Broiler chicken health, welfare and fluctuating asymmetry in organic versus conventional production systems

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Abstract

The aim of this study was to test three predictions: (1) that a combination of animal-based parameters indicates better health and welfare in broilers from organic versus conventional farms, (2) that broilers from organic farms show less fluctuating asymmetry (FA) than broilers from conventional farms, and (3) that, at the level of the individual bird, the relationship between FA and welfare is negative and strongest in conventional broilers. On 140 slaughter-age birds randomly selected from seven organic and seven conventional flocks in Belgium we measured standardised FA and five conventional animal-based welfare indicators (tonic immobility duration, latency-to-lie, and condition of the foot pad, hock, and breast). The caeca from the birds from four organic and four conventional flocks were removed for assessing the presence of two bacterial pathogens (Salmonella and Campylobacter) and the concentration of the health-promoting lactic acid bacteria. Finally, the blood serum concentration of the acute phase protein alpha-1-acid glycoprotein (AGP), a non-specific indicator of immunological stress, was determined.

Salmonella was found in the caeca content of two (2.5%) birds only (both from the same conventional farm), whereas 44 (55.7%) birds were infected with Campylobacter. The prevalence of Campylobacter, the concentration of lactic acid bacteria, the duration of tonic immobility, and the condition of the breast and foot pad did not differ between both production systems. Apart from a higher concentration of AGP, organic birds had better scores for hock condition and a longer latency-to-lie indicating better leg health. In addition, organic birds scored better on the aggregated welfare index (i.e. the average of the five standardised welfare indicators). As both production systems differed in many aspects (e.g. slower-growing genotypes, slaughter age, feed, stocking density, group size) it is impossible to assign differences in welfare/health indicators to a single factor. Whatever the causes may be our findings suggest that, despite the potentially elevated risk of immunological challenge, broiler chicken welfare is generally superior in organic farms as compared with conventional farms in Belgium. Regarding the validity of FA as welfare indicator, the prediction of lower FA in the population with highest aggregated welfare score was confirmed but, at the level of the individual, no associations between FA and the aggregated welfare index were found irrespective of whether data from organic and conventional broilers were analysed separately or combined.

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1. Introduction

Organic farming has grown rapidly in European agriculture during the last decades. The establishment of organic farming next to conventional intensive farming has been made possible by the willingness of an increasing number of consumers to pay premium prices for organic food and by government subsidies for more sustainable production systems (Bennet, 1996). Apart from environmentally benign production of high quality food, high standards of animal health and welfare have always been an important goal for organic husbandry (Lund and Röcklinsberg, 2001; Vaarst et al., 2004). Moreover, consumers generally associate organic labels with good animal welfare (Harper and Henson, 2001; Miele and Evans, 2005). Nevertheless, there is some scepticism and uncertainty about how effective the animal welfare goal has been realised in organic husbandry partly because of the lack of comparative scientific studies and partly because of the seemingly different view on animal welfare. Indeed, in organic farming, animal welfare is interpreted somewhat differently from what is common in conventional agriculture. Whereas animal welfare is commonly regarded as the physical and emotional state of the animal, in organic farming it is predominantly viewed with regards to naturalness, i.e. animals should be able to express their species-specific natural behaviour (Waiblinger et al., 2004; Lund, 2006). Standards have therefore been enforced so that husbandry and housing are appropriate to each species’ physiological characteristics and natural behaviour. These standards are formulated in the Basic Standards of the International Federation of Organic Agriculture Movements (IFOAM, 2002), and for the European Union in EU Regulations such as No. 1804/1999 (EC, 1999). For example, organic broilers should have access to an outdoor area from the age of six weeks onwards, are reared for 12 instead of six weeks (allowing a more natural speed of growth), and are stocked at a much lower density. Although these standards clearly go beyond legislation for conventional husbandry and provide several preconditions for good living conditions for broilers, they are no guarantee for good animal welfare (Sundrum, 2001). The interactions between housing conditions, the management and the animals are so complex and unpredictable, that the effect of environmental conditions on animal welfare is rarely systematic which implies that it ought to be assessed by measures taken on the animals. Moreover, the emphasis on natural behaviour and integrity of the animals in organic husbandry may conflict with other health (e.g. greater exposure to parasites, predation and changing microclimate in outdoor than indoor systems) and welfare (e.g. possible cold stress in systems with an outdoor run) challenges (Berg, 2001). Restrictions on the use of dietary supplements (vitamins and synthetic amino acids) and of conventional veterinary medicinal inputs, have been identified in a survey to have a potential deteriorating effect on animal welfare (Hovi et al., 2002). Furthermore, Lund (2006) argued that the organics movement’s primary goals deal with ecological sustainability and this ecocentric focus on systems does not provide an obvious basis for animal welfare which deals with the well-being of the individual.

