The value of a retrospective analysis of slaughter records for the welfare of broiler chickens

Xavier Averós,*,† Bernardino Balderas,† Enrique Cameno,* and Inma Estevez*,†,1

*Department of Animal Production, NEIKER-Basque Institute for Agricultural Research and Development, Basque Research and Technology Alliance (BRTA), Arkaute, Spain, 01192; †Servicio Andaluz de Salud, Granada, Spain, 18016; and 1IKERBASQUE, Basque Foundation for Science, Bilbao, Spain, 48013

ABSTRACT The effects of transport risk factors on deaths on arrival (DOAs; %) and carcass rejections (%) on broiler chickens transported to a slaughterhouse in Southern Spain were assessed using information routinely collected at the slaughterhouse. A total of 2,284 flocks and 10,198,663 broiler chickens, transported in 2,103 commercial transports from 217 different farms to a single slaughterhouse, were studied using the information of veterinary service and slaughter records. Studied risk factors were transport condition at arrival score (good/bad) and plumage condition score (good/bad) at arrival at the slaughterhouse, mixing loads (yes/no), transport duration (min), and season of the year (spring/summer/autumn/winter). Generalized linear mixed models were used to determine the effects of risk factors and their interactions on DOA and carcass rejections. The interactive nature of risk factors affecting both variables was revealed. Mixing loads interacted with transport duration ($P = 0.0001$), amplifying the negative consequences of long transports on DOA. Mixing loads also interacted with transport conditions at arrival ($P < 0.0005$), with the impact of bad transport conditions at arrival being particularly negative in the case of mixed loads. These facts raise questions about the suitability of mixing loads both from the animal welfare and economic standpoints. Transport duration interacted with season ($P = 0.0003$), with the detrimental effect of long distances on DOA being particularly evident during summer and winter, which highlights the need for alternative management measures when hot or cold temperatures are foreseen during transport. Long transports also increased carcass rejections ($P < 0.0001$). Mixing loads and bad plumage condition exacerbated the overall, detrimental effect of autumn transports on DOA ($P = 0.0084$ and $P = 0.0009$, respectively) with respect to summer transports. Similar results were obtained for the interactions between mixing loads and transport season ($P = 0.0043$) and transport conditions at arrival and transport season ($P = 0.0014$) on carcass rejections. Overall, results highlight the value of slaughter records to produce information useful to reduce the impact of transport risk factors, improve broiler chicken welfare, and improve slaughterhouse economic results.

Key words: broiler chicken, DOA, mixed load, transport, welfare

INTRODUCTION

World broiler chicken production was estimated at $2.4 \times 10^{10}$ birds in 2018 (FAOSTAT, 2020), representing 76% of the total live animals in production. This reflects the importance of poultry for world population. Transport to slaughter is a critical step in the chicken production chain that may have negative consequences on bird welfare if associated management and handling procedures are inadequate (Mitchell and Kettlewell, 2009). As mentioned by Schwartzkopf-Genswein et al. (2012), animal welfare concerns include the potential for animals to experience stress, injury, fatigue, mortality, and morbidity due to transportation aspects such as poor handling and exposure to variable climatic conditions or mixing flocks from different farms. Poor transport conditions have obvious negative effects on bird welfare and will, most likely, translate into increased deaths on arrival (DOAs; Bayliss and Hinton, 1990; Warriss et al., 1992; Jacobs et al., 2017a) and increase carcass rejections (Nijdam et al., 2004). Therefore, knowledge on how to control for risk factors will lead to improved welfare conditions and increased economic profit for all actors in the broiler chicken production chain.
DOA and carcass rejections may be the result of poor transport conditions but may also originate during the pretransport history of broiler chickens (Haslam et al., 2008). A clear relationship exists between on-farm welfare conditions and slaughter problems, shown by the correlation between on-farm mortality and DOA that different studies have described (Haslam et al., 2008; Chauvin et al., 2011; Whiting et al., 2007; BenSassi et al., 2019). Therefore, assessment at slaughter may offer valuable information about prior on-farm welfare conditions (Grilli et al., 2015). Broiler chickens’ on-farm health and welfare depends, among others, on bedding conditions and more specifically on bedding water content (Dawkins et al., 2004). Wet litter will result in plumage dirtiness, which is highly correlated with DOA and carcass rejections at slaughter houses (De Jong et al., 2014; Jacobs et al., 2017b; BenSassi et al., 2019). Given that plumage dirtiness is also highly correlated with plumage condition at the end of transport (Jacobs et al., 2017b), the latter visual indicator can be proposed as a reliable estimator of the broiler chicken pretransport welfare status.

Regarding transport conditions, duration is a well-known welfare risk factor in all production species. Long transports are stressful for broiler chickens (Zhang et al., 2009) and may lead to increased DOA (Bayliss and Hinton, 1990; Warriss et al., 1992; Vecerek et al., 2006; Whiting et al., 2007; Chauvin et al., 2011; Vieira et al., 2011; Caffrey et al., 2017). Long transport durations will also exacerbate the negative consequences that poor transport departing conditions, such as those caused by inadequate bird catching and loading at the farm, have on DOA (Jacobs et al., 2017b). Climatic conditions during transport are also critical for broiler chickens, as both high temperatures, particularly in combination with high relative humidity, and low temperatures will result in thermal stress and lead to increased DOA and carcass bruising (Nijdam et al., 2004; Schwartzkopf-Genswein et al., 2012). The detrimental consequences of thermal conditions above or below thermoneutrality will additionally be amplified during long transports (Nijdam et al., 2004). Beyond temperature, other adverse weather conditions affecting DOA are wind and rain (Chauvin et al., 2011). In consequence, season of the year, through a combined action of previously mentioned climatic variables, will have a large influence on the welfare of birds as they are transported to slaughter. Different studies have described higher DOA during summer and winter (Petracci et al., 2006; Vecerek et al., 2006; Haslam et al., 2008), although no consensus exists regarding the influence of low temperatures (Warriss et al., 2005), and the same has also been described for carcass bruising (Nijdam et al., 2004). Bird dirtiness differs according to season (Wilkins et al., 2003) and is also associated to weather conditions during transport, with rain particularly (Jacobs et al., 2017b).

