Proposed bursa of fabricius weight to body weight ratio standard in commercial broilers

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ABSTRACT Several causes may induce change and atrophy in the bursa of Fabricius (BF). Databases on BF standards are available from published studies, however, updated references are needed to adjust the BF standards to present changes in highly specialized broiler genetic lines. The aim of this study was to evaluate BF-related measurements (weight and dimensions) under controlled conditions that would mimic field situations. Chickens were kept in isolation, thus avoiding exposure to disease agents by vaccination or field infections. This study was conducted using male Cobb 500 commercial broilers from the same hatch and source. Absence of disease was confirmed throughout the study. Despite the presence of individual variations, a minimum bursa-to-body weight ratio standard of 0.11 is proposed in broilers from 7 to 42 days of age.

Key words: chicken, broiler, bursa of Fabricius

2015 Poultry Science 94:2088–2093
http://dx.doi.org/10.3382/ps/pev230

INTRODUCTION

The bursa of Fabricius (**BF**) is a primary lymphoid organ in birds and plays a key role in the differentiation of B-lymphocytes (Schat and Skinner, 2014). Its development begins during incubation and reaches maximum size between 8 to 10 weeks of age, when the regression process starts, and is completed by 6 to 7 months of age (Olah et al., 2014). Several pathologic conditions (infectious diseases, mycotoxins, etc.) can directly impact bursa size, such as chicken infectious anemia virus (**CAV**) (Haridy et al., 2012), infectious bursal disease (**IBD**) virus (McMullin, 2004), Marek's disease virus (Chang et al., 2011), and reovirus (Wang et al., 2007) as well as mycotoxin contamination in feed (Hoerr, 2008). Bursa size standards should ideally be set before describing abnormal features such as atrophy. Glick (1956) and Wolfe et al. (1962) addressed BF size and development in meat-type or egg-type chicken genetic lines kept in "normal" conditions and by keeping birds free from any infectious disease, examining the influence of age, sex, genetic line, and husbandry conditions on the bursa weight and bursa-to-body weight (**BB**) ratio. However, these authors did not issue standards (reviewed in Cazaban et al., 2015, in press) so the only available references on BF size or weight studies in normal conditions were published over 50 years ago. There is a need to issue updated standards that would take into account the genetic selection in broilers today.

The objective of the study was to establish standards of bursa weight and bursa-to-body weight ratio in a commercial broiler line.

MATERIALS AND METHODS

Animals

Three hundred one-day-old male Cobb 500 broiler chicks were purchased from a single commercial hatchery (Granja el Pilar, Tarragona, Spain). All chicks used in the experiment were hatched on the same day and came from the same 40-week-old breeder flock. Gender sorting was done at the hatchery and only males were selected for this study. A first-quality sorting was performed at the hatchery and another on arrival at the SyBA experimental farm (Seguridad y Bienestar Animal, Les Franqueses del Vallès, Spain). A total of 210 one-day-old chicks were used in this study. Surplus chicks were humanely euthanatized using embutramide (T61®, MSD Animal Health, Madrid, Spain) by intravenous route.

Received March 16, 2015.
Accepted June 29, 2015.
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Housing

The birds were kept in biosecure facilities under conditions that aimed at mimicking field conditions. On arrival, the birds were placed in two pens of 24 m^2 each in a naturally-ventilated room. Birds' density was kept constant at around 12 to 13 chickens/m^2 throughout the study. Chickens were raised in one of the pens for the first 2 weeks of the rearing period, and then, from the third week of age onwards, they were distributed into 2 pens. Feed and water were provided ad libitum. Starter and grower-finisher feed diet was available from 1 to 21 and 22 to 42 days of age, respectively. Standard lighting and environmental temperature were recorded using data loggers to monitor them throughout the study.

Vaccination and Medication

Customary to the hatchery's practice, the chicks were vaccinated at the hatchery against Marek's disease (Cryomarex HVT, Merial Laboratorios, Barcelona, Spain) by subcutaneous injection and infectious bronchitis (Avipro IB H120, Lohmann Animal Health, Cuxhaven, Germany) by aerosol spray. To prevent coccidiosis, monensin (125 ppm) was incorporated in the grower-finisher diets. No other medications were administered for the remainder of the study.