Because neither husbandry standards nor allowing natural behaviour guarantee good welfare in terms of good physical and mental health, both consumers, producers, and certifying organisations would benefit from animal-based assessments of how well the animal welfare goal in organic husbandry is fulfilled in practice (Knierim et al., 2004). However, such data are extremely scant and restricted to parameters of physical health (Lund and Algers, 2003). The first objective of this study is therefore to compare organic versus conventionally reared broiler birds with regards to a wide variety of animal-related health and welfare indicators.

The second objective of this study concerns the investigation of fluctuating asymmetry (FA) as an indicator of animal welfare. FA is the recommended and most commonly used measure of developmental instability (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). It describes random departures from perfect symmetrical development in traits that are genetically coded to be bilaterally symmetric (Palmer, 1994). The magnitude of these departures is thought to be a reflection of the failure of the organism to maintain developmental homeostasis resulting from an inability to counter the effects of genetic and environmental stressors. It follows that the magnitude of these departures might be an objective, integrated, and animal-based measure of animal welfare as has been suggested by Møller et al. (1995, 1999). Others, however, have pointed out that the empirical evidence for this claim is unsatisfactory and sometimes inconsistent (Campo et al., 2000, 2002; Tuyttens, 2003). Recently, several authors have argued that at least some of these inconsistencies might be due to low statistical power and the use of inadequate methodologies for measuring and analysing FA (Tuyttens, 2003; Van Nuffel et al., submitted for publication; Knierim et al., in press). For example, FA measured in single traits only very weakly reflects developmental
instability which biases estimates of associations between FA and other welfare variables downwards (Whitlock, 1996, 1998). In many studies, real FA was not separated from measurement error or from other types of bilateral asymmetry such as directional asymmetry or antisymmetry which have a genetic basis and no a priori definable optimal state (Palmer and Strobeck, 1986, 1992). Not only estimating FA may be problematic, the independent validation of whether the presumed stress treatment is truly deleterious for the welfare of the animals is inadequate or even, lacking in many studies. Other studies, with analyses at the level of the individual rather than the population, have only investigated associations between FA and single welfare indicators such as duration of tonic immobility, intensity of tibial dyschondroplasia, or gait quality. Although there is consensus amongst researchers that in order to assess animal welfare a variety of welfare indicators should be used, the association between a composite measure of FA and an aggregated index of welfare has never been investigated to our knowledge. Finally, even if FA and welfare are measured precisely and accurately on an appropriate number of individuals, a significant negative correlation between both variables may only be expected when a sufficient amount of developmental stress differentiates individuals that can cope with their living conditions from those that cannot (Palmer, 1994; Leung and Forbes, 1997; Lens et al., 2002; Tuyttens et al., 2005).

Apart from comparing a variety of animal-based health and welfare indicators between organic and conventional birds, the aim of this study is to test two predictions in order to validate FA as an indicator of broiler chicken welfare. At the population level we predict that if an aggregated welfare index (based on various conventional broiler chicken welfare indicators) indicates a difference in welfare between organic versus conventional birds, the group with highest welfare will have lowest composite FA. At the level of the individual bird, we predict that the relation between composite FA and the aggregated welfare index is negative and strongest in the population with poorest welfare.