Mixing loads is a relatively common practice during commercial transportation to slaughter houses in Spain, that consists in loading animals from different farms into the same truck. This is done to optimize truck occupation and transport costs but may be negative from a health and welfare perspective. In slaughter pigs, mixing loads has been found to increase the percentage of carcass problems and to exacerbate the negative impact of longer distances on mortality at the end of transport (Gosilve et al., 2006). A similar effect might be expected during broiler chicken transport to slaughter, but no information exists up to date on the impact of mixing loads and how this interacts with other risk factors.

All the aforementioned information is routinely collected during veterinary inspections at the end of transport or can be easily extracted from slaughterhouse records. These data are often overlooked and not sufficiently exploited by industry. Therefore, a retrospective analysis of risk factors contained in slaughter records may be helpful to reveal relationships between them and how they may impact the welfare of broilers assessed on the basis of DOA and carcass rejections. This will result in relying on valuable information to base technical decisions with benefits for both broiler chicken welfare and the economic return of the production chain, ultimately providing these data with an extra added value.

The aim of this study was to determine the influence of transport condition and plumage condition scores at arrival at the slaughterhouse, mixing loads, transport duration, and season of the year on DOA and carcass rejections in broiler chickens transported to a slaughterhouse in Southern Spain, based on the information routinely collected by the official veterinarians at the slaughterhouse.

**MATERIALS AND METHODS**

Information on 2,103 commercial transports, carrying 2,284 flocks and 10,198,663 broiler chickens from 217 different farms to a single slaughterhouse located in Southern Spain, was collected by the slaughterhouse official veterinary inspection service as part of their routine job. Transports were all carried out during 2017, following the requirements of the Council Regulation (EC) No 1/2005 relative to the protection of animals during transport. At the farm, the loading procedure was similar for all transports. Inside the farm, broilers were caught and introduced into plastic crates. Once complete, plastic crates were placed in metal modules, which were loaded onto the truck. Truck capacity was 10 metal modules and 18 plastic crates/modules. Upper protective canvas tops were used in all transports, while hauliers were asked to use side protective canvas tops in case of cold and/or rain. Upon arrival at the slaughterhouse, broiler chickens waited for 4 h on average before slaughter.

Slaughter records were collected manually and recorded on paper. For the purpose of this study, records were transferred to a database. Each transport was individually identified, and farms were given a unique code to be able to identify different transports from a single farm within the year. In case collected information for a specific transport was unclear, missing or erroneous, or reported unusually high DOA and/or carcass rejection values attributable to reasons other than transport,
it was discarded. After filtering information, 2,154 and 1,649 flocks were finally available for further analysis and development of DOA and carcass rejections models, respectively. For each transported flock, information on transport duration (minutes between the first chicken was loaded onto the truck until the last chicken was unloaded at the slaughterhouse) and transport season (spring, summer, autumn, winter) was collected. Mean temperature and relative humidity during 2017 in the transport area were 18.9°C and 42.4% in spring, 24.1°C and 40.3% in summer, 11.0°C and 63.3% in autumn, and 8.5°C and 65.7% in winter. Mixed loads were identified by a 2-level variable according to whether the truck transported 1 or 2 flocks. The transport conditions of each flock were visually scored, from outside the truck, by official veterinarians at arrival at the slaughterhouse, using a 2-level scale (Good/Bad) that depended on whether they were able to detect the next visual indicators: trapped birds, signs of hyperthermia (i.e., birds with their head out of the plastic crate and panting) or hypothermia (i.e., birds grouped in the center of the plastic crate to protect from cold), signs of overcrowding, and/or lack of proper trailer canvas tops to protect birds from adverse climatic conditions. Visual inspection included all transport modules and plastic crates. For a visual indicator to be declared as detected, it had to be observed on at least 50% of inspected birds. Transport condition was scored as bad if at least one of the visual indicators of bad transport conditions was detected. Similarly, plumage condition of each flock was also visually assessed at arrival at the slaughterhouse using a 2-level scale (Good/Bad) according to whether birds presented or not yellowish plumage coloration attributable to wet bedding at the farm. At least 50% of inspected birds had to have a bad plumage condition for the plumage condition of the flock to be declared as bad. If a truck was composed of mixed loads, each flock was scored separately. For each flock, the number of broiler chickens loaded onto the truck, birds that are slaughtered, and rejected carcasses at slaughter were recorded. Deaths on arrival (DOA; n) for each flock were the number of chickens found dead at unloading. Carcass rejections (n) relate to those removed from the production line after slaughter because of emaciation, ascites/edema, septicemia, cellulitis, lesions, trauma, and/or hematomas among others, as established by the Spanish Agency for Food Safety and Nutrition (AECOSAN, 2015).

