Influence of flock treatment with the antibiotic tylosin on poultry meat quality: results of a preliminary experiment

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Abstract

The veterinary antibiotic tylosin was administered to broilers at sub-therapeutic and therapeutic levels to study its effect on the quality of poultry breast meat. No statistically significant differences were observed in moisture content, pH, drip loss, colour and extent of lipid oxidation between the breast meat from treated and not treated birds. However, the cooking loss of the meat from the birds administered tylosin was significantly higher than that from the not treated ones. Additionally, the mean shear force of the breast meat was significantly lower for the sub-therapeutically treated broilers than for the not treated and the therapeutically treated ones. It was concluded that the level at which tylosin was administered to the broilers affected the quality of the breast meat, particularly its textural properties.

Additional keywords: breast muscle, colour, cooking loss, drip loss, lipid oxidation, moisture content, shear force, tenderness

Introduction

Antimicrobials and other veterinary drugs are used in animal husbandry not only to treat diseases but also to improve growth performance and health status of the animals. In the case of monogastric animals like pigs and broilers, antimicrobials are mainly administered to control the gut flora. In that way, producers try to prevent the onset

of sub-clinical infections and the misuse of the nutrients provided by the feed. Antibiotics, especially feed antibiotics, are administered to suppress the pathogenic bacteria causing sub-clinical infections and to reduce the production of bacterial toxins, the bacterial consumption of nutrients and the consequent suppression of a 'good' gut flora. A continuous use of antibiotics as growth promoters may, however, induce the proliferation of resistant pathogenic bacteria and the transfer of resistance traits to other organisms, with potentially grave consequences to both animal and human health, and the environment (Barton, 2000; Cromwell, 2002; Salyers, 2002).

In agreement with the 1999 Precautionary Principle of the European Commission (Anon., 2000), EU member states have banned some antimicrobial veterinary drugs that are widely used elsewhere. Moreover, they are preparing the implementation of a ban on the use of growth-promoting antibiotics in animal husbandry from January 2006 onwards (Casewell *et al.*, 2003). This ban means a considerable transformation for the whole of the European meat supply chain – from consumers and retailers to meat processors and animal growers. However, on the very short run hardly any scientific studies are being planned to assess the impact of growth-promoting antibiotics on the objective and perceived quality of meat or meat products. This is quite surprising, given that the direction and intensity of this impact may greatly affect purchasing behaviour and retail prices, thereby indirectly bearing on the economic sustainability of European Union's food supply chains.

The attributes judged to be the most important for the overall quality evaluation of fresh poultry meat by consumers are meat and skin colour, absence of visual defects (bruises and haemorrhages), tenderness, flavour and juiciness (Issanchou, 1996; Berri, 2000). Meat colour is thought to be determined mostly by rearing and veterinary practices, i.e., by the type of feed and feed additives administered, and the health status of the birds (Fletcher, 2002). The deposition of pigments in the skin of broilers has been shown to be genetically determined and to depend on dietary sources of the pigments, health status and meat processing operations. Also poultry diseases, particularly coccidiosis, have been demonstrated to substantially (negatively) affect pigmentation. A good health status of the flock is then crucial to the achievement of the desired uniform pigment absorption and deposition in individual birds. The absence of signs of bruising and haemorrhages is equally influenced by the flocks' health status, besides factors related to rearing, pre-processing and processing activities. So it is not surprising that administering anticoccidial drugs and antimicrobial growth promoters has been established as a major factor influencing the positive development of broiler skin pigmentation and the absence of visual defects (Fletcher, 2002). On the other hand, poultry meat's tenderness and juiciness are believed to be not only influenced by transporting, slaughtering and processing operations but also by the specific rearing system (Castellini *et al.*, 2002). Finally, also the preparation of meat by consumers or cooks is believed to play a role in the overall perceived quality, as it affects the flavour and juiciness of the cooked product (Issanchou, 1996; Berri, 2000; Fletcher, 2002).

Ultimate muscle pH, shear force, cooking loss and water-holding capacity of the meat are assumed to provide good estimates for its colour, tenderness and juiciness. On the other hand, fatty acid composition, extent of lipid oxidation and total and haem-iron can provide a reasonable indication of flavour development during cooking

(Castellini *et al.*, 2002; Fletcher, 2002). Apart from its effect on skin appearance, not much is known about the potential influence on poultry meat quality of the veterinary regime of the broilers. One of the few related studies published so far addresses the growth-promoting effect of antibiotics when administered in sub-therapeutic regimes to pigs, which is stated to result also in meat of a better quality, with less fat and increased protein content (Hughes & Heritage, 2004). If the continuous administering of antibiotics in low dosage to farm animals during growth (i.e., a sub-therapeutic regime) leads to changes in the protein and fat content of their meat, then it may probably also have a significant effect on one or more other meat quality aspects. How, and to what extent this effect takes place in broilers is yet unclear. Therefore, the general aim of this preliminary study was to establish the effect of the antimicrobial veterinary drug tylosin administered to broilers on the objective quality of fresh poultry meat.