Experimental Design and Sampling

Housing and handling of chickens complied with the animal care and use guidelines that are in force at Centre de Recerca en Sanitat Animal (CReSA, Universitat Autònoma de Barcelona [UAB], Bellaterra, Spain). Observation for clinical signs and mortality was conducted daily until the end of the study. Thirty chickens were randomly sampled on a weekly basis from hatch until slaughter age of 42 days as follows: necropsies were conducted at the Centre de Recerca en Sanitat Animal (CReSA, Universitat Autònoma de Barcelona [UAB], Bellaterra, Spain), where all the measurements were taken.

Body weight and bursa weight were used to calculate the BB ratio, according to the following formula:

$$\text{BB ratio} = [\text{bursa weight (g)}/\text{body weight (g)}] \times 100.$$

The two diameters (height and width) of each BF were measured using a vernier caliper. This served to calculate the BF volume, according to the following formula, where r is the radius:

$$\text{Volume} = (4/3) \times \Pi \times r^3.$$

Each BF was cut longitudinally into 2 parts: one part was placed in 10% neutral buffered formalin for histopathological lesion scoring using the European Pharmacopoeia 6.0 scale (Monograph of Avian Infectious Bursal Disease Vaccine (Live), ref. 01/2008:0587). The scoring ranges from 0 (no lesions) to 5 (100% of follicles show nearly complete lymphoid depletion).

Serology

Serum samples were kept frozen at -20°C until study completion, and then submitted to a diagnostic laboratory (Seysa, Valmojado, Spain). IBD antibodies were tested using a commercial ELISA test kit (BioChek, Reeuwijk, the Netherlands); CAV and reovirus antibodies were tested in the last sampling (d 42) only.

Mycotoxin Detection

Feed samples were collected from the starter and grower-finisher diets and were sent to Laboratorio de Diagnostico General (Barcelona, Spain) to be tested for the presence of the following mycotoxins: aflatoxin, fumonisin B1, ochratoxin A, trichotecenes (T2-toxin), and zearalenone.

Statistical Analysis

BF weight, BF size, and BB ratio were subjected to statistical analysis between sampling days using the ANOVA test at a confidence level of 5% ($P = 0.05$). The coefficient of variation (**CV**) was calculated to show data dispersion by dividing the standard deviation (**SD**) by the mean. Correlation between BF weight and body weight was also assessed.

RESULTS

Health of the Birds

No clinical signs were recorded. No gross lesions were found at post mortem examination.

Gross and Microscopic Lesions of the BF

Relevant histopathological lesions of the BF were not observed (score 0), except for one animal sampled at D21 that showed a focal heterophilic granuloma, consistent with a mild bacterial infection of the BF (score 1).

Serology

Mean maternally derived IBD antibody titer was less than 4,000 at one-day-old, with a CV of 51%. From 14 days of age onwards, two-thirds of sera were tested seronegative (titer < 391). All tested sera were seronegative from d 21 up to d 42 (data not shown).

Elisa testing for CAV and reovirus performed at d 42 were negative to both antigens (titers < 724 and 1,352, respectively) (data not shown).

Table 1. Mycotoxins assay in starter and grower-finisher diets (in mg/kg).

Mycotoxins	Starter diet	Finisher diet	EU standards*
Aflatoxin	0.006	0.006	0.02
Fumonisin	<0.420	<0.420	20
Ochratoxin	<0.002	<0.002	0.1
Trichothecenes (T-2)	<0.025	<0.025	5
Zearalenone	0.029	0.014	2

*Standards were set by Directive 2002/32/EC on undesirable substances in animal feed and by Commission Recommendation 2006/576 /EC on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

Mycotoxins Assay in Feed

Table 1 summarizes the results of the mycotoxins assays that were done in the 2 diets compared to the accepted maximum concentrations in Europe. The assays done in the 2 diet samples confirmed the absence, or very low detectable levels, of the 5 mycotoxins that were investigated.

Considering the previous results: (i) good general health status, (ii) absence of gross and microscopic lesions of the BF, (iii) absence of antibody response to IBDV, CAV, and Reovirus, and (iv) absence of detectable contamination of the feed by 5 mycotoxins, it was decided not to subject the BF to further virology analyses.

