Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Apr - Jun 2013 / v.15 / n.2 / 119-122

Effects of pre-slaughter fasting on broiler welfare, meat quality, and intestinal integrity

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■Keywords

Broiler industry, stress, intestinal morphology, intestinal morphometrics.

Submitted: February/2012
Approved: March/2013

ABSTRACT

The Brazilian Ministry of Agriculture (MAPA) regulations establish 12 hours as the maximum pre-slaughter fasting period for broilers; however, many processing plants have considered this time is not sufficient, and consequently return the birds to the farms, with consequent economic losses and welfare problems. Therefore, it is necessary to investigate the possible effects of longer pre-slaughter fasting times. The objective of the present study was to evaluate the effect of pre-slaughter fasting times longer than those established by MAPA on broiler welfare, breast meat quality, and intestinal integrity. Forty 42-d-old broilers were submitted to different pre-slaughter fasting times: group I: 6 hours, group II 9h, group III 12h, and group IV 15h. Bird welfare was assessed before slaughter. After sacrifice, intestinal samples were collected to assess their morphology and morphometrics, and the *Pectoralis major* muscle was analyzed for pH and color. There was no influence ($p>0.05$) of treatments on breast muscle pH or color.There were no significant changes in intestinal morphometrics ($p<0.05$). Bird behavior was affected ($p<0.05$), suggesting that welfare was impaired as fasting time increased, but no differences in the analyzed parameters were detected between broilers fasted for 12 or 15 hours. It was concluded that the behavioral differences between birds fasted for 12 and 15 hours are not sufficient to assert that those fasted for 15 hours were in worse welfare conditions.

INTRODUCTION

Pre-slaughter feed fasting, which consists of feed withdrawal a few hours before slaughter, is an important step in broiler processing as it affects meat yield and quality.Its objective is to reduce carcass contamination during processing, and therefore, the need of reprocessing (Duke *et al*., 1997; Northcutt *et al*., 1997).

The increasing trade of deboned parts and processed chicken products brought to light other concerns, such as the effect of fasting on meat quality traits, particularly pH, tenderness, cooking loss, and meat chemical composition (Ali *et al*., 1999; Beraquet, 1999; Berri, 2000). On the other hand, the scientific and technical communities are also increasingly concerned with animal welfare. This concept has changed in the last few decades because more people are now living in cities than in the country and their purchase power has grown, and therefore public demands on environmental protection and on the provision of proper welfare of livestock (Edwards, 2004; Nääs, 2005; Moura *et al*., 2006). This has led to further studies on pre-slaughter stress and to changes in the production systems by food companies, aiming at preventing any negative public perception of their products.

The intestinal epithelium is especially influenced by the absence of food. For instance, villus height is significantly reduced after 24h fasting (Yamauchi *et al*., 1996; Maiorka *et al*., 2002). In the study of Shamoto & Yamauchi (2000), broiler villus height was reduced after 72h fasting, and significantly increased three hours after refeeding.

Considering that the regulation of the Brazilian Ministry of Agriculture (MAPA) demands no more than 12h fasting, the knowledge on the effects of longer fasting periods on broiler welfare, meat quality, and intestinal wall integrity should be very useful.

MATERIALS AND METHODS

In the experimental poultry house of the School of Veterinary Medicine and Animal Science (FMVZ) of UNESP, Botucatu, Brazil, 200 male Cobb broilers were housed at an average density of 30kg/m^2. Water was supplied ad libitum until catching, and feed was offered until the beginning of the fasting period.

At 42 days of age, 40 birds were taken in specific transport crates to FMVZ experimental processing plant, and were distributed in four groups of 10 birds submitted to four different pre-slaughter fasting times:group I: 6 hours, group II: 9 hours, group III: 12 hours, and group IV: 15 hours. During lairage, broiler behavior was monitored every three hours. At the end of the fasting times, birds were electrically stunned and bled by cutting the jugular vein and carotid artery. Carcasses were scalded, eviscerated, and chilled.

