (Bjerrum et al., 2006; Gong et al., 2007; Yin et al., 2009). Factors that are known to influence the gut microbial profile of animals include broiler breed (Turnbaugh et al., 2006), initial microbial profile at day of hatch (Ballou et al., 2016), diet composition (Torok et al., 2013), feed additives (Singh et al., 2013), age (Niu et al., 2015) and health status of the animal (Wu et al., 2014; Wu et al., 2014; Stanley et al., 2014). In addition, differences in nutrient requirements and nutrient digestibility may have an effect on the gut microbiota population as undigested nutrients in the small intestine result in the proliferation of certain bacterial species (Apajalahti and Vienola, 2016). Research on how the gut microbiome differs according to the sex of the animal is limited in poultry. It has been speculated that the differences in the growth performance between male and female broilers may be associated with differences in the gut bacterial profile between the 2 sexes as the gut microbiome plays a role in nutrient digestion and absorption (Turnbaugh et al., 2006; Rintilä and Apajalahti, 2013).

Earlier studies made use of culture-dependent approaches to identify the composition of poultry gut microbiota (Barnes, 1979; Mead, 1989). However, a large number of bacteria remain unidentified due to the lack of knowledge of appropriate culturing conditions. Culturing and biochemical techniques have resulted in the misclassification of some bacteria (Torok et al., 2008). Development in 16S rRNA gene sequencing has allowed for a more in-depth look into microbial composition, structure and diversity by using next-generation sequencing technology and sophisticated bioinformatics analysis (Choi et al., 2015). Lee et al. (2017) investigated the effect of sex on the gut microbiome and found that bacterial communities of broiler chickens vary depending on the sex of the host. Female broilers had an increased relative abundance of the phyla Bacteroidetes, Firmicutes and Proteobacteria, of which the genera *Alistipes*, *Holdemania* and *Clostridium* were particularly high. *Shigella* and *Moraxellaceae* were also more abundant in female broiler chickens. Similarly, male broiler chickens had a higher abundance of the phyla Bacteroidetes and Firmicutes compared to other bacterial taxa, but only 2 genus level taxa (*Bacteroides* and

*Blautia*) were detected as enriched bacteria that discriminate male and female groups. At the species level, increased numbers of *Alistipes massiliensis*, *Clostridium citroniae*, *Clostridium maritimum*, and *Shigella sonnei* were found in female chickens, whereas there were higher levels of *Blautia producta* in male chickens. *Bacteroides*, which were found at high levels in male chickens, are known to have the ability to degrade indigestible fibre in the GIT (Lee et al., 2017). Therefore, it is suggested that the performance differences between male and female birds could be related to the difference in their ability to degrade certain feed components such as fibre. Although there was a difference between gut bacterial communities between males and females in the study, the information about the reason for these differences for chickens is scarce in the literature. Ley et al. (2005) found that the amount of carcass fat could affect the GIT bacterial populations in mice. In broilers, males have leaner carcasses than females which could be related to the possible differences in the intestinal bacterial profile between sexes (Lumpkins et al., 2008). Other possible reasons for differences include a variation in the GIT conditions such as pH, temperature and mucin composition and amount, which might alter the number and types of bacteria that are able to proliferate and grow within the intestines (Lumpkins et al., 2008).

Torok et al. (2013) found that the gender of broilers significantly influenced the total eubacterial numbers within the caeca and ileum, with males having increased numbers of eubacteria compared to females. The number of caecal *Lactobacilli* was also significantly influenced by gender with males having higher numbers of *L. salivarius* and *L. crispatus*. One of the major differences between males and females is the amount of circulating hormones and it has been hypothesised that this could be a contributing factor to the sexual dimorphism of the gut microbiome (Ren and Sylvia, 2018). Yurkovetskiy et al. (2013) found that castration of male mice changes their gut microbiome to be more similar to that of a female indicating that hormones may have an effect in determining the gut microbiome. Org et al. (2016) examined the effect of sex hormones on the gut microbiome in mice by performing a gonadectomy on three strains of mice. Across all three strains, *Ruminococcaceae* populations were found to be significantly different in control males and gonadectomy males. Furthermore, following gonadectomy, when the mice were administered with testosterone, differences in the *Ruminococcaceae* population were no longer present in 2 strains of the male mice. In females, the *Akkermansia* population was found to be reduced in gonadectomy females compared to the control females. It is clear that more research is needed to determine the differences in the gut microbiome between males and females in poultry as well as the reasons for these differences and how they have an effect on growth performance and efficiency.