Morphometric Study of the BF

Tables 2 and 3 display all measurements of the BF throughout the study.

The mean BF weight increased steadily as birds grew older. However, higher variation of individual weights was also observed as the birds aged. A multiplication factor of 3.2 and of 2.1 was noticed between the heaviest and the lightest BF weights on d 35 (5.96 and 1.86 g, respectively) and d 42 (7.39 and 3.58 g, respectively).

BF volume is represented in Figure 1.

Figure 2 displays the results of BB ratio.

The BB ratios obtained showed a wide range of figures (a multiplication factor of 1.8 to 2.9 was found between the highest and lowest figures on a given sampling day). Nevertheless, a minimum BB ratio figure of 0.11 to 0.13 was consistently obtained except at the start (d 1) when the lowest figure was 0.07.

In an attempt to correlate the weight of the bursa to the body weight, a correlation analysis was carried out. (Figure 3).

A significant correlation was found between BF weight and body weight ($r^2 = 0.87$; $P < 0.001$); the association decreased in the late samplings where bursa and body weights were more dispersed than at the younger age. For instance, the BF of some 2 kg broilers was heavier than the BF of some 3 kg broilers (approximately 6 and 4 g, respectively).

Table 2. Bursa of Fabricius (BF) weight (g) in male Cobb 500 broilers.

		Age (days)						
		1	7	14	21	28	35	42
BF weight (g)	Mean	0.04^{a}	$0.24^{a,b}$	$0.67^{b,c}$	1.47^{c}	2.54^{d}	3.85^{e}	4.85^{f}
	SD	0.01	0.05	0.16	0.38	0.51	0.95	1.04
	Max.	0.07	0.33	1.01	2.34	4	5.96	7.39
	Min.	0.03	0.15	0.4	0.82	1.68	1.86	3.58
	CV (%)	25.0	20.8	23.9	25.9	20.1	24.7	21.4

CV, coefficient of variation; SD, standard deviation.

Different superscript letters in a row mean statistical differences ($p < 0.05$) between days.

Table 3. Bursa of Fabricius (BF) height (mm) and width (mm) in male Cobb 500 broilers.

		Age (days)						
		1	7	14	21	28	35	42
BF height (mm)	Mean	4.75^{a}	9.28^{b}	12^{c}	16.39^{d}	20.66^{e}	25.69^{f}	27.43^{g}
	SD	0.5	1.08	1.39	1.94	2.48	2.65	2.47
	Max.	5.8	11.5	14.6	19.9	29.2	32.8	32
	Min.	0.03	0.15	0.4	0.82	1.68	1.86	3.58
	CV (%)	10.5	11.6	11.6	11.8	12.0	10.3	9.0
BF width (mm)	Mean	4.61^{a}	7.44^{b}	9.38^{c}	14.65^{d}	18.71^{e}	20.89^{f}	23.67^{g}
	SD	0.48	0.83	1.34	1.85	2.47	2.94	2.5
	Max.	5.5	8.7	11.8	18.2	27.8	26.6	28.3
	Min.	0.03	0.15	0.4	0.82	1.68	1.86	3.58
	CV (%)	10.4	11.2	14.3	12.6	13.2	14.1	10.6

CV, coefficient of variation; SD, standard deviation.

Different superscript letters in a row mean statistical differences ($P < 0.05$) between days.