Statistical analysis of data was carried out with the SAS statistical software (SAS 9.4). To determine whether flock transport condition and plumage condition scores at arrival were affected by transport duration, season, and mixed loads, both variables were treated as binary response variables. With this, the effects of transport duration, season, and mixed loads were modelled using logistic regressions by means of the GLIMMIX procedure, which were obtained separately for flocks contributing to DOA and carcass rejections data sets. The effects of the described risk factors on the welfare of broiler chickens, assessed through DOA and carcass rejections, were evaluated. For this, separate models were developed for DOA and carcass

### Table 1. Number of flocks that were finally included in the dead on arrival (DOA) and carcass rejections models, segmented according to each level of each studied risk factor.

| Variable                        | DOA (n = 2,154 flocks) | Carcass rejections (n = 1,649 flocks) |
|---------------------------------|------------------------|---------------------------------------|
| Mixed loads                     |                        |                                       |
| Not mixed                       | 1,987                  | 1,514                                 |
| Mixed                           | 167                    | 135                                   |
| Transport conditions at arrival |                        |                                       |
| Good                            | 1,846                  | 1,399                                 |
| Bad                             | 308                    | 250                                   |
| Season                          |                        |                                       |
| Spring                          | 542                    | 357                                   |
| Summer                          | 548                    | 471                                   |
| Autumn                          | 572                    | 564                                   |
| Winter                          | 492                    | 257                                   |
| Plumage condition at arrival    |                        |                                       |
| Good                            | 469                    | 396                                   |
| Bad                             | 1,685                  | 1,253                                 |

### Table 2. Transport duration, dead on arrival (DOA), and carcass rejections summary statistics for the DOA and carcass rejections models.

| Variable                        | DOA model (n = 2,154 flocks) | Carcass rejections model (n = 1,649 flocks) |
|---------------------------------|------------------------------|---------------------------------------------|
| DOA (%)                         | 0.26                         | 0.77                                        |
| Transport duration for flocks included in the DOA model (min) | 178                          | 165                                         |
| Carcass rejections (%)          | 0.50                         | 0.77                                        |
| Transport duration for flocks included in the carcass rejections model (min) | 105.01                       | 99.65                                       |
rejections using generalized linear mixed models analysis with the GLIMMIX procedure. In both models, flock was considered the experimental unit. DOA and carcass rejections were modelled as count data, which have traditionally been modelled with a Poisson distribution. To avoid data overdispersion generally associated with the Poisson distribution, data were modelled using a negative binomial distribution, which usually provides better estimation models for this type of data (Little et al., 2006) and has been previously applied in similar studies with successful results (Chauvin et al., 2011). The number of loaded and slaughtered broiler chickens were, respectively, offset variables for DOA and carcass rejections. Therefore, DOA and carcass rejections were expressed as percentages.

Main fixed effects were the same for both models and included transport duration, season of transport, whether transport was a mixed load, the transport condition score, and the plumage condition score at arrival. Initial models included all possible two-way interactions between main fixed effects. Final models were developed using a stepwise backward procedure, in which nonsignificant interactions were gradually removed from the model using the highest $P$ value as the removal criterion. Final models retained all significant two-way interactions ($P < 0.05$). Both models included an intercept with random variation according to the specific journey to the slaughterhouse. In addition, the DOA model accounted for unequal random transport variance according to season, while the carcass rejections model accounted for unequal random transport variance according to plumage condition. Least square means were computed in case of statistically significant effects ($P < 0.05$), with $P$ values adjusted for multiple comparisons by Tukey range tests. For significant interactions, tests of simple effects (Winer, 1971) were performed to detect differences between the levels of a risk factor within each level of the other risk factor.

### RESULTS

Table 1 shows, for the 2,154 and 1,649 flocks finally included in the respective DOA and carcass rejections models, how many flocks contributed to each level of each studied risk factor. This information offers an idea of the amount of information available during the data-modelling step. Flocks transported during mixed transports represented 7.8% of total flocks included in the DOA model and 8.2% of flocks included in the carcass rejection model. Transport conditions of 14.3% and 15.2% flocks were scored as “bad” at arrival in the

| Variable                                      | Odds ratio estimate | 95% Confidence limits |  
|-----------------------------------------------|---------------------|-----------------------|  
| Dead on arrival (DOA) data set (n = 2,154 flocks) |                     |                       |  
| Transport conditions at arrival               |                     |                       |  
| Mixed loads                                   |                     |                       |  
| ‘Unmixed vs mixed loads’                      | 1.305               | 0.729                 | 2.338                 | 0.370                 |  
| Transport duration                            | 1.001               | 0.999                 | 1.002                 | 0.254                 |  
| Season                                        |                     |                       |                       |                       |  
| ‘Winter vs summer’                            | 0.032               | 0.012                 | 0.089                 | <0.001                |  
| ‘Autumn vs summer’                            | 0.201               | 0.130                 | 0.312                 |                       |  
| ‘Spring vs summer’                            | 1.686               | 1.267                 | 2.245                 |                       |  
| Plumage condition at arrival                  |                     |                       |                       |                       |  
| Mixed loads                                   |                     |                       |                       |                       |  
| ‘Unmixed vs mixed loads’                      | 1.429               | 0.977                 | 2.088                 | 0.065                 |  
| Transport duration                            | 1.001               | 1.000                 | 1.002                 | 0.063                 |  
| Season                                        |                     |                       |                       |                       |  
| ‘Winter vs summer’                            | 1.370               | 1.012                 | 1.854                 | <0.001                |  
| ‘Autumn vs summer’                            | 0.674               | 0.516                 | 0.881                 |                       |  
| ‘Spring vs summer’                            | 2.551               | 1.820                 | 3.577                 |                       |  
| Carcass rejections data set (n = 1,649 flocks) |                     |                       |                       |                       |  
| Transport conditions at arrival               |                     |                       |                       |                       |  
| Mixed loads                                   |                     |                       |                       |                       |  
| ‘Unmixed vs mixed loads’                      | 1.422               | 0.757                 | 2.671                 | 0.273                 |  
| Transport duration                            | 1.001               | 0.999                 | 1.003                 | 0.198                 |  
| Season                                        |                     |                       |                       |                       |  
| ‘Winter vs summer’                            | 0.029               | 0.007                 | 0.120                 | <0.001                |  
| ‘Autumn vs summer’                            | 0.177               | 0.112                 | 0.280                 |                       |  
| ‘Spring vs summer’                            | 1.934               | 1.404                 | 2.665                 |                       |  
| Plumage condition at arrival                  |                     |                       |                       |                       |  
| Mixed loads                                   |                     |                       |                       |                       |  
| ‘Unmixed vs mixed loads’                      | 1.412               | 0.927                 | 2.152                 | 0.108                 |  
| Transport duration                            | 1.002               | 1.003                 | 1.003                 | 0.011                 |  
| Season                                        |                     |                       |                       |                       |  
| ‘Winter vs summer’                            | 1.181               | 0.817                 | 1.707                 | <0.001                |  
| ‘Autumn vs summer’                            | 0.685               | 0.519                 | 0.905                 |                       |  
| ‘Spring vs summer’                            | 3.076               | 2.041                 | 4.637                 |                       |  