#### 4.4. Expression of genes encoding nutrient uptake

It is thought that a potential difference in nutrient uptake in the intestine between male and female broilers could result in a difference in growth rate and thus a difference in final BW between the 2 sexes (Kaminski and Wong, 2017). The uptake of nutrients is controlled by transporter proteins which are located at the brush border of the intestinal epithelia. The majority of amino acids are transported by the peptide transporter 1 (*PepT1*) either as free amino acids or peptides (Smith et al., 2013). Glutamate, which is the main energy source for intestinal epithelial cells, and aspartate, which plays an important role in gluconeogenesis and ATP synthesis, are transported across the brush border by the excitatory amino acid transporter 3 (*EAAT3*) (Brosnan and Brosnan, 2013; Kanai et al., 2013). Glucose and galactose are transported from the lumen of the small intestine across the brush border membrane

and into the enterocyte primarily by the sodium-dependent glucose transporter 1 (*SGLT1*), while glucose transporter-5 (*GLUT5*) mediates the passive transport of fructose into enterocytes (Mueckler and Thorens, 2013; Wright, 2013). At the basolateral membrane, there are transporters that regulate the efflux of nutrients from the cell into the blood or vice versa. The amino acid transporters alanine, serine, cysteine and threonine transporter-1 (*ASCT1*), cationic amino acid transporter-1 (*CAT1*), large neutral amino acid transporter-1 (*LAT1*), and Y + L amino acid transporter-2 ( $y + nbsp$ ; *LAT2*) are involved in the efflux of neutral, cationic, and branched chain amino acids into the blood (Fotiadis et al., 2013; Kanai et al., 2013). For carbohydrates, the monosaccharide transporter glucose transporter-2 (*GLUT2*) transports glucose, galactose, fructose, mannose, and glucosamine out of the cell into the blood (Mueckler and Thorens, 2013).

Few studies have been conducted to determine the differences in the expression of nutrient transporters between males and females in poultry. Weintraut et al. (2016) profiled the mRNA expression of amino acid and monosaccharide transporters in the small intestine of male and female turkeys. The results from this study showed that there was a higher expression of all genes investigated, except *GLUT2* and *SGLT1*, in females. The *GLUT2* was expressed at the same level in both genders, and *SGLT1* was expressed greater in males. Kaminski and Wong (2017) profiled the mRNA expression of an aminopeptidase and selected amino acid and monosaccharide transporters in the small intestine of male and female Aviagen Line A chickens at day of hatch, day 7 and 14. The expression of solute carrier family 7, member 9 ( $b^0, + AT$ ), *EAAT3*, *ASCT1*,  $y + nbsp$ ; *LAT2*, and *GLUT2* mRNA was greater in male than female chickens. There was a sex  $\times$  age interaction for  $b^0, + AT$ , *PepT1*, *SGLT1*, *ASCT1*, and  $y + nbsp$ ; *LAT2* mRNA, with greater mRNA abundance in males compared to females, at day of hatch but no difference was detected at day 7 and 14 between genders. A 3-way sex  $\times$  age  $\times$  tissue interaction was observed for *GLUT2* expression. At day of hatch, males showed higher expression of *GLUT2* in the duodenum and ileum and at day 7 in the jejunum. However, no difference between males and females was observed at day 14. An increase in the expression of  $b^0, + AT$ , *EAAT3*, *ASCT1*,  $y + nbsp$ ; *LAT2*, and *GLUT2* in males at the day of hatch could lead to increased uptake of amino acids, peptides and glucose into the intestinal enterocytes. Zeng et al. (2011) examined the effect of sex on the expression of different nutrient transporters in embryonic chicks from Wenshi Yellow-Feathered chicks (WYFC) and White Recessive Rock chicks (WRRC). The mRNA abundance of  $b^0, + AT$  was greater in WRRC females than males. In WYFC, the mRNA abundances of *CAT1*, cationic amino acid transporter-4 (*CAT4*), and large neutral amino acid transporter-2 (*LAT2*) were greater in males than females, whereas  $y + nbsp$ ; *LAT2* was greater in females than males. The latter result differs from the results of Kaminski and Wong (2017), which showed greater expression of  $y + nbsp$ ; *LAT2* in males than females. This difference in results could be due to the different breeds used in the 2 studies.