2. Methods

2.1. Indicators of health and welfare

From October 2003 until April 2004, seven organic and seven conventional flocks from in total 12 different Belgian farms were sampled (Table 1). Organic broiler farms differed markedly from conventional broiler farms with respect to several management and housing conditions (Table 1). For example, flock size was much smaller in organic farms ($\bar{x} = 4179$ broilers, SD = 817 broilers) as compared to conventional farms ($\bar{x} = 18891$, SD = 2886 broilers). Stocking density (indoor) was also much lower in organic farms ($\bar{x} = 10.0$, SD = 1.1 broilers/m$^2$) than in conventional farms ($\bar{x} = 18.9$, SD = 1.2 broilers/m$^2$). Birds were slaughtered, and hence, collected for our measurements at a much older age at organic ($\bar{x} = 80.6$, SD = 1.5 days) than at conventional farms ($\bar{x} = 40.6$, SD = 1.1 days). All conventional farms reared Cobb broilers, while most organic farms used the Kabir hybrid. The Kabir hybrid is a slow-growing hybrid weighing ca. 2 kg at day 81 (Castellini et al., 2002), selected in Israel for better resistance to environmental stress and disease and better adaptation to tough conditions (see www.kabir.co.il). One organic farm, however,

<table>
<thead>
<tr>
<th>Farm</th>
<th>Type</th>
<th>Date of visit</th>
<th>Hybrid</th>
<th>Flock size</th>
<th>Area barn (m$^2$)</th>
<th>Stocking density (birds/m$^2$)</th>
<th>Age of sample (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td>Conventional</td>
<td>29-09-2003</td>
<td>Cobb</td>
<td>22,580</td>
<td>1280</td>
<td>17.6</td>
<td>39</td>
</tr>
<tr>
<td>Farm B</td>
<td>Organic</td>
<td>13-10-2003</td>
<td>Kabir</td>
<td>4300</td>
<td>430</td>
<td>10.0</td>
<td>80</td>
</tr>
<tr>
<td>Farm C</td>
<td>Conventional</td>
<td>20-10-2003</td>
<td>Cobb</td>
<td>18,990</td>
<td>980</td>
<td>19.4</td>
<td>42</td>
</tr>
<tr>
<td>Farm D (flock 1)</td>
<td>Conventional</td>
<td>28-10-2003</td>
<td>Cobb</td>
<td>14,900</td>
<td>850</td>
<td>17.5</td>
<td>41</td>
</tr>
<tr>
<td>Farm E</td>
<td>Conventional</td>
<td>30-10-2003</td>
<td>Cobb</td>
<td>19,980</td>
<td>1000</td>
<td>20.0</td>
<td>41</td>
</tr>
<tr>
<td>Farm F (flock1)</td>
<td>Organic</td>
<td>10-11-2003</td>
<td>Kabir</td>
<td>4800</td>
<td>480</td>
<td>10.0</td>
<td>81</td>
</tr>
<tr>
<td>Farm G</td>
<td>Organic</td>
<td>17-11-2003</td>
<td>Cobb</td>
<td>17,785</td>
<td>860</td>
<td>20.7</td>
<td>41</td>
</tr>
<tr>
<td>Farm H</td>
<td>Organic</td>
<td>15-12-2003</td>
<td>Kabir</td>
<td>4800</td>
<td>480</td>
<td>10.0</td>
<td>81</td>
</tr>
<tr>
<td>Farm I</td>
<td>Organic</td>
<td>11-02-2004</td>
<td>Kabir</td>
<td>4300</td>
<td>430</td>
<td>10.0</td>
<td>83</td>
</tr>
<tr>
<td>Farm D (flock 2)</td>
<td>Conventional</td>
<td>16-02-2004</td>
<td>Cobb</td>
<td>16,000</td>
<td>840</td>
<td>19.0</td>
<td>39</td>
</tr>
<tr>
<td>Farm F (flock2)</td>
<td>Organic</td>
<td>01-03-2004</td>
<td>Kabir</td>
<td>4300</td>
<td>480</td>
<td>9.0</td>
<td>81</td>
</tr>
<tr>
<td>Farm J</td>
<td>Conventional</td>
<td>01-03-2004</td>
<td>Cobb</td>
<td>22,000</td>
<td>1200</td>
<td>18.3</td>
<td>41</td>
</tr>
<tr>
<td>Farm K</td>
<td>Organic</td>
<td>18-03-2004</td>
<td>Non-commercial</td>
<td>4350</td>
<td>500</td>
<td>8.7</td>
<td>78</td>
</tr>
<tr>
<td>Farm L</td>
<td>Organic</td>
<td>22-03-2004</td>
<td>Kabir</td>
<td>2400</td>
<td>200</td>
<td>12.0</td>
<td>80</td>
</tr>
</tbody>
</table>