1Models are calculated separately for DOA and carcass rejection data sets.
2It was modelled the probability of finding a flock with bad transport conditions at arrival.
3It was modelled the probability of finding a flock with bad plumage condition at arrival.

Table 3. Odds ratio estimates of the effects of mixed loads, transport duration, and season on the transport conditions and plumage condition scores at arrival to the slaughterhouse of broiler flocks.
respective DOA and carcass models. On the other hand, flocks were balanced across the different seasons for DOA (22.8, 26.6, 25.2, and 25.4% during winter, autumn, spring, and summer, respectively) and were slightly more unbalanced for carcass rejections (15.6, 34.2, 21.6, and 28.6% during winter, autumn, spring, and summer, respectively). Plumage condition scored as bad at the slaughterhouse in most of flocks contributing to DOA and carcass rejections models (77.2 and 76.0%, respectively).

Summary statistics for the continuous risk factor transport duration (min), the response variables DOA (%), and carcass rejections (%) are shown in Table 2, distinguishing between DOA and carcass rejection models. Mean transport duration was about 200 min for flocks included in the DOA and carcass rejections models. Values for the rest of transport duration descriptive statistics were also equivalent between models.

The odds ratio estimates of the effects of mixed loads, transport duration, and season on transport condition and plumage condition scores at arrival, obtained separately for the DOA and carcass rejections’ data sets, are shown in Table 3. A highly significant seasonal effect was observed in all cases. The risk of finding a flock with bad transport conditions at arrival was much lower during winter and autumn than during summer, but was moderately higher during spring than summer, and this effect was found in both DOA and carcass rejections’ data sets. On the other hand, the risk of finding a flock with bad plumage condition at arrival was much lower during winter and autumn than during summer but was moderately higher during spring than summer, and this effect was found in both DOA and carcass rejections’ data sets.

| Variable | DOA (%) | P value |
|----------|---------|---------|
| Intercept | -5.7574 | <0.0001 |
| Mixed loads | Not mixed | -1.3892 | <0.0001 |
|          | Mixed | 0       |
| Transport conditions at arrival | Good | -1.0520 | 0.0006 |
|          | Bad | 0       |
| Season | Winter | 0.2897 | <0.0001 |
|          | Autumn | 1.2386 | 0.0001 |
|          | Spring | 0.2868 | 0.2998 |
|          | Summer | 0       |
| Plumage condition at arrival | Good | 0.1068 | 0.3884 |
|          | Bad | 0       |
| Duration | 0.0008 | <0.0001 |
| Duration × Mixed loads | Not mixed | 0.0035 | <0.0001 |
|          | Mixed | 0       |
| Duration × Season | Winter | 0.0007 | 0.0003 |
|          | Autumn | -0.0012 | 0.0004 |
|          | Spring | -0.0004 | 0.0005 |
|          | Summer | 0       |
| Mixed loads × Transport conditions at arrival | Not mixed × Good | 1.0508 | 0.0005 |
|          | Not mixed × Bad | 0       |
|          | Mixed × Good | 0       |
|          | Mixed × Bad | 0       |
| Mixed loads × Season | Not mixed × Winter | -0.5879 | 0.0084 |
|          | Not mixed × Autumn | -0.8011 | 0.2347 |
|          | Not mixed × Spring | -0.5009 | 0.2837 |
|          | Not mixed × Summer | 0       |
|          | Mixed × Winter | 0       |
|          | Mixed × Autumn | 0       |
|          | Mixed × Spring | 0       |
|          | Mixed × Summer | 0       |
| Season × Plumage condition at arrival | Winter × Good | 0.1888 | 0.0009 |
|          | Winter × Bad | 0       |
|          | Autumn × Good | -0.1866 | 0.1053 |
|          | Autumn × Bad | 0       |
|          | Spring × Good | -0.2776 | 0.1421 |
|          | Spring × Bad | 0       |
|          | Summer × Good | 0       |
|          | Summer × Bad | 0       |

1P value corresponds to the statistical significance of the main risk factor or interaction.
winter. This also occurred for both DOA and carcass rejections data sets. In addition, a slight but significant positive association between transport duration and the risk of finding a flock with bad plumage condition at arrival was found in the carcass rejection data set.