The results from studies aimed at determining the effect of sex on nutrient transporter gene expression are conflicting and therefore, we cannot make a definite conclusion as to whether differences in nutrient absorption between males and females have an effect on the performance differences between the sexes. Presumably, it is true that AA absorption is not limited by the levels of the AA transporters, but the levels of the transporters reflect the efficiency of AA absorption overall. On the other hand, the higher absorption through upregulated AA transporters can affect the upstream digestion of protein or bound AA. In this case, we cannot conclude whether upregulated AA transporters are the reason or result of higher absorption of AA. Another factor to consider is the

effect of FI on absorption rate and in order to accurately determine the effect of sex on nutrient absorption, FI needs to be equal between the sexes. Currently there are no studies that have been performed based on equal FI. Nutrient transporter gene expression is also affected by the interactions between sex and breed as well as the age of the birds and needs to be considered when making comparisons. Most of the differences were reported in very young birds when growth differences between males and females are small and it is therefore difficult to claim that the differences found in nutrient transporter gene expression would have an effect on BW. Furthermore, the roles of nutrient transporter upregulation in young male chicks may need to be investigated to see whether such upregulation contributes to the later growth differences due to the physiological changes of younger birds. More research will need to be conducted in future to better understand the effect of sex on nutrient transporter gene expression taking into account FI, sex and breed interactions, bird age and whether upregulated AA transporters are the reason or result of higher absorption of AA.

## 5. Conclusions

Many studies have shown differences between male and female broiler chickens in terms of growth performance. The underlying reasons for these differences may include differences in nutrient digestibility, gut microbiota population as well as nutrient transporter gene expression. These differences may contribute to increased variation in the results obtained from nutrition-based research trials. Most nutritional experiments are performed using male-only broilers, which can help to reduce variation in the results and ensure a more consistent response. However, the use of male-only birds may introduce possible bias in determining the nutrient requirements of as-hatched broilers, as males only represent half of the birds used in the industry. Practical sexing methods are also needed if single-sex birds are to be used. The potential difference in nutrient requirements between male and female broilers means precision feeding to more accurately meet the requirements of the different sexes which could lead to improved performance. In addition, separate sex rearing may provide a more suitable environment to enhance the performance of each sex. The influence of separate and mixed-sex rearing on the results of nutritional studies needs to be further evaluated. It would be of benefit to obtain more recent results for the modern broiler breeds by determining the physiological and nutritional differences between male and female birds.

## Author contributions

**Ashley England:** Writing – original draft, **Kosar Gharib-Naseri:** Writing – review & editing, **Sarbast Kheravii:** Writing – review & editing, **Shubiao Wu:** Writing – review & editing, Supervision.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

## Acknowledgements

Sustainable Commercial Egg Production Scholarship of The University of New England (UNE) for funding Ashley England.