Salmonella, Campylobacter, lactic acid bacteria and AGP were determined for farms printed in italics only.
used a not commercially available genotype based on a parental stock of Hubbard JA57 hens mated with a New Hampshire cock with the aim of achieving 2.4 kg slaughter-weight.

From each flock 10 slaughter-age broilers were randomly selected and transported to a test arena at the ILVO research institute. The following five conventional welfare indicators were measured or scored on each chicken before they were euthanized by cervical dislocation: (1) duration of tonic immobility as a test of fear (the longer the more fearful; Jones, 1987), (2) latency-to-lie in shallow water as a test of leg-condition (the shorter the worse the ability to carry its bodyweight; Berg and Sanotra, 2002), (3) condition of foot pad, (4) condition of hock, and the (5) condition of breast. The latter three conditions were scored — according to the methodology refined and agreed upon by the Provincial Centre for Applied Poultry Research (Geel, Belgium) and some other European poultry research stations — on a four-point scale (score 0 = very good condition, score 3 = very bad condition). The scoring system has been described in detail by De Sutter and Vervaet (2005). The relevancy of lesions on the foot pad, hock and breast for the welfare of broiler chickens has been confirmed by Berg (2004) and Mayne (2005).

An aggregated index of welfare was calculated as follows. Each score or measurement was standardised such that its mean=0 and SD=1, so that each score was given equal weight in multiple-trait analyses. The sign of the standardised latency-to-lie measures was reversed so that each measure was negatively related to welfare. Subsequently, these scores were summed and divided by five.

Additional health parameters were quantified for birds from four organic and four conventional flocks (Table 1). The caeca from the birds (n=80) were removed within 24 h after slaughtering from the intestinal packages. They were kept on ice for the determination of the absence/presence of two bacterial pathogens (Salmonella and Campylobacter) and of the concentration of a health-promoting bacterial group, namely lactic acid bacteria. The removed caeca were exteriorly disinfected with ethanol and the content collected in tubes, put on ice and stored at −80 °C until further analysis. For the analysis of the presence of Salmonella, 0.5 g of caecal content was homogenised (1–5 min) with a stomacher in 9.5 ml buffered peptone water. This homogenate was further processed for Salmonella analysis and confirmation as described previously (Heyndrickx et al., 2002). For the analysis of the presence of Campylobacter, 1 ml of the caecal homogenate was diluted in 9 ml Preston broth and processed as described previously (Herman et al., 2003).

For the enumeration of lactic acid bacteria, 1 g of caecal content was serially diluted in 9 ml autoclaved dilution buffer (1 g/l bacteriological agar, 0.5 g/l cysteine, HCl, 4.5 g/l KH₂PO₄, 6 g/l Na₂HPO₄ and 1 ml/l Tween 80) and appropriate dilutions spiral plated (Eddy Jet IUL Instruments) on MRS (Oxoid) plates acidified with sodium acetate (pH 6.2). After incubation in an anaerobic chamber (Bugbox, Ruskin Technology Ltd.) at 37 °C for 3 days, the total lactic acid bacteria count (in cfu/g caecum sample) was determined. Finally, the blood serum concentration of the acute phase protein alpha-1-acid glycoprotein (AGP) was determined. Blood samples (approximately 2 ml) of individual birds were collected in serum tubes, centrifuged for 10 min at 2700 g at 4 °C and the supernatant was stored at −20 °C until testing. The AGP level was determined using a commercial Single Radial Immunodiffusion Plate kit (Cardiotech Services Inc., Louisville, KY), following the instructions of the manufacturer.