The DOA model and results are shown in Table 4. Figure 1 illustrates the interaction of transport duration and mixed loads on DOA and reflects that increasing transport duration generally resulted in increased DOA, although values were higher in mixed loads, particularly during shorter transports. The results of the interaction between transport duration and season are presented in Figure 2, showing that the detrimental effects of transport duration were particularly evident during winter transports. The interaction between mixing loads and bad transport conditions at arrival resulted in a significantly higher DOA (Table 5). DOA also differed according to season and whether transports were mixed or not (Table 6). Differences corresponding to the interaction between transport season and plumage condition score at arrival on DOA are also shown in Table 6.

The carcass rejection model and the statistical significance of included risk factors and interactions are shown in Table 7. Figure 3 illustrates the effect of transport duration on carcass rejection. The interaction between transport season and mixed loads shows that mixed loads in autumn and spring resulted in a higher percentage of carcass rejection than summer transports (Table 8). The interaction between transport season and transport conditions at arrival score on carcass rejections (Table 8) shows that a high percentage of carcass rejections were detected in winter and spring in flocks scored with good transport conditions at arrival, while for flocks with bad transport conditions at arrival, this percentage was higher during autumn and spring.

**DISCUSSION**

Data on 1-year records of broiler chicken transports to one slaughterhouse in Southern Spain were collected, and with this, the interactive nature of risk factors affecting the welfare of broiler chickens was determined.
Mixing loads amplified the negative consequences of long transports and bad transport conditions at arrival on DOA. The detrimental effect of long transports on DOA was remarkable during summer and winter, and long transports also increased carcass rejections. Seasonal differences were particularly remarkable when transport conditions were suboptimal (mixing loads, personal differences were particularly remarkable when long transports also increased carcass rejections. 

Table 5. Interaction between the transport conditions score at arrival and mixed loads on the dead on arrival (DOA; %) of broilers transported to slaughter. 1

| Variable         | Good transport conditions at arrival | Bad transport conditions at arrival | P value |
|------------------|--------------------------------------|------------------------------------|---------|
| Not mixed        | 0.186 ± 0.005                        | 0.186 ± 0.010                      | 0.0005  |
| Mixed            | 0.199 ± 0.019b                       | 0.569 ± 0.147                      |         |

1Within each line (i.e., for not mixed and mixed transports independently), different letters indicate the existence of significant differences between good and bad transport conditions at arrival (P < 0.05). P value refers to the overall interaction effect.

The detrimental effect of long transports on DOA and carcass rejections, effects that are amplified for mixed transports. No studies have to date evaluated the impact of mixing loads on broiler chickens. This is a relatively common commercial practice in Spain that intends to optimize transport costs and is also common in other species (Gosálvez et al., 2006). Results, however, suggest that mixing has a negative impact on broiler chickens’ welfare and ultimately leads to increased DOA and carcass rejections, similar to the results already described for pigs (Gosálvez et al., 2006). Indeed, mixing loads will increase DOA even during short transports (Figure 1), which highlights the negative consequences of this practice. All these negative consequences raise concerns about the suitability of mixing loads from both the welfare and economic perspectives.

Increased transport duration also resulted in a higher percentage of carcass rejections (Figure 3), although in this case, no interaction was detected with other risk factors. Our results confirm the interactive nature of transport risk factors affecting broiler chicken welfare (Mitchell and Kettlewell, 2009; Schwartzkopf-Genswein et al., 2012) and, in particular, the impact of transport duration (e.g., Warriss et al., 1992; Vecerek et al., 2006; Chauvin et al., 2011; Caffrey et al., 2017). Our results reveal the important impact that mixing loads has on DOA and carcass rejections, effects that are amplified for mixed transports. No studies have to date evaluated the impact of mixing loads on broiler chickens. This is a relatively common commercial practice in Spain that intends to optimize transport costs and is also common in other species (Gosálvez et al., 2006). Results, however, suggest that mixing has a negative impact on broiler chickens’ welfare and ultimately leads to increased DOA and carcass rejections, similar to the results already described for pigs (Gosálvez et al., 2006). Indeed, mixing loads will increase DOA even during short transports (Figure 1), which highlights the negative consequences of this practice. All these negative consequences raise concerns about the suitability of mixing loads from both the welfare and economic perspectives.

Table 6. Interaction between the season of the year and mixed loads and between season of the year and plumage condition at arrival on the DOA (%) of broilers transported to slaughter. 1

| Variable                      | Spring | Summer | Autumn | Winter | P value |
|-------------------------------|--------|--------|--------|--------|---------|
| Mixed loads                   | 0.133 ± 0.008a | 0.205 ± 0.010abc | 0.226 ± 0.010a | 0.194 ± 0.010b | 0.0084  |
| Mixed                         | 0.247 ± 0.054a | 0.231 ± 0.046abc | 0.568 ± 0.118a | 0.393 ± 0.073a |         |
| Plumage condition at arrival  | 0.166 ± 0.025a | 0.229 ± 0.029abc | 0.344 ± 0.039a | 0.320 ± 0.036ab | 0.0009  |
| Good condition at arrival     | 0.197 ± 0.021a | 0.206 ± 0.021abc | 0.373 ± 0.040a | 0.238 ± 0.023a |         |
| Bad condition at arrival      | 0.197 ± 0.021a | 0.206 ± 0.021abc | 0.373 ± 0.040a | 0.238 ± 0.023a |         |

1Within each line (i.e., for not mixed and mixed transports independently in the case of mixed loads, and for good and bad condition at arrival independently in the case of plumage condition) different letters indicate the existence of significant differences between mixed transports and bad transport conditions at arrival on DOA (P < 0.05). P value refers to the overall interaction effect.
factors. To our knowledge, no other study has dealt with
the effects of transport duration on carcass rejection in
broiler chickens, although Caffrey et al. (2017) suggested
that longer transports might be particularly problematic
for birds suffering some disease or with injuries caused
during catching and loading. Long journeys are more
likely to result in increased occurrence of lesions, trauma,
and hematomas, which are direct causes of carcass rejec-
tion, what would explain our