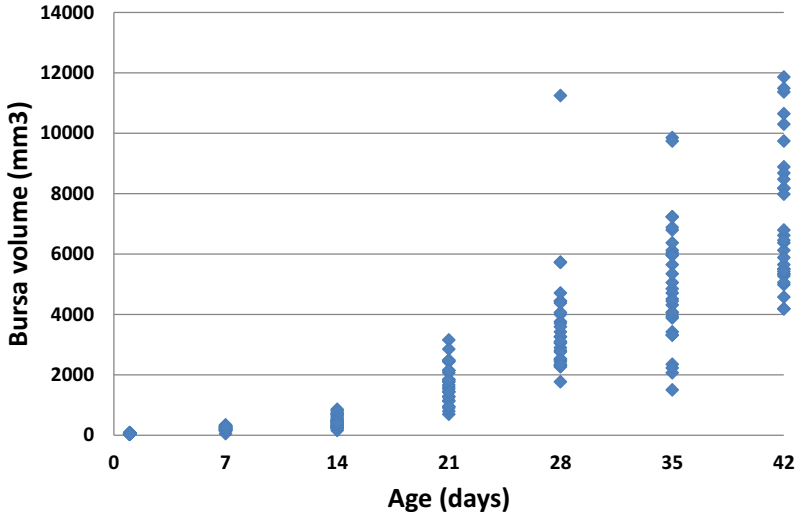

Figure 1. Bursa of Fabricius (BF) volume in male Cobb 500 broilers.

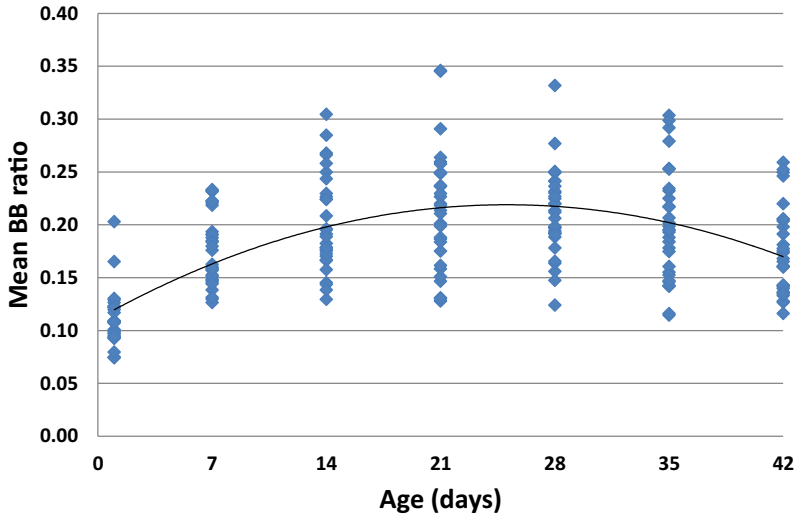

Figure 2. Bursa-to-body weight (BB) ratios in male Cobb 500 broilers.

DISCUSSION

To the authors' knowledge, this is the only available study carried out on one of the current genetic chicken lines (namely, male Cobb 500) which aimed at setting size standards for healthy BF. This more updated information should be helpful to field practitioners who frequently use the BF as an indicator of pathologic or stressful conditions in chickens. The few existing databases are outdated and not useful. In this experiment, housing conditions were similar to commercial field conditions and broiler husbandry practices.

The use of unvaccinated chicks would be more suitable for the purpose of this study; however, the chicks used in this experiment were purchased from an independent hatchery where a vaccination program (against Marek's disease and infectious bronchitis) is routinely implemented. Nevertheless, the commercial Marek's disease (HVT) and infectious bronchitis (H120) vaccine strains used were safe and they were not expected to impact bursa integrity either positively or negatively and the use of these vaccinated chicks more closely reflected field conditions.

Mean IBD antibody titer at hatch was moderate (less than 4,000). As expected, all chicks were ELISA antibody-positive at hatch; the rate of IBD seropositivity progressively dropped. From d 21 up to the study completion, all serum samples became antibody negative. The steady decrease in titers confirmed the absence of field infection by IBDV throughout the study. Broilers did not receive any live Gumboro vaccine which may induce lesions of the bursa and have an impact on its size (Jungbaeck and Nutolo, 2001).

Particular emphasis was placed on immunosuppressive conditions and factors that could directly impact BF size: IBD (BF histopathology, weekly serology throughout the study), CAV infection (serology at study completion), reoviruses infection (serology at study completion), Marek's disease (daily observation, then necropsy), and mycotoxins (feed analysis). The