The five welfare indicators as well as the aggregated welfare index and the additional health parameters were compared between the two production systems using two way mixed effects ANOVA, with treatment as fixed effect and farm (nested within treatment) as random effect. Health parameters were ln-transformed to obtain normality of residuals and additivity of effects. For the prevalence of Campylobacter, comparisons between the two production systems were performed by a logistic two-way ANOVA model with treatment and sex as factors. We obtained robust estimates correcting for independence of data within farms using Generalised Estimation Equations in proc GENMOD in SAS (Littell et al., 1996). Results are presented on a logit scale.

2.2. Fluctuating asymmetry

For estimating FA, birds were slaughtered and both sides of the following 13 presumed bilateral symmetric traits on 70 intact carcasses were measured twice to the nearest 0.01 mm using a digital calliper: wattle length, beak length, eye length, nostril length, nostril — auditory canal length, radius length, mid toe (3rd phalanx) length, outer toe (4th phalanx) length, back toe length, tarsometatarsus length, tarsometatarsus width at spur, tarsometatarsus width 1 cm above the spur, tarsometatarsus width at the joint with the tibiotarsus. For full details and illustration about these measurements we refer to Van Nuffel et al. (submitted for publication) and http://www.ilvo.vlaanderen.be/documents/fluctasbroilers.pdf.

A reduced number of traits was measured twice on the subsequent 70 carcasses. The most suitable
combination of morphological traits for estimating the degree of FA was selected on the basis of the following criteria (see Van Nuffel et al., submitted for publication): (i) a high signal : noise ratio (or FA : measurement error), (ii) a significant amount of fluctuating asymmetry, (iii) absence of directional asymmetry, (iv) absence of antisymmetry, and (v) absence of correlations in the signed FA values. We carried out mixed regression analysis with REML parameter estimation to separate real asymmetry (signed FA; left minus right trait length) from measurement error (Van Dongen et al., 1999). The significance of FA was obtained from a likelihood ratio test while directional asymmetry was tested by an F-test, adjusting the denominator degrees of freedom by Satterthwaite’s procedure (Van Dongen et al., 1999). Whereas FA and antisymmetry cannot be separated statistically with high power, distributions of FA with a negative kurtosis are generally considered indicative for the presence of antisymmetry (Palmer and Strobeck, 1992; Van Dongen, 1998). Unlike FA, directional asymmetry and antisymmetry are considered inappropriate for the estimation of the underlying developmental instability, due to their presumed heritable component (Palmer and Strobeck, 1992; Palmer, 1994). To examine whether signed trait asymmetries were correlated at the individual level, we calculated unbiased individual FA estimates (i.e. deviations of the random slopes from the fixed effects slope in the mixed regression model; Van Dongen et al., 1999) and estimated individual-level association with Pearson’s correlation coefficients. Between-trait correlation for signed FA values would suggest that these traits developed dependently during ontogeny, which may hamper proper interpretation of between-trait correlations in unsigned FA (Leamy et al., 1997).

Levels of asymmetry were compared between the two production systems using a mixed model approach, combining all traits that met the predefined criteria. Production system, sex and their interaction were treated as fixed effects, while farm (nested within production system) and trait were added as random effects (including the trait×farm and trait×production system interaction). For 20 broilers, the sex was not determined. We therefore, treated sex as a continuous factor where males were set equal to 0.5 and females equal to −0.5. Broilers of which sex was unknown were set equal to zero. In this way, the measurements of these 20 individuals could be included at each stage of the analysis. Since within each individual repeated measures on different traits were combined in a single analysis, correlations in the residual values were also incorporated in the model. Model selection started with the residual variance–covariance structure (based on the Bayesian Information Criterion comparing an ‘unstructured’, ‘compound symmetry’ and ‘variance component only’ correlation structure), after which the random effects structure was tested using likelihood ratio tests. Fixed effects were tested using F-tests. In order to avoid problems of pseudo-replication, the random effect farm was retained in the model regardless of its statistical significance such that the test of the fixed production system effect was based on the appropriate denominator mean squares and degrees of freedom.

The associations between average individual FA (standardised) and the aggregated welfare index were analysed using Spearman rank correlations. In these analyses data were either combined or analysed separately for organic and conventional farms.

