



## Effect of apple pomace degraded by *Pleurotus ostreatus* on growth and meat quality parameters of broiler chickens

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### Abstract

The objective of this work was to compare the growth and physicochemical parameters of the meat of broiler chickens (*Gallus gallus*), fed with a supplemented diet (SD) consisting of 3% pleurotin, compared to those obtained when using a traditional diet (CD). Pleurotin is the remaining material from the biodegradation of substrates such as apple pomace used to cultivate the *Pleurotus ostreatus* fungus. The experience lasted 48 days and in the first eight days, the birds were divided into two groups of 24 individuals, one group for each treatment. On day 12, each initial group was subdivided into four experimental units (replicates) of 6 chickens each. The growth parameters measured were total weight gain (TWG), final live weight (FLW), carcass weight (CW), average daily gain (ADG) in weight, and feed conversion ratio (FCR). Physicochemical parameters such as pH, water content (WC), CIE color, total fat (TF), and fatty acid composition (FAC) were also measured. Statistical analysis was carried out through the t-Student independent samples test. FLW was on average 7.6% significantly higher with SD than with CD, however, no difference was detected in CW. The total ADG was 7.4%, significantly higher with SD while the FCR was 32.0% lower with SD than with CD. No significant differences were found in any of the meat quality parameters measured for the same piece. Despite these latest results in terms of meat quality, it is concluded that the increase found in total ADG and FLW, and an improvement in FCR values with SD favor the use of this amendment in chicken rearing. In addition, the introduction of the by-product of the juice industries of the Río Negro Valley in other production cycles, such as edible mushrooms production and a supplement for bird diets (pleurotin), promotes a circular bioeconomic mechanism.

**Key words:** pleurotin, supplemented diet, feed conversion ratio.

## Efecto del bagazo de manzana degradada por *Pleurotus ostreatus* sobre parámetros de crecimiento y calidad de carne de pollos parrilleros

**Resumen.** El objetivo de este trabajo fue comparar parámetros de crecimiento y fisicoquímicos de la carne de pollos parrilleros (*Gallus gallus*) alimentados con una dieta suplementada (SD) con 3% de pleurotina versus una dieta tradicional (CD). La pleurotina es el remanente de la biodegradación del sustrato (orujo de manzana) utilizado para el cultivo de *Pleurotus ostreatus*. La experiencia duró 48 días. A los 8 días, las aves se dividieron en dos grupos de 24 individuos, un grupo para cada tratamiento. El día 12, cada grupo se subdividió en cuatro unidades experimentales (réplicas) de 6 pollos cada una. Los parámetros de crecimiento medidos fueron: ganancia de peso total (TWG), peso vivo final (FLW), peso de la carcasa (CW), ganancia total de peso promedio diaria (ADG) y conversión alimenticia (FCR). Se midieron como parámetros fisicoquímicos: pH, contenido de agua (WC), color CIE, grasa total (TF) y composición de ácidos grasos (FAC). Los resultados obtenidos mostraron que el FLW fue en promedio 7,6% significativamente más alta con SD que con CD. La ADG total resultó 7,4%, significativamente mayor con SD. La FCR fue 32% menor con SD. No se detectó diferencia en el CW. Asimismo, no se hallaron diferencias significativas en ninguno de los parámetros de calidad medidos para la misma pieza. Se concluye que el incremento en la ADG total y la FLW y la disminución de los valores de FCR con SD, favorecería el empleo de pleurotina en la crianza de pollos. La introducción de subproductos de las industrias jugueras del Valle de Río Negro en otros ciclos productivos, como lo son la producción de hongos comestibles y de un suplemento para dietas de aves (la pleurotina), promueve un mecanismo bioeconómico circular.

**Palabras clave:** pleurotina, dieta suplementada, conversión alimenticia

## INTRODUCTION

One of the main residues of the juice industry in the Alto Valle and Valle Medio del Río Negro, Argentina, is apple and pear pomace. Due to its lignocellulosic and acid nature, it is a material that is difficult to degrade and its accumulation causes alterations in the environment such as the leaching of fermentation products, the attraction of pests, emission of odors, etc. (Sadh et al. 2018, Toledo et al. 2018).

Fungi of the *Pleurotus* genus have a lignocellulolytic metabolism that facilitates the degradation of the pomace. For this reason, they are used as a substrate for the cultivation of oyster mushrooms (*Pleurotus* sp. fungi).

Formerly, the substrate degraded by fungi was called “spent” substrate, some of its components are exhausted when they are used as a source of nutrients by the cultivated mushroom. The spent mushroom substrate (SMS) is a lignocellulosic biomass that contains fungal mycelium and a biodegraded substrate. Currently, it is considered a valuable waste, as a source of raw material for new processes, but it is still named SMS by some authors (Koutrotsios et al. 2014, Mohd Hanafi et al. 2018). Other authors call the remnant obtained from biodegradation pleurotin (Bermúdez Savón et al. 2014) and according to its characteristics and rich composition in bioactive phytochemicals such as antioxidant, antimicrobial, immunomodulatory and anti-inflammatory compounds (Mutlag et al. 2017), it could be used as an additive in dietary supplements for animal feed, contributing to the good health status of the animals and the quality of their meat (Lai et al. 2015, Hsieh et al. 2021).

Intensive poultry farming, according to the figures provided by Statista (2022), in 2021 about 105 million metric tons (MMT) of broiler meat was estimated to be produced in the whole world. The USA was the top producer of chicken meat in the world with 20.4 MMT, Brazil in second place with 14.5 MMT, and Argentina in ninth place with 2.3 MMT.

Thanks to the technological advances experienced in recent years (genetic improvements, automation, health plans, etc.), broiler chicken reaches the weight required for slaughter (2.27 kg) in less than 50 days (Kumar et al. 2019). Poultry products can be considered one of the most important sources of cheap protein, where white meat (poultry meat) is very cheap as compared with red meat (cow meat). Poultry production is characterized also by a higher conversion rate of feed to meat in comparison with other animals, where the production of one kg of poultry meat needs from 2.00 to 2.50 kg of feed (Utami and Wahyono 2018).

Antibiotic supplementation has been widely used in recent decades to stabilize intestinal microbiota, improve production parameters, and prevent avian diseases. However, the usefulness of this strategy has been questioned due to the emergence and spread of antibiotic-resistant bacteria in meat or promoting abdominal fat accumulation in broilers (Hassan et al. 2020, Pesciaroli et al. 2020). Therefore, there is renewed interest in finding viable alternatives to antibiotics (Nunes et al. 2012, Mehdi et al. 2018, Nair et al. 2018).

Fernandes et al. (2019) had shown that apple pomace pectic polysaccharides and xyloglucans can present hydrophobic features. Arabinoxylans are the major non-starch polysaccharide fractions in wheat, which increase digesta viscosity, reduce the digestibility of nutrients and decrease the feed efficiency and growth