## References

- Awad EA, Zulkifli I, Farjam AS, Chwen LT, Hossain MA, Aljoubori A. Effect of low-protein diet, gender and age on the apparent ileal amino acid digestibilities in broiler chickens raised under hot-humid tropical condition. *Indian J Anim Sci* 2016;86:696–701.
- Abdullah AY, Al-Beitawi NA, Rjoup MMS, Qudsieh RI, Ishmais MAA. Growth performance, carcass and meat quality characteristics of different commercial crosses of broiler strains of chicken. *J Poultry Sci* 2010;47:13–21.
- Apajalahti J, Vienola K. Interaction between chicken intestinal microbiota and protein digestion. *Anim Feed Sci Technol* 2016;221:323–30.
- Ballou AL, Ali RA, Mendoza MA, Ellis JC, Hassan HM, Croom WJ, Koci MD. Development of the chick microbiome: how early exposure influences future microbial diversity. *Front Vet Sci* 2016;3:2.
- Barnes EM. The intestinal microflora of poultry and game birds during life and after storage. *J Appl Bacteriol* 1979;46:407–19.
- Benyi K, Tshilate TS, Netshipale AJ, Mahlako KT. Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. *Trop Anim Health Prod* 2015;47:1225–31.
- Bjerrum L, Engberg RM, Leser TD, Jensen BB, Finster K, Pedersen K. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. *Poultry Sci* 2006;85:1151–64.
- Brosnan JT, Brosnan ME. Glutamate: a truly functional amino acid. *Amino Acids* 2013;45:413–8.
- Bruijnijns MRN, Blok V, Stassen EN, Gremmen HGJ, Moral “Lock-in” in responsible innovation: the ethical and social aspects of killing day-old chicks and its alternatives. *J Agric Environ Ethics* 2015;28:939–60.
- Castellini C, Mugnai C, Pedrazzoli M, Dal Bosco A. Productive performance and carcass traits of Leghorn chickens and their crosses reared according to the organic farming system. *Proc. Atti XII European Poultry Conference* 2006:10–4. Verona.
- Choi KY, Lee TK, Sul WJ. Metagenomic analysis of chicken gut microbiota for improving metabolism and health of chickens—a review. *Asian-Australas J Anim Sci* 2015;28:1217.
- Chrystal PV, Moss AF, Khoddami A, Naranjo VD, Selle PH, Liu SY. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. *Poultry Sci* 2017;55:939–45.
- Da Costa MJ, Colson G, Frost TJ, Halley J, Pesti GM. Straight-run vs. sex separate rearing for two broiler genetic lines Part 2: economic analysis and processing advantages. *Poultry Sci* 2020;99:2127–36.
- Da Costa MJ, Zaragoza-Santacruz S, Frost TJ, Halley J, Pesti GM. Straight-run vs. sex separate rearing for 2 broiler genetic lines Part 1: live production parameters, carcass yield, and feeding behavior. *Poultry Sci* 2017b;96:2641–61.
- de Albuquerque R, Marchetti LK, Fagundes ACA, Bittencourt LC, da Trindade Neto MA, de Lima FR. Efeito de diferentes densidades populacionais e do sexo sobre o desempenho e uniformidade em frangos de corte. *Braz J Vet Res Anim Sci* 2006;43:581–7.
- Deaton J, Reece F, Kubena L, May J. Rearing broiler sexes separate versus combined. *Poultry Sci* 1973;52:16–9.
- Doeschate RT, Scheele C, Schreurs V, Van Der Klis J. Digestibility studies in broiler chickens: influence of genotype, age, sex and method of determination. *Br Poultry Sci* 1993;34:131–46.
- Doran TJ, Morris KR, Wise TG, O’Neil TE, Cooper CA, Jenkins KA, Tizard MLV. Sex selection in layer chickens. *Anim Prod Sci* 2018;58:476–80.
- Fanatico A, Pillai PB, Emmert J, Owens C. Meat quality of slow-and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. *Poultry Sci* 2007;86:2245–55.