Materials and methods

A 6-week experiment was conducted with 60 male chicks that were randomly assigned to three experimental treatments representing three levels of a veterinary antibiotic. Following slaughtering, several quality parameters of the breast meat from these birds were compared. The animal ethical committee of Wageningen University and Research Centre in Lelystad, The Netherlands approved the experiment.

Experimental details

The chicks, of the Ross breed, were one day old when the experiment started. Each experimental treatment comprised 2 × 10 birds, randomly divided over two adjacent 1-m² indoor pens, the bottoms of which were covered with litter. The 6 indoor pens were positioned next to each other to ensure that all birds were reared under the same conditions, except for the experimental treatments, and that a potential confounding effect of the use of different pens on treatment results would be prevented.

Three treatments were compared. Two groups of 10 birds were offered the antibiotic in a therapeutic regime, two groups in a sub-therapeutic regime, and two groups served as control and were not offered the antibiotic.

The veterinary antibiotic used was tylosin tartrate (Tylan® Soluble AF1300, Batch number A90772DE, expiry date 11/2005, Elanco, Greenfield, Indianapolis, USA). Tylosin was chosen as it is frequently used in broiler rearing, both as a growth promoter and as an antimicrobial agent. Tylosin is particularly effective against infections with *Clostridium perfringens*, a bacterium causing *necrotic enteritis* in birds (Anon., 1999a, b).

The antibiotic in the sub-therapeutic regime was offered during 24 days prior to slaughtering and in the therapeutic regime during 5 days prior to slaughtering, as recommended by the manufacturer for each of the purposes intended. The dosages were 150 mg and 530 mg tylosin per litre of drinking water, respectively. On the last day before slaughtering no tylosin was offered.

All birds were fed *ad libitum* with the same commercial poultry diet during their entire life period. Also the drinking water was available without restrictions. However,

the consumption of feed or drinking water was not recorded. The animals stayed in good condition during the experimental period and no additional medical treatment was necessary. All birds were slaughtered at the same time, exactly 6 weeks after the start of the experiment.

Sampling and data collected

The 20 broilers of each treatment were weighed together at the end of the experiment, just before slaughtering. The birds were then stunned with CO_2 and killed by manual exsanguinations. Their breasts were plucked, excised (including muscles and ribs), labelled and stored for 24 h at 4 °C. The next day, the *pectoralis major* muscle of each breast was excised and weighed individually. The meat's pH was measured 24 h post mortem with a digital pH-meter (Portamess 913, Knick, Berlin, Germany) equipped with a combined pH and a reference electrode (Inlab®427, Mettler Toledo, Schwerzenbach, Switzerland) at two different points of one of the breast muscles of each bird (Dunn *et al.*, 1993). Drip loss was measured once using the disk filter paper method (Kauffman *et al.*, 1986). The raw meat's colour parameters L*, a* and b* were measured at two points on the skin side of the muscle, using a laser analyser (cm525i, Minolta, Tokyo, Japan) and the Cielab Color System (Anon., 1978). The second breast muscle of each bird was stored for 24 h at 4 °C awaiting subsequent determination of moisture content and extent of lipid oxidation.

Following pH, drip loss and colour measurements, each of the breast muscle samples was placed in a polyethylene bag, which was vacuum sealed and immersed in a pre-heated (75 °C) re-circulating water bath for 30 minutes to ensure that the core temperature reached 70 °C (Cyril *et al.*, 1996). After cooling down under running cold tap water for 30 minutes, the cooked breasts were removed from their bags, dried with a paper towel and re-weighed (post-cooking weight). Cooking loss was calculated as the difference in percentage terms between pre- and post-cooking weight (Honickel, 1998). Three 1-cm³ meat cubes were cut perpendicularly to the fibre direction from the middle part of each cooked breast for measuring shear force with a Warner-Bratzler apparatus equipped with a texture analyser (TA-HDi, Stable Micro Systems, Surrey, UK). Shear force values determined in this way express the tenderness of meat after cooking (Anon., 1995).