Considering the effect of season alone, DOA was
higher during autumn, followed by winter, spring, and
summer, thus matching the results obtained by Caffrey et al. (2017). Nevertheless, DOA was particularly high
during winter and summer as compared to spring for
long transports. This result evidences the negative
impact of more extreme outdoor temperatures on broiler
chickens when they are transported to slaughter over
long distances. Some disagreement exists in literature
regarding the effect of season on DOA, so that Vecerek et al. (2006) described higher DOA during both summer
and winter months, but Warriss et al. (2005), Petracci et al. (2006), and Haslam et al. (2008) reported increased
DOA during summer months as compared to the rest of
seasons, but of course this will depend on the location of
the study and weather patterns of the year of the study.

On the other hand, when including external temperature
in their models, Caffrey et al. (2017) found higher DOA
as temperatures were colder; with this inclusion, these
authors found that DOA were higher in autumn than
in spring. It should be however noticed that the
mentioned study was carried out in Canada. Fast-growing broiler chickens may experience reduced
thermotolerance caused by intensive genetic selection
(Sandercock et al., 1995; 2006). Therefore, both high
and low outdoor temperatures during long transports
are detrimental for broiler chicken DOA, due to hyper-
thermia or hypothermia. Our results con
fi
rm that,
when climatic conditions go beyond the chicken thermoneutral range such as the cases of summer and winter
transports, particular attention should be paid to longer
transports as they will incur in a higher risk of DOA.
Alternative management strategies should be taken in
these cases to maintain transport conditions as close as
possible to bird thermoneutrality. This would result in
a reduction of welfare problems originated by thermal
stress and reduce DOA.

As expected, transports that were scored with bad
conditions at arrival (chickens trapped in cages, showing
signs of hyperthermia or hypothermia, overcrowded,
and/or lacking a proper trailer canvas topper) resulted

### Table 7. Parameter estimates and standard error of the estimate for risk factors included in the carcass rejections (%) model.

| Variable                                      | Carcass rejections (%) | Estimate | SE     | P value¹ |
|-----------------------------------------------|------------------------|----------|--------|----------|
| Intercept                                     |                        | -5.7905  | 0.1601 | <0.0001  |
| Mixed loads                                   |                        | 0.0815   | 0.1410 | <0.0001  |
| Mixed                                        |                        | 0        |        |          |
| Transport conditions at arrival               |                        |          |        |          |
| Good                                          |                        | 0.3367   | 0.0820 | 0.0982   |
| Bad                                           |                        | 0        |        |          |
| Season                                        |                        |          |        |          |
| Winter                                        |                        | 0.1329   | 0.5443 | 0.0004   |
| Autumn                                        |                        | 1.2026   | 0.2497 |          |
| Spring                                        |                        | 0.9302   | 0.2490 |          |
| Summer                                        |                        | 0        |        |          |
| Plumage condition at arrival                  |                        |          |        |          |
| Good                                          |                        | -0.0715  | 0.0418 | 0.0875   |
| Bad                                           |                        | 0        |        |          |
| Duration                                      |                        |          |        |          |
| Mixed loads × Winter                          |                        | 0.0012   | 0.0002 | <0.0001  |
| Not mixed × Winter                            |                        | -0.4414  | 0.1963 |          |
| Not mixed × Autumn                            |                        | -0.7471  | 0.2025 | 0.0043   |
| Not mixed × Spring                            |                        | -0.6540  | 0.2384 |          |
| Not mixed × Summer                            |                        | 0        |        |          |
| Mixed × Winter                                |                        | 0        |        |          |
| Mixed × Autumn                                |                        | 0        |        |          |
| Mixed × Spring                                |                        | 0        |        |          |
| Mixed × Summer                                |                        | 0        |        |          |
| Transport conditions at arrival × Season       |                        |          |        |          |
| Good × Winter                                 |                        | 0.4753   | 0.5125 | 0.0014   |
| Good × Autumn                                 |                        | -0.6489  | 0.1610 |          |
| Good × Spring                                 |                        | -0.2634  | 0.1134 |          |
| Good × Summer                                 |                        | 0        |        |          |
| Bad × Winter                                  |                        | 0        |        |          |
| Bad × Autumn                                  |                        | 0        |        |          |
| Bad × Spring                                  |                        | 0        |        |          |
| Bad × Summer                                  |                        | 0        |        |          |

¹P value corresponds to the statistical significance of the main risk factor or interaction.
in higher DOA (Table 4). Hyperthermia and hypothermia have already been discussed, and regarding these, the absence of a proper trailer canvas topper will expose birds to adverse weather. Trapped birds and cage over-crowding are the result of inadequate catching, crating, and loading practices, with consequences over subsequent transport and slaughter, and even leading to elevated DOA (Nijdam et al., 2005). Given the link between bad transport conditions scored at arrival and increased DOA, the use of these scores to identify problems and trigger targeted, corrective measures during future transports appears advisable to reduce DOA, and lead to improved welfare and higher economic turnover. These results also confirm that the impact of poor transport conditions scored at arrival will be further aggravated when loads are mixed (Table 5), suggesting a synergistic interaction between them, and the need to avoid them as much as possible through a careful planning of transport logistics. Mixing loads will exacerbate the detrimental effect of autumn transports on DOA and carcass rejections with respect to summer transports (see tables 6 and 8). This may appear surprising but has already been described for slaughter pigs (Guardia et al., 1996) and has been attributed to the fact that companies try to adapt their transport logistics when weather conditions are expected to be extreme, such as the case of summer conditions. With the exception of longer transports that are in any case problematic, this logistics adaptation may explain the overall good results found during summer with respect to autumn.