- Fotiadis D, Kanai Y, Palacín M. The SLC3 and SLC7 families of amino acid transporters. *Mol Aspect Med* 2013;34:139–58.
- Galli R, Preusse G, Uckermann O, Bartels T, Krautwald-Junghans M-E, Koch E, Steiner G. Ovo sexing of domestic chicken eggs by Raman spectroscopy. *Anal Chem* 2016;88:8657–63.
- Gehle MH, Powell TS, Arends LG. Effect of different feeding regimes on performance of broiler chickens reared sexes separate or Combined 1,2,3. *Poultry Sci* 1974;53:1543–8.
- Gong J, Si W, Forster RJ, Huang R, Yu H, Yin Y, Yang C, Han Y. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. *FEMS Microbiol Ecol* 2007;59:147–57.
- Goo D, Kim JH, Choi HS, Park GH, Han GP, Kil DY. Effect of stocking density and sex on growth performance, meat quality, and intestinal barrier function in broiler chickens. *Poultry Sci* 2019;98:1153–60.
- Gous R, Moran Jr E, Stilborn H, Bradford G, Emmans G. Evaluation of the parameters needed to describe the overall growth, the chemical growth, and the growth of feathers and breast muscles of broilers. *Poultry Sci* 1999;78:812–21.
- Gous RM. Nutritional and environmental effects on broiler uniformity. *World’s Poultry Sci J* 2017;74:21–34.
- Gruhn K, Zander R. Comparative investigations of the digestion performance between colostomized hens and roosters with wheat and maize. *Arch Geflügelk (Germany, FR)*. 1989.
- Hernandez F, Lopez M, Martinez S, Megias MD, Catala P, Madrid J. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. *Poultry Sci* 2012;91:683–92.
- Hess CW, Dembnicki EF, Carmon JL. Type-of-rearing and location effects on broiler body weights. *Poultry Sci* 1960;39:1086–91.
- Kaleta EF, Redmann T. Approaches to determine the sex prior to and after incubation of chicken eggs and of day-old chicks. *World’s Poultry Sci J* 2008;64:391–9.
- Kaminski NA, Wong EA. Differential mRNA expression of nutrient transporters in male and female chickens. *Poultry Sci* 2017;97:313–8.
- Kanai Y, Cléménçon B, Simonin A, Leuenberger M, Lochner M, Weisstanner M, Hediger MA. The SLC1 high-affinity glutamate and neutral amino acid transporter family. *Mol Aspect Med* 2013;34:108–20.
- Kidd MT, Corzo A, Hoehler D, Miller ER, Dozier WA. Broiler responsiveness (Ross × 708) to diets varying in amino acid density. *Poultry Sci* 2005;84:1389–96.
- Kim E, Corzo A. Interactive effects of age, sex, and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. *Poultry Sci* 2012;91:908–17.
- Krautwald-Junghans M, Cramer K, Fischer B, Förster A, Galli R, Kremer F, Mapesa E, Meisner S, Preisinger R, Preusse G. Current approaches to avoid the culling of day-old male chicks in the layer industry, with special reference to spectroscopic methods. *Poultry Sci* 2017;97:749–57.
- Lamoreux W, Proudfoot F. Effects on body weight and feed conversion of broiler chickens from three commercial crosses reared with the sexes separated or intermingled. *Can J Anim Sci* 1969;49:23–8.
- Lan Y, Verstegen MWA, Tamminga S, Williams BA. The role of the commensal gut microbial community in broiler chickens. *World’s Poultry Sci J* 2005;61:95–104.
- Lang B, Collins W, Palmer D, Skoglund W. Relationship of sex separation to individual variation in body weight and to experimental error. *Poultry Sci* 1960;39:1578–9.
- Laseinde E, Oluyemi J. Effect of sex separation at the finisher phase on the comparative growth performance, carcass characteristics and breast muscle development between male and female broiler chicks. *Niger J Anim Prod* 1994;21:1–18.