Welfare was assessed in the lairage area of the experimental processing plant.Birds were remained in the transport crates at a density of 10 per cage during the welfare assessment. Crates were protected from direct sunlight, and environmental temperature ranged between 17 and 26°C. Welfare was assessed by observation of bird behavior, and the activities performed during 10 minutes were recorded for each fasting time (6, 9, 12, and 15 hours). Observations were plotted in frequency histogram, which showed the respective proportion of time used for the following behaviors: thermoregulation (panting), active or inactive (standing or sitting), distressed, excessive chirping. It must be noted that sitting, standing, and distressed can only be performed by the bird one at the time. Distressed in this study was considered when the birds does not keep standing or sitting, demonstrating discomfort with the situation, according to the methodology adapted from Campos (2000).

Breast meat pH was determined directly using a pH meter (Sentron, model 1001) coupled to a probe (Sentron, type LanceFET, model 1074001) with thin penetrating tip inserted 0.5-1.0cm below the surface of the left side of the breast muscle (*Pectoralis major*). This measurement was performed in all carcasses of all treatments two hours *post-mortem.*

Breast meat color was measures using a spectrophotometer (Konica Minolta CR 400, CIELab system) to determine L* (luminosity), a* (redness), and b* (yellowness) values inThree different points of the ventral surface and in the middle of the cranial section of the *Pectoralis major.* Breast meat was exposed to the air for 30 min before color measurements, as proposed by Van Laack *et al*. (2000).

Segments measuring approximately 2 cm were collected from the duodenum, jejunum, and cecum and immersed in Bouin solution for 24 h, according to the specifications of McManus & Mowry (1960). After being fixed and sectioned, the material was dehydrated by immersion in graded series of alcohol, cleared by three passages in xylol, and embedded in Polyfinparaplast. Tissues were cut with the aid of an automatic microtome (Leica, RM-2145) to obtain 4-µm thick cuts in a semi-serial sequence of a 30µm cut. Tissue sections were stained with periodic acid Schiff (PAS) and Masson trichrome stains, according to the methodology of McManus & Mowry (1960) and Behmer *et al*. (2003). Mucosa, submucosa, muscularis mucosa (circular internal and longitudinal external layers) and serosa thickness of the collected intestinal segments were determined by image analysis with the aid of an image-capture software program (Leica Qwin Lite 3.0). Five measurements were performed per layer.

Welfare data were submitted to analysis of variance for non-parametric data using the SAEG (1998) statistical package, and means were compared to the Mann Whitney test at 5% significance level.

The other results were submitted to analysis of variance using SAS (2004) statistical package. Means were compared by the test of Tukey, using the General Linear Models (GLM) procedure) at 5% significance level.

RESULTS AND DISCUSSION

Fasting time influenced ($p<0.05$) the evaluated behaviors, suggesting worse welfare as fasting time increased (Table 1). Other studies reported that broiler stress is directly proportional to fasting time

Table 1 - Broiler behavior in lairage.

		Standing		Sitting		Panting		Feather-pecking		Chirping		Distressed	
Time	N. birds	n	%	n	%	n	%	n	%	n	%	n	%
6h	40	8	20 a	32	80 b	0	0 c	0	0 c	2	5 b	0	0 c
9h	30	4	13 b	22	73 ab	0	0 c	0	0 c	3	10 b	4	13 b
12h	20	2	10 b	12	60 a	3	15 a	1	5 b	4	20 b	6	30 a
15h	10	0	0 c	5	50 a	1	10 b	3	30 a	7	70 a	5	50 a

Means followed by different letters in the same row are statistically different by the test of Mann Whitney ($p<0.05$).

(Mendes, 2001; Northcutt *et al*., 2003; Castro *et al*., 2008). In the present study, with 15 h of fasting, no birds were standing, there was a higher incidence of feather pecking, and chirping was more intense, suggesting that birds were distressed. However, the percentage of birds sitting and distressed was not different between 12 and 15 h of fasting. Behavioral adjustments occur faster than physiological adjustments, and therefore behavioral changes are reliable indicators of animal welfare. Agonistic behaviors, such as feather-pecking, as defined by Cast (1997), Martrenchar *et al*. (2000), and Marx *et al*. (2001), are associated to stress situations. Birds increasingly displayed agonistic behavior as fasting time increased, corroborating the hypothesis that welfare worsens as fasting time increases. However, this parameter was not different ($p>0.05$) between broilers fasted for 12 or 15 h.