Bad plumage condition score at arrival, attributable to poor bedding condition (de Jong et al., 2014), also tended to increase the percentage of carcass rejections. Wet bedding, that depends among others on the litter material, relates to the development of pathogenic microorganisms and disease in broiler chickens’ farms (Dunlop et al., 2016) and on-farm disease is, in its turn, strongly associated to increased carcass rejection at slaughter (Haslam et al., 2008). Birds with bad

Table 8. Interaction between the season of the year and mixing loads on carcass rejections at slaughter (%).1

| Variable                                  | Spring       | Summer       | Autumn       | Winter       | P value |
|-------------------------------------------|--------------|--------------|--------------|--------------|---------|
| Mixed loads                               |              |              |              |              |         |
| Mixed                                     | 0.553 ± 0.024| 0.479 ± 0.021| 0.546 ± 0.038| 0.446 ± 0.113| 0.0043  |
| Mixed                                     | 0.980 ± 0.185a| 0.441 ± 0.002b| 1.062 ± 0.167a| 0.639 ± 0.181ab|         |
| Transport conditions at arrival           |              |              |              |              |         |
| Good transport conditions at arrival      | 0.764 ± 0.077a| 0.544 ± 0.03b| 0.651 ± 0.048ab| 0.801 ± 0.056a| 0.0014  |
| Bad transport conditions at arrival       | 0.710 ± 0.078a| 0.388 ± 0.039b| 0.890 ± 0.135c| 0.356 ± 0.181c|         |

1Within each line (i.e., for not mixed and mixed transports independently in the case of mixed loads, and for good and bad transport conditions at arrival independently in the case of transport conditions at arrival) different letters indicate the existence of significant differences between transport seasons (P < 0.05). P value refers to the overall interaction effect.
plumage condition may be those more sensitive to the action of additional stressors and therefore those with a higher probability of dying during autumn transports, as reflected by DOA concentrating during autumn with respect to other seasons (Table 6). The interaction between transport conditions score at arrival and transport season on carcass rejections does not offer clear results (Table 8) but also points toward a modulation of the seasonal effect of transport according to journey conditions.

In conclusion, this study confirms the relevance of transport risk factors on the DOA and carcass rejections and their interactive nature. The detrimental impact of mixing loads on DOA and carcass rejections makes this practice questionable both in animal welfare and economic terms. Mixing loads amplify the negative consequences of other risk factors such as long journey duration and bad transport conditions scored at arrival, the latter being originated during the loading process and/or transport. The detrimental effect of long journeys on DOA was particularly evident during summer and winter, when more extreme outdoor temperatures were more probable. This highlights the need for alternative management practices during transport during more extreme weather conditions, to maintain birds as close as possible to thermoneutrality and prevent their negative impact over welfare and economic returns. Mixing loads, as a stressful procedure, and bad plumage condition scores, reflecting suboptimal farm departing conditions, would exacerbate the overall, detrimental effect that autumn transports have on broiler chickens with respect to summer transports.

ACKNOWLEDGMENTS

The authors thank the INIA for the Spanish funding for the “Integrated EU Mobile Broiler Data: Optimising Broiler Chicken Management, Health and Welfare through use of integrated EU data” Project (IMBDatA, ERA-NET grant number 291815, ANIHWA), and the Spanish Ministry of Science, Innovation and Universities for the “Development of new technologies (apps) for animal welfare certification and risk assessment in intensive pig and poultry production” Project (Techwel, grant number RTI2018-096034-R-C22). Funding sources had no involvement in the study design, the collection, analysis and interpretation of data, the writing of the report, and in the decision to submit the article for publication.

Conflict of interest statement: The authors did not provide a conflict of interest statement.