### Table 2
Test statistics comparing health and welfare indicators between broiler birds from conventional versus organic production systems

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Estimate (SE) organic</th>
<th>Estimate (SE) conventional</th>
<th>dF</th>
<th>Test statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency-to-lie (s)</td>
<td>547 (39)</td>
<td>256 (39)</td>
<td>10</td>
<td>t = 5.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tonic immobility (s)</td>
<td>108 (30)</td>
<td>182 (30)</td>
<td>10</td>
<td>t = −1.72</td>
<td>0.116</td>
</tr>
<tr>
<td>Condition breast</td>
<td>0.42 (0.12)</td>
<td>0.62 (0.11)</td>
<td>10</td>
<td>t = −1.27</td>
<td>0.234</td>
</tr>
<tr>
<td>Condition hock</td>
<td>0.30 (0.18)</td>
<td>1.64 (0.18)</td>
<td>10</td>
<td>t = −5.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Condition foot pad</td>
<td>1.03 (0.27)</td>
<td>1.58 (0.27)</td>
<td>10</td>
<td>t = −1.43</td>
<td>0.183</td>
</tr>
<tr>
<td>Aggregated welfare index</td>
<td>0.33 (0.10)</td>
<td>−0.36 (0.10)</td>
<td>10</td>
<td>t = 4.73</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lactic acid bacteria (ln(conc.))</td>
<td>6.55 (0.24)</td>
<td>5.99 (0.24)</td>
<td>10</td>
<td>t = 1.51</td>
<td>0.136</td>
</tr>
<tr>
<td>AGP* (ln(conc.))</td>
<td>6.21 (0.11)</td>
<td>5.45 (0.11)</td>
<td>4</td>
<td>t = 4.65</td>
<td>&lt; 0.010</td>
</tr>
<tr>
<td>Campylobacter (logit prevalence)</td>
<td>0.00 (0.92)</td>
<td>−0.13 (0.82)</td>
<td>z</td>
<td>z = 0.11</td>
<td>0.912</td>
</tr>
</tbody>
</table>

* AGP is the acute phase protein alpha-1-acid glycoprotein.

3. Results

3.1. Indicators of health and welfare

Birds from organic farms had a longer latency-to-lie and better scores for hock condition as compared to birds from conventional farms (Table 2). Duration of tonic immobility, condition of the breast, and condition of the foot pad tended to be better among organic broilers than among conventional broilers but the differences were not statistically significant. The aggregated welfare index was highly significantly different between birds from both production systems, with birds from organic farms having better welfare than birds from conventional farms (Table 2).
Salmonella was found in the caeca content of two (2.5%) birds only (both from the same conventional farm), whereas 44 (55.7%) birds were infected with Campylobacter. The occurrence of Campylobacter did not differ between both production systems, nor did the concentration of lactic acid bacteria in the caecum. However, as compared to conventionally produced birds, organic birds had a higher concentration of AGP (Table 2).

3.2. Fluctuating asymmetry

Based on the measurements on the first 70 carcasses, nine of the 13 traits were found to be unsuitable for estimating FA (Table 3). Three traits exhibited directional asymmetry: wattle length ($F_{1,9}=27.75$, $P<0.001$), backtoe length ($F_{1,9}=18.02$, $P=0.002$), and radius length ($F_{1,9}=18.02$, $P=0.012$). Another five traits (eye length, nostril length, nostril — auditory canal length, midtoe length, and tarsometatarsus width 1 cm above the spur) were discarded because of a negative kurtosis value indicative of antisymmetry. Tarsometatarsus width at the joint was also removed because it was correlated in its signed FA with tarsomatatarsus width at spur. Hence only the four remaining traits (beak length, tarsometatarsus length at spur, and outertoe length) were measured in the subsequent 70 chicken carcasses. Based on the full dataset on 140 birds it was checked once again whether all traits had signed asymmetries with a normal distribution and a mean of zero, thus exhibiting true FA. One more trait (outertoe length) was discarded because it exhibited directional asymmetry ($F_{1139}=7.20$, $P=0.008$). Hence the statistical analyses were based on three traits. Based on the measurements of all 140 birds, repeatability of the true FA estimates was low for the three traits (tarsometatarsus length: 55.1%, tarsometatarsus width at spur: 29.7%, beak length: 62.2%).