There was no effect of treatment on breast meat pH (6h: 6.19 ± 0.04; 9h: 6.27 ± 0.04; 12h: 6.21 ± 0.04; 15h: 6.28 ± 0.04; $p> 0.1$), a* (6h: 2.98 ± 0.39; 9h: 2.73 ± 0.39; 12h: 2.82 ±0.39; 15h: 3.26 ± 0.39; $p> 0.1$) e b* (6h: 1.04 ± 0.41; 9h: 2.47 ± 0.41; 12h: 1.34 ± 0.41; 15h: 1.38 ± 0.41; $p> 0.1$) values. However, L* value was higher with 9h of fasting compared with the other fasting times (6h: 44.77 ± 1.00a; 9h: 49.07 ± 1.00b; 12h: 45.00 ± 1.00a; 15h: 45.24 ± 1.00ab; $p< 0.05$). Nevertheless, all determined L* values are within the range considered normal in literature, according to the chicken meat classification of Allen *et al*. (1998) in pale (L*>50.0) or dark (L*<45.0) or of Qiao *et al*. (2001), in pale (L*>53), dark (L*<46), or normal (46>L*<53). Differently from the present results, Castro *et al*. (2008), evaluating the effect of different fasting times (3, 6, 9, 12, 15, or 18h) on the breast meat of broilers reared in conventional systems, observed that L* values decreased as fasting time increased, despite the lack of statistical difference ($p>0.05$).

Table 2 - Breast meat pH and color (L*, a*, b*) values of broilers submitted to different *ante-mortem* fasting times.

	Treatments (fasting times)			
	6h	**9h**	**12h**	**15h**
pH	6.19 ± 0.04	6.27 ± 0.04	6.21 ± 0.04	6.28 ± 0.04
L*	44.77 ± 1.00 a	49.07 ± 1.00 b	44.92 ± 1.00 a	45.24 ± 1.00 ab
a*	2.98 ± 0.39	2.73 ± 0.39	2.82 ± 0.39	3.26 ± 0.39
b*	1.04 ± 0.41	2.47 ± 0.41	1.34 ± 0.41	1.38 ± 0.41

Means followed by the same letter are not different by the test of Tukey ($P<0.05$).

There was no effect ($p>0.05$) of treatments (6h, 9h, 12h, 15h *ante-mortem* fasting) on duodenum (6h: 944.6; 9h: 932.5; 12h: 1010.0 and 15h: 957.9), jejunum (6h: 946.7; 9h: 944.2; 12h: 991.9 and 15h: 992.1) or cecum (6h: 419.9; 9h: 447.1; 12h: 443.1; 15h: 445.7) villi height (Fig 1, 2, and 3; Table 3). It must be noted that mucosa thickness affects villus height. Mucosa thickness in the evaluated intestinal segments is presented in Table 4. These results are consistent with the findings of Thompson & Applegate (2006), who observed that there are villi height, crypt depth, and cell migration and proliferation changes in the intestinal segments of birds submitted to long fasting times (< 24 h), but these changes are not evident when short fasting times (< 24 h) are applied. Duodenum and jejunum villi heights measured in the present study was consistent with those reported by Mitchel and Carlisle (1992) and Okamoto *et al*. (2009) of 938 µm, in average, in the jejunum and by Smith *et al*. (1990), of 1310 µm and 850 µm in the duodenum of broilers selected for fast growth and non-selected broilers, respectively.

The absence of villi height differences among broilers submitted to different pre-slaughter fasting times in the present experiment indicates that there was a balance between two cytological events (cell renewal and cell loss due to sloughing), suggesting that digestion, absorption, and intestinal integrity were not influenced by any of the fasting times, including 15 h of fasting (Figures 1, 2, and 3)

Table 3 - Mucosa thickness (µm) of the duodenum, jejunum, and cecum of broilers submitted to different *ante-mortem* fasting times.

	Treatment			
Mucosa	**6h**	**9h**	**12h**	**15h**
Duodenum	944.6	932.5	1010.0	957.9
Jejunum	946.7	944.2	991.9	992.1
Cecum	419.9	447.1	443.1	445.7

Means followed by different letters in the same row are statistically different by the test of Tukey ($p<0.05$)

CONCLUSIONS

It is concluded that 15 hours of pre-slaughter fasting does not impair breast meat quality and does not affect the morphology and the morphometrics of the duodenum, jejunum, and cecum of broilers, particularly when compared to 12 hours of fasting. The worse welfare of broilers detected after 12 hours of fasting demonstrates that increasing pre-slaughter fasting times increases bird distress.

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