REFERENCES

AECOSAN. 2015. Spanish Agency for Food Safety and Nutrition. Accessed March 2020. http://www.aecosan.msssi.gob.es/AECOSAN/docs/documentos/seguuridad_alimentaria/gestion_riesgos/Procedimiento_deteccion_post_mortem_bienestar_pollos_engorde.pdf.
Bayliss, P. A., and M. H. Hinton. 1990. Transportation of broilers with special reference to mortality rates. Appl. Anim. Behav. Sci. 28:93-118.
BenSassi, N., X. Averos, and I. Estevez. 2019. The potential of the transect method for early detection of welfare problems in broiler chickens. Poult. Sci. 98:522-532.
Caffrey, N. P., I. R. Dohoo, and M. S. Cockram. 2017. Factors affecting mortality risk during transportation of broiler chickens for slaughter in Atlantic Canada. Prev. Vet. Med. 147:199-208.
Chauvin, C., S. Hillion, L. Balaine, V. Michel, J. Peraste, I. Petetin, C. Lupo, and S. Le Bouquin. 2011. Factors associated with mortality of broilers during transport to slaughterhouse. Animal 5:287-293.
Council Regulation (EC) No 1/2005 2005. On the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97. Off. J. L 3:1-44.
Dawkins, M. S., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342-344.
de Jong, J. C., H. Gummink, and J. van Harn. 2014. Wet litter not only induces footpad dermatitis but also reduces overall welfare, technical performance, and carcass yield in broiler chickens. J. Appl. Poult. Res. 23:51-58.
Dunlop, M. W., A. F. Moss, P. J. Groves, S. J. Wilkinson, R. M. Stuetz, and P. H. Selle. 2016. The multidimensional causal factors of ‘wet litter’ in chicken-meat production. Sci. Total Environ. 562:766-776.
FAOSTAT. 2020. Statistical Database of the Food and Agriculture Organization. Accessed Feb. 2020. http://www.fao.org/faostat/en/#home.
Gosálvez, L. F., X. Averós, J. J. Valdelvira, and A. Herranz. 2006. Influence of season, distance and mixed loads on the physical and carcass integrity of pigs transported to slaughter. Meat Sci. 73:553-558.
Grilli, C., A. R. Loschi, S. Rea, R. Stoechi, L. Leoni, and F. Conti. 2015. Welfare indicators during broiler slaughtering. Br. Poult. Sci. 56:1-5.
Guardia, M. D., M. Gispert, and A. Diestro A. 1996. La mortalidad en ganado porcino durante el periodo previo al sacrificio en mataderos comerciales. Invest. Agrar. Prod. Sanid. Anim. 11:171–179.
Haslam, S. M., T. G. Knowles, S. N. Brown, L. J. Wilkins, S. C. Kestin, P. D. Warriss, and C. J. Nicol. 2008. Prevalence and factors associated with it, of birds dead on arrival at the slaughterhouse and other rejection conditions in broiler chickens. Br. Poult. Sci. 49:685-696.
Herenda, D., and O. Jakel. 1994. Poultry abattoir survey of carcass condemnation for standard, vegetarian, and free range chickens. Can. Vet. J. 35:293-296.
Jacobs, L., E. Delezie, L. Duchateau, K. Goethals, and F. A. M. Tuyttens. 2017a. Broiler chickens dead on arrival: associated risk factors and welfare indicators. Poult. Sci. 96:259-265.
Jacobs, L., E. Delezie, L. Duchateau, K. Goethals, and F. A. M. Tuyttens. 2017b. Impact of the separate pre-slaughter stages on broiler chicken welfare. Poult. Sci. 96:266-273.
Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. Generalized linear mixed models. Pages 525–566 in SAS® for Mixed Models. 2nd ed. SAS Institute Inc., Cary, NC.
Mitchell, M. A., and P. J. Kettlewell. 2009. Welfare of poultry during transport—a review. Pages 90–100 in Proceedings of 8th Poultry Welfare Symposium, Cervia, Italy, 18–22 May 2009.
National Chicken Council. 2017. Animal welfare guidelines and audit checklist for broilers. NCC-Welfare-Guidelines-Broilers.pdf. Accessed Feb. 2020. www.nationalchickencouncil.org.
Nijdam, E., E. Arens, E. Lamboujj, E. Decuyper, and J. A. Stegeman. 2004. Factors influencing bruises and mortality of broilers during catching, transport, and lairage. Poult. Sci. 83:1610–1615.
Nijdam, E., E. Delezie, E. Lamboujj, M. J. A. Naburs, E. Decuyper, and J. A. Stegeman. 2005. Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. Poult. Sci. 84:467–474.
Petracci, M., M. Bianchi, C. Cavanu, P. Gaspari, and A. Lavazza. 2006. Preshlaughter mortality in broiler chickens, turkeys, and spent hens under commercial slaughtering. Poult. Sci. 85:1660–1664.
Sandercock, D. A., M. A. Mitchell, and M. G. Macleod. 1995. Metabolic heat production in fast and slow growing broiler chickens during acute heat stress. Br. Poult. Sci. 36:868.
Sandercock, D. A., R. R. Hunter, M. A. Mitchell, and P. M. Hocking. 2006. Thermoregulatory capacity and muscle membrane integrity are compromised in broilers compared with layers at the same age or body weight. Br. Poult. Sci. 47:322–329.

Schwartzkopf-Genswein, K. S., L. Fauconato, S. Dadgar, P. Shand, L. A. González, and T. G. Crowe. 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality; a review. Meat Sci. 92:227–243.

Vecerek, V., S. Grbalova, E. Voslarova, B. Janackova, and M. Malena. 2006. Effects of travel distance and the season of the year on death rates of broilers transported to poultry processing plants. Poult. Sci. 85:1881–1884.

Vieira, F. M. C., I. J. O. Silva, J. A. Barbosa Filho, A. M. C. Vieira, and D. M. Broom. 2011. Preslaughter mortality of broilers in relation to lairage and season in a subtropical climate. Poult. Sci. 90:2127–2133.

Warriss, P. D., E. A. Bevis, S. N. Brown, and J. E. Edwards. 1992. Longer journeys to processing plants are associated with higher mortality in broiler chickens. Br. Poult. Sci. 33:201–206.

Warriss, P. D., A. Pagazautundua, and S. N. Brown. 2005. Relationship between maximum daily temperature and mortality of broiler chickens during transport and lairage. Br. Poult. Sci. 46:647–651.

Welfair Certification. 2020. Accessed April 2020. https://www.animalwelfair.com/.

Whiting, T. L., M. E. Drain, and D. P. Rasali. 2007. Warm weather transport of broiler chickens in Manitoba. II. Truck management factors associated with death loss in transit to slaughter. Can. Vet. J. 48:148–154.

Wilkins, L. J., S. N. Brown, A. J. Phillips, and P. D. Warriss. 2003. Cleanliness of broilers when they arrive at poultry processing plants. Vet. Rec. 153:701–703.

Winer, B. J. 1971. Statistical Principles in Experimental Design. 2nd ed. McGraw Hill, Inc., New York, NY.

Zhang, L., H. Y. Yue, H. J. Zhang, L. Xu, S. G. Wu, H. J. Yan, Y. S. Gong, and G. H. Qi. 2009. Transport stress in broilers: I. Blood metabolism, glycolytic potential, and meat quality. Poult. Sci. 88:2033–2041.