- Le Bihan-Duval E, Mignon-Grasteau S, Millet N, Beaumont C. Genetic analysis of a selection experiment on increased body weight and breast muscle weight as well as on limited abdominal fat weight. *Br Poultry Sci* 1998;39:346–53.
- Lee KC, Kil DY, Sul WJ. Cecal microbiome divergence of broiler chickens by sex and body weight. *J Microbiol* 2017;55:939–45.
- Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005;102:11070–5.
- Lopez KP, Schilling MW, Corzo A. Broiler genetic strain and sex effects on meat characteristics. *Poultry Sci* 2011;90:1105–11.
- Lumpkins BS, Batal AB, Lee M. The effect of gender on the bacterial community in the gastrointestinal tract of broilers. *Poultry Sci* 2008;87:964–7.
- Madiilindi MA, Mokobane A, Letwaba PB, Tshilate TS, Banga CB, Rambau MD, Bhebhe E, Benyi K. Effects of sex and stocking density on the performance of broiler chickens in a sub-tropical environment. *S Afr J Anim Sci* 2018;48.
- Mead G. Microbes of the avian cecum: types present and substrates utilized. *J Exp Zool* 1989;252:48–54.
- Mehaffey JM, Pradhan SP, Meulenet JF, Emmert JL, McKee SR, Owens CM. Meat quality evaluation of minimally aged broiler breast fillets from five commercial genetic strains. *Poultry Sci* 2006;85:902–8.
- Moter V, Stein HH. Effect of feed intake on endogenous losses and amino acid and energy digestibility by growing pigs 1,2. *J Anim Sci* 2004;82:3518–25. 2004.
- Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. *Mol Aspect Med* 2013;34:121–38.
- Musigwa S, Morgan N, Swick RA, Cozannet P, Wu S-B. Energy dynamics, nitrogen balance, and performance in broilers fed high-and reduced-CP diets. *J Appl Poultry Res* 2020;29:830–41. 2020.
- Niu Q, Li P, Hao S, Zhang Y, Kim SW, Li H, Ma X, Gao S, He L, Wu W. Dynamic distribution of the gut microbiota and the relationship with apparent crude fiber digestibility and growth stages in pigs. *Sci Rep* 2015;5:9938.
- Olawumi SO, Fagbuauro SS. Productive performance of three commercial broiler genotypes reared in the derived savannah zone of Nigeria. *Int J Agric Res* 2011;6:798–804.
- Org E, Mehrabian M, Parks BW, Shipkova P, Liu X, Drake TA, Lulis AJ. Sex differences and hormonal effects on gut microbiota composition in mice. *Gut Microb* 2016;7:313–22.
- Otsuka M, Miyashita O, Shibata M, Sato F, Naito M. A novel method for sexing day-old chicks using endoscope system. *Poultry Sci* 2016;95:2685–9.
- Peak SD, Walsh TJ, Benton CE, Brake J, Van Horne PLM. Effects of two planes of nutrition on performance and uniformity of four strains of broiler chicks. *J Appl Poultry Res* 2000;9:185–94.
- Phelps P, Bhutata A, Bryan S, Chalker A, Ferrell B, Neuman S, Ricks C, Tran H, Butt T. Automated identification of male layer chicks prior to hatch. *World’s Poultry Sci J* 2003;59:33–8.
- Ravindran V, Wu Y, Hendriks W. Effects of sex and dietary phosphorus level on the apparent metabolizable energy and nutrient digestibility in broiler chickens. *Arch Anim Nutr* 2004;58:405–11.
- Ren C, Sylvia K. Sexual dimorphism in the gut microbiome. *IU J Undergrad Res* 2018;4:12–6.
- Rinttilä T, Apajalahti J. Intestinal microbiota and metabolites—implications for broiler chicken health and performance 1. *J Appl Poultry Res* 2013;22:647–58.
- Samadi, Liebert F. Estimation of nitrogen maintenance requirements and potential for nitrogen deposition in fast-growing chickens depending on age and sex. *Poultry Sci* 2006;85:1421–9.