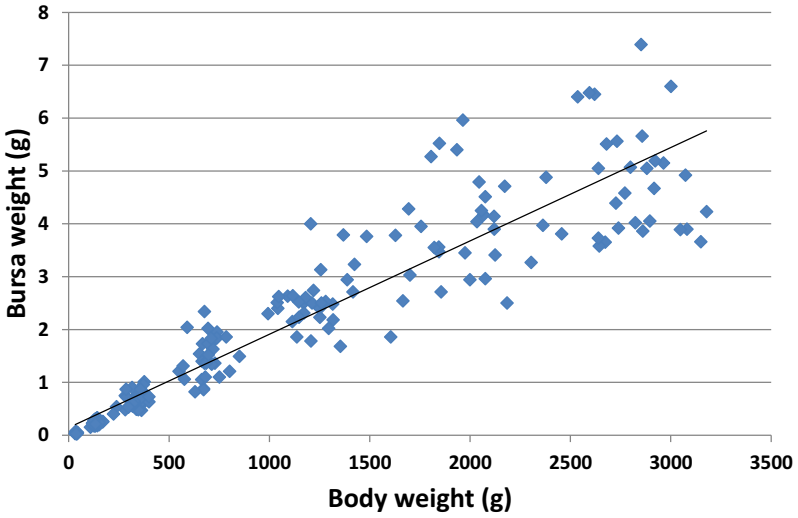

Figure 3. Correlation between bursa weight and body weight in male Cobb 500 broilers during the study.

specific assays confirmed that the chickens under test remained free from all of these conditions or factors. To rule out Marek's disease contamination, particular care was taken to look for flaccid neck ("floppy broiler") syndrome (vasculitis in the central nervous system). No clinical signs, tumors, skin lesions, or sciatic nerve enlargement were observed.

The absence of histopathological lesions of the BF confirmed the absence of field contamination throughout the study. Frequently, BF histopathological lesions are scored using the in-house lesion scoring scale in force in the laboratory. In the present study, BF lesions were assessed using the European Pharmacopoeia lesion scoring scale despite the fact that this scale is designed for the safety assessment of live IBD vaccines that are submitted to the regulatory authorities in the European Union; however, no live IBD vaccine was used in the present study. It is more up-to-date than the historical scale (Muskett et al., 1979).

Values of BF weight and volume steadily increased until the end of the study with dispersion widening as the birds aged, showing significant individual variation. Such individual variability had already been stressed by the historical studies; interestingly, intense and continuous genetic selection towards higher output and more uniformity in carcass or egg yield over the last 50 years has not removed individual variability in biological parameters. As a conclusion, it seems that such a strong individual variability between bursa weights is unavoidable, including when all possible variability factors are removed within the frame of an experiment (one single genetic line, one single gender, no Gumboro vaccination, neither IBDV nor other common field virus infections, no mycotoxins contamination in feed).

The lowest calculated BB ratio was consistently around 0.11 to 0.13 throughout the study, except at study initiation (d 1) where it was 0.07. This could represent a new standard of minimal BB ratio in male Cobb 500 commercial broilers, kept under ideal conditions (that is to say with minimum stress, and no diseases): a BB ratio of 0.11 or above from 7 to 42 days of age. Such a new reference is lower than the previous BB standards of McMullin (2004), for instance, who proposed 0.30. This author did not specify any breed, or any age, however. Interestingly, the proposed minimum figure currently seems to be lower compared with older studies; this could be related to the increasing body weight yield in the modern commercial breeds of broilers compared to the BF weight, hence a lower BB ratio.

In conclusion, this study showed that an ideal BB ratio potential of 0.11 or above could be observed in healthy male Cobb 500 commercial broilers from 7 to 42 days of age that were housed in isolated conditions. Despite efforts to remove any possible source of variation, the recorded figures were quite widely dispersed due to the remaining individual variability. Further studies are required to confirm the robustness of such findings in male Cobb 500 broilers by reproducing a similar experiment. A similar set of data would also be needed in female Cobb 500 broilers, and in other common commercial meat- or egg-type genetic lines in the field. This would help to update 50-year-old databases, and assist poultry field veterinarians and technicians in interpreting post mortem findings on BB ratio and modulate related conclusions, which are almost always drawn from a limited number of observations.

ACKNOWLEDGMENTS

This study has been financially sponsored by Ceva Sante Animale, reference C/320/11.

The authors would like to thank Fernando Lozano and John Elattrache for the English revision.

CONFLICT OF INTEREST

There is no conflict of interest.

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