Moisture content of the meat was determined gravimetrically, measuring the weight loss following heating a 5-g sample from each stored raw breast muscle for 15 h at 105 °C in an electric oven (Abeni & Bergoglio, 2001). The extent of lipid oxidation of the raw breast meat was determined using the thiobarbituric acid reactive substance (TBA-RS) method of Juncher *et al.* (2000). Seven grams of minced meat from each breast muscle sample were homogenized for 60 seconds in a 25-ml solution of tri-chloroacetic acid (7.5 % trichloroacetic acid, 0.1% propylgallate, 0.1% EDTA and 1 ml sulphanilamide). The liquid was then filtered through a folding filter (S&S 595½, Ø 185 mm, Schleicher & Schuell GmbH, Dassel, Germany), and 3 ml of the filtrate was mixed with 3 ml 0.02 M thiobarbituric acid solution. This mixture was heated for 30 minutes at 100 °C and cooled down until room temperature. The absorbance at 538 nm of the resulting samples was measured with a spectrophotometer (Varian, type

Cary 50, Roosendaal, The Netherlands), and the respective malondialdehyde (MDA) content determined using an appropriate calibration curve. TBA-RS values were expressed in mg MDA per kg raw meat.

Statistical analysis

A statistical analysis was conducted of the data set obtained from the breast meat analyses. As the 60 animals were housed under identical conditions, the 20 birds per treatment – although distributed over two pens – were regarded as a single group. Furthermore, the *ad libitum* feeding and the unrestricted supply of water, which contained the antibiotic, made us consider the individual birds of a group as independent animals. Means and standard deviations of the measured quality parameters were calculated for each of the three treatment groups (group size = 20). Student's two sample t-test was used to separate statistically different (P = 0.05) treatment means of the breast meat quality parameters for the three treatments.

Results and discussion

Growth performance

The average weights of the not treated, the sub-therapeutically and the therapeutically treated birds at the time of slaughtering (i.e., total live weight at the end of the experiment divided by 20) were 2575 g, 2850 g and 2627 g, respectively. As the birds had not been weighed individually at any time during the experiment, the effect of tylosin on live weight could not be statistically analysed. Nevertheless, at the end of the rearing period the sub-therapeutically treated birds weighed, on average, about 11% more than the not treated birds, and about 8% more than the therapeutically treated ones. The difference in average weight between the therapeutically treated and the not treated birds was only about 2%. As all other variables that could have influenced growth performance, like feed, race, health status or rearing system, were controlled during the experiment, and were the same for each treatment, it was concluded that the sub-therapeutic tylosin treatment promoted growth. Given that the effectiveness of antimicrobial veterinary drugs as growth promoters of farm animals, including broilers, has already been established (Gaskins *et al.*, 2002; Dibner & Richards, 2005), this conclusion is warranted.

Breast meat quality aspects

The quality parameters of the breast meat for the experimental treatments are summarized in Table 1. The mean values for pH, drip loss, colour (L*, a*, b*), moisture content and extent of lipid oxidation did not differ significantly (P < 0.05) between the treatments. However, the mean cooking loss values of the meat samples of the therapeutic and sub-therapeutic treatments differed significantly (P < 0.05) from the values recorded for the samples from the not treated birds. Moreover, the mean shear force

Table 1. Means and standard deviations of the quality parameters of the breast meat of the broilers in each experimental treatment (n = 20).

Quality parameter	Not treated			Sub-therapeutic			Therapeutic		
pH	5.92	±	0.09	5.91	±	0.09	5.94	±	0.08
Drip loss (g × 100)	4.39	±	0.79	4.15	±	1.01	4.06	±	0.97
Colour									
L*	55.04	±	2.12	54.69	±	2.15	54.74	±	1.84
a*	6.36	±	0.67	6.70	±	0.87	6.84	±	0.90
b*	12.58	±	0.96	12.94	±	1.28	13.08	±	0.79
Cooking loss (%)	6.78	±	0.99	7.86[ab]	±	0.95	8.57[ab]	±	1.35
Shear force (kg cm^{-2})	3.39	±	1.02	2.59[ab]	±	0.49	3.40[bc]	±	0.39
Moisture content (%)	73.06	±	0.63	72.90	±	0.89	73.36	±	0.76
TBA-RS (mg MDA per kg)	0.99	±	0.32	0.88	±	0.19	1.06	±	0.38

[a] Statistically different from control group in the same row ($P < 0.05$).

[b] Means of sub-therapeutic and therapeutic treatments in the same row differ statistically from each other ($P < 0.05$).

[c] Statistically different from sub-therapeutic group in the same row ($P < 0.05$).

Means in the same row without superscripts are not statistically different ($P < 0.05$).

values for the meat from the sub-therapeutically treated birds differed significantly ($P < 0.01$) from the mean values for both the not treated and the therapeutically treated ones.

The mean pH values for the breast muscles from the three treatments are characteristic of those found in broiler breast muscle (Dunn *et al.*, 1993; Dransfield & Sosnicki, 1999; Castellini *et al.*, 2002). As the pH of poultry meat is determined mainly by the animal's pre-slaughtering and slaughtering conditions (Bate-Smith & Bendall, 1949), which were controlled in this study, no statistically significant differences in pH values were expected between the treatments. No statistically significant differences in drip loss from the raw breast muscle between the three treatments were found either, which might be explained by the fact that the post-mortem conditions (storage time and temperature, and pH) for the three treatments were similar (Offer & Trinick, 1983; Honickel, 1998). As stated earlier, colour variation in poultry meat depends on primary production factors (breed, age, and nutritional status), pre-slaughtering and slaughtering conditions, and subsequent storage (Honickel, 1998; Berri, 2000; Fletcher, 2002). Since these factors were controlled in this study, and as no statistically significant differences in pH were observed, we did not expect to find any statistically significant difference in the colour parameters' values between the treatments either, as was indeed the case.