- Shafey TM, Alodan MA, Hussein EOS, Al-Batshan HA. The effect of sex on the accuracy of predicting carcass composition of Ross broiler chickens. *J Anim Plant Sci* 2013;23:975–80.
- Shim MY, Tahir M, Karnuah AB, Miller M, Pringle TD, Aggrey SE, Pesti GM. Strain and sex effects on growth performance and carcass traits of contemporary commercial broiler crosses. *Poultry Sci* 2012;91:2942–8.
- Siaga R, Baloyi JJ, Rambau MD, Benyi K. Effects of stocking density and genotype on the growth performance of male and female broiler chickens. *Asian J Poultry Sci* 2017;11:96–104.
- Singh P, Karimi A, Devendra K, Waldroup PW, Cho KK, Kwon YM. Influence of penicillin on microbial diversity of the cecal microbiota in broiler chickens. *Poultry Sci* 2013;92:272–6.
- Smith CA, Sinclair AH. Sex determination: insights from the chicken. *Bioessays* 2004;26:120–32.
- Smith DE, Cléménçon B, Hediger MA. Proton-coupled oligopeptide transporter family SLC15: physiological, pharmacological and pathological implications. *Mol Aspect Med* 2013;34:323–36.
- Smith R, Gyles N, Gilbreath J. The influence of producing sexes separately on growth, feed utilization, and dressed grade of broilers. *Proc. Poult Sci* 1954;33:1082–1082.
- Torok VA, Dyson C, McKay A, Ophel-Keller K. Quantitative molecular assays for evaluating changes in broiler gut microbiota linked with diet and performance. *Anim Prod Sci* 2013;53.
- Torok VA, Ophel-Keller K, Loo M, Hughes RJ. Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Appl Environ Microbiol* 2008;74:783–91.
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JL. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–31.
- Udeh I, Ezebor PO, Akporahuarho PN. Growth performance and carcass yield of three commercial strains of broiler chickens raised in a tropical environment. *Growth* 2015;5.
- Weintraut M, Kim S, Dalloul R, Wong E. Expression of small intestinal nutrient transporters in embryonic and posthatch turkeys. *Poultry Sci* 2016;95:90–8.
- Weissmann A, Reitemeier S, Hahn A, Gottschalk J, Einspanier A. Sexing domestic chicken before hatch: a new method for in ovo gender identification. *Theriogenology* 2013;80:199–205.
- Wright EM. Glucose transport families SLC5 and SLC50. *Mol Aspect Med* 2013;34:183–96.
- Wu S, Gharib-Naseri K, England A, Kheravii S. Meat chicken sexing methods and the use of single or mixed-sex chickens in research. 2021.
- Yin Y, Lei F, Zhu L, Li S, Wu Z, Zhang R, Gao GF, Zhu B, Wang X. Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. *ISME J* 2009;4:367.
- Young LL, Northcutt JK, Buhr RJ, Lyon CE, Ware GO. Effects of age, sex, and duration of postmortem aging on percentage yield of parts from broiler chicken carcasses. *Poultry Sci* 2001;80:376–9.
- Yurkovetskiy L, Burrows M, Khan AA, Graham L, Volchkov P, Becker L, Antonopoulos D, Umesaki Y, Chervonsky AV. Gender bias in autoimmunity is influenced by microbiota. *Immunity* 2013;39:400–12.
- Zelenka J, Liska I. Effect of sex and age on amino acids digestibility in a feed mixture. In: *Proceedings 7th European poultry Conference*; 1986.
- Zeng P, Li X, Wang X, Zhang D, Shu G, Luo Q. The relationship between gene expression of cationic and neutral amino acid transporterTs in the small intestine of chick embryos and chick breed, development, sex, and egg amino acid concentration. *Poultry Sci* 2011;90:2548–56.
- Zerehdaran S, Vereijken Aj, Arendonk Jv, Van der Waaij E. Effect of age and housing system on genetic parameters for broiler carcass traits. *Poultry Sci* 2005;84:833–8.