<table>
<thead>
<tr>
<th>Trait</th>
<th>FA:ME</th>
<th>DA</th>
<th>AS</th>
<th>Signal: (P-value)</th>
<th>Correlations (P-value) (kurtosis) noise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beak length</td>
<td>0.002</td>
<td>&gt;0.05</td>
<td>0.308</td>
<td>1.699</td>
<td></td>
</tr>
<tr>
<td>TMT length</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>0.421</td>
<td>1.160</td>
<td></td>
</tr>
<tr>
<td>TMT width at spur</td>
<td>0.033</td>
<td>&gt;0.05</td>
<td>1.743</td>
<td>0.549</td>
<td></td>
</tr>
<tr>
<td>TMT width at joint</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>0.359</td>
<td>0.333</td>
<td></td>
</tr>
<tr>
<td>Outer toe length</td>
<td>0.002</td>
<td>&gt;0.05</td>
<td>4.832</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>TMT width at 1 cm above spur</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>-0.669</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midtoe length</td>
<td>0.003</td>
<td>&gt;0.05</td>
<td>-0.102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye length</td>
<td>0.029</td>
<td>&gt;0.05</td>
<td>-0.102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nostril — auditory canal length</td>
<td>0.031</td>
<td>&gt;0.05</td>
<td>-0.451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nostril length</td>
<td>0.002</td>
<td>&gt;0.05</td>
<td>-0.497</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backtoe length</td>
<td>0.003</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wattle length</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius length</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Firstly, we tested which traits showed a significant amount of FA ($P<0.05$). Subsequently, traits with a significant amount of directional asymmetry (DA, $P<0.05$) and traits with a negative kurtosis value, indicating antisymmetry (AS), were removed. Finally, the remaining traits were ranked according to their signal-to-noise ratio and traits correlated ($P<0.05$) in their signed FA value with a higher ranked trait were discarded (such correlations are shown with a solid line). Traits meeting all four selection criteria are in bold.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Procedure to select the most suitable combination of traits for estimating fluctuating asymmetry (FA) based on measurements of 70 carcasses</th>
</tr>
</thead>
</table>

A summary of the model selection for the mixed model comparing levels of asymmetry between production systems is provided in Table 4. There appeared to be no correlation in the residual asymmetries since both the unstructured and compound symmetry correlation structures did not improve the model fit significantly over a variance component only structure where the between-
trait correlation is assumed to be zero. We therefore continued our analyses assuming no correlation. Nevertheless, the results on random and fixed effects appeared very similar with each of the three correlations structures relevant here. There was no variation in asymmetry among farms nested within production system, nor was there any indication of an interaction between trait and both production system or farm. The between-trait variation was highly significant. Asymmetry in broilers from organic farms was on average about 0.05 mm lower compared to conventional ones, and this effect was statistically significant (Table 4).

Individual asymmetry, based on all three traits, was not significantly associated with the aggregated welfare index. This was true for the combined data ($r=0.09, P=0.322$) as well as for the organic ($r=0.02, P=0.903$) and conventional ($r=-0.02, P=0.857$) systems separately.

4. Discussion

The latency-to-lie test as well as the assessments of hock condition indicate that organic broilers suffered less from leg problems as compared to broilers from conventional farms. Bestman and Maurer (2006) reported that the use of slow-growing hybrids has reduced skeletal lesions in organic broilers and such hybrids usually remain mobile during the whole fattening period (see also Kestin et al., 2001). In an experiment comparing two genotypes in combination with three different ground types in an organic housing system, Bassler (2005) also found that leg condition was worse in the fast-growing genotype as compared to the slow-growing genotype. Tonic immobility duration, breast condition and foot pad condition did not differ significantly between organic versus conventional birds. These five commonly used animal-based indicators of broiler welfare combined into an aggregated welfare index suggest better overall welfare in organic than conventional production systems. The difference in FA – a novel but promising integrated measure of animal welfare (Moller et al., 1999) – also suggests better welfare among broilers from organic than conventional farms.