Broiler's rearing conditions (bird density and the amount of movement the animals are allowed), race and age at slaughtering have been identified as the main factors affecting moisture content of poultry meat (Abeni & Bergoglio, 2001; Castellini et al., 2002). Again, since these factors were controlled during the experiment, no statistically significant differences were found in moisture content of the breast meat among the three treatments.

The oxidative stability of lipids in poultry meat has been associated with the peroxidative processes taking place after slaughtering, which in turn have been linked to the oxidative capacity of muscle meat and its haem-iron content (Castellini et al., 2002). It is also known that exercise increases the amount of haem-iron, particularly in the more oxidative muscles (O'Brien et al., 1992), and that physical fitness increases the oxidative capacity of muscle meat (Petersen et al., 1997). As the housing and rearing conditions were the same for all animals in this study, we did not expect to find any statistically significant difference in the oxidative stability between the meat from the two treatments or the control, as was indeed the case. The low TBA-RS values obtained for the breast samples are in agreement with the limited space and movement during growth and with the slaughtering age, which was lower than in similar studies carried out elsewhere (e.g. Castellini et al., 2002).

The number and size of muscle fibres is related to changes throughout the animal's growth. It has been established that the muscle fibres of broilers from fast-growing lines have a larger diameter than those from broilers from slower growing ones. Larger fibre diameters may cause a lower fibre packing density in the muscle, thus increasing the tenderness of meat (Dransfield & Sosnicki, 1999). The results of the shear force measurements in this study could lead to a parallel hypothesis. As suggested above, the difference in live weight observed between the sub-therapeutically treated animals on the one hand and the therapeutically and not treated ones on the other could be attributed to a growth-promoting effect of the tylosin in a sub-therapeutic regime. The moisture content of the breast meat did not statistically differ between the three treatments. This could lead to the hypothesis that a higher live weight might translate itself in a higher protein content of this muscle as fat usually constitutes only about 2% of the breast meat (Castellini et al., 2002). More muscle protein results in fibres with a larger diameter (Offer & Trinick, 1983; Dransfield & Sosnicki, 1999), explaining the significantly higher tenderness of the breast meat from the sub-therapeutically treated birds compared with the therapeutically and the not treated ones (Table 1).

The results obtained from the cooking loss measurements are somewhat more puzzling. It is thought that the water in meat is held by capillarity. Part of it is held in the inter-filament space within myofibrils (the structural unit of muscle fibres), part in the extracellular space and the rest between the myofibrils within a fibre (Offer & Trinick, 1983). Muscle meat composed of fibres with a large diameter is less cohesive and has a less compact structure than meat with smaller fibres (Dransfield & Sosnicki, 1999). So it is reasonable to assume that this large-fibre meat will also have a lower water-holding capacity, given that the space within and between its myofibrils will be bigger. Let us assume that our hypothesis on the link between the growth-promoting effect of tylosin administered at a sub-therapeutic level, muscle fibre diameter and the observed tenderness of the corresponding meat is correct. It is then reasonable to

expect that this meat will also exhibit a higher cooking loss than the meat from the not treated birds. This expectation was indeed confirmed by the cooking results (Table 1). However, we could not find any convincing argument for the cooking loss of the meat from the therapeutically treated birds being significantly higher than that of the meat from the two other treatments. A possible explanation may be related to an element of stress introduced by the administering of a very high dosage of an antibiotic to healthy animals a few days before they were slaughtered. If stress played a role, one would also expect other effects of it, e.g. on the quality parameters drip loss and shear force. But this was not the case.

Implications

The results of this preliminary study indicate that the implementation of the 2006 EU ban on the use of antimicrobial growth promoters in animal husbandry could affect the eating quality of fresh poultry meat, provided that the differences observed in texture and cooking loss turn out to be large enough to be perceived by consumers. This issue should be further investigated through the performance of sensory and consumer acceptance studies. Such studies should include the preparation of poultry meat as this is most often carried out in household kitchens and not only as prescribed by analytical methods.

The EU ban will almost certainly result in an increase of the poultry meat price. So it would be interesting to investigate whether (1) the results of our study can be repeated or extended, and (2) consumers are willing to pay a premium price for a perceived (and objective) safety improvement, especially if that also implies compromising on relevant quality characteristics, such as cooking losses.

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