Having access to an outdoor run, living twice as long, and prophylactic medicines being restricted, we expected broilers from organic farms to be at an increased risk for some infections and diseases. Indeed, the serum concentration of AGP – a major acute phase protein in poultry and a non-specific indicator of inflammation (Chamanza et al., 1999) – was higher in the organic birds than in the birds from the conventional farms. However, the difference between the AGP level observed in broilers from organic versus conventional farms – albeit statistically significant – is possibly too small to conclude that organic birds experience more immunological stress, as the concentration of AGP may increase several fold in cases of inflammation (Chamanza et al., 1999). Acute phase proteins are part of the complement system, which is part of the innate immune system that protects the body against infections. An alternative explanation, therefore, is that slower-growing chicken lines (such as those used in organic farming) allocate relatively more energy on health and longevity than on growth and reproduction in comparison with fast-growing chicken strains. The higher level of AGP observed in organic broilers and such hybrids usually remain mobile during the whole fattening period (see also Kestin et al., 2001). In an experiment comparing two genotypes in combination with three different ground types in an organic housing system, Bassler (2005) also found that leg condition was worse in the fast-growing genotype as compared to the slow-growing genotype. Tonic immobility duration, breast condition and foot pad condition did not differ significantly between organic versus conventional birds. These five commonly used animal-based indicators of broiler welfare combined into an aggregated welfare index suggest better overall welfare in organic than conventional production systems. The difference in FA – a novel but promising integrated measure of animal welfare (Moller et al., 1999) – also suggests better welfare among broilers from organic than conventional farms.

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goal of improved animal welfare also appears to be fulfilled in practice despite the potentially elevated risk of immunological challenge. The question of which (combination of) husbandry or management aspects are responsible for this welfare improvement, is not truly relevant for the objective of our observational study, namely to compare two entire production systems as they are currently practiced in Belgium. The use of different hybrids (slow-growing), the higher slaughter age, the reduced stocking density, the smaller flock size, the access to an outdoor area are all an inherent part of the organic broiler production system. These factors by definition co-vary with treatment because they determine whether or not the system can be classified as organic. Consequently, it cannot be confirmed, nor falsified, whether the observed differences in welfare status between both production systems were caused by a single factor. For example, perhaps the observed differences were related to the fact that slower-growing hybrids are used in organic systems whereas fast-growing hybrids are used in conventional systems. If so, this would not invalidate our conclusion as the use of slower-growing hybrids is one of the very features that differentiates commercial organic broiler production systems from conventional ones. Because of the same reason, it would be flawed to test our hypothesis by comparing conventional production systems with organic systems in which fast-growing strains are used since this is not common practice in reality.

Concerning the validity of FA as an integrated indicator of animal welfare, our results are not conclusive. At the population level, we found that FA in broilers from the organic production systems was lower than in broilers from conventional systems. While this agrees with the prediction that FA is lowest in the population of broilers with highest welfare, the possibility cannot be excluded that this difference is caused by a confounding factor. For example, it cannot be ruled out that FA is lowest in organic broilers because slower-growing strains are used which have been reported to have lower FA than fast-growing strains (Møller et al., 1995; Yalçin et al., 2001). It is not known whether this difference between strains is purely genetic or is related to the fact that slow-growing strains usually suffer fewer welfare problems than fast-growing strains (Rodenburg et al., 2004b; van Horne et al., 2004).

At the level of the individual, we found no association between FA and the aggregated welfare index irrespective of whether data from organic and conventional broilers were analysed separately or combined. Hence, our second prediction that the relation between composite FA and the aggregated welfare index is negative and strongest in the population with poorest welfare was not confirmed by the present study. It should be noted, however, that as fluctuating asymmetry is a measure of variation, it is less reliable as an indicator of underlying developmental instability at the level of the individual than at population level. At the level of the individual, single trait FA is a variance measure based on two data points only and therefore unreliable. As there is insufficient evidence about which traits are most sensitive to stress, the power of individual based analyses can only be increased by measuring FA of many traits on many individuals. In our study however only three of the 13 traits could be retained for the analyses, which is probably insufficient for documenting a relationship between individual FA and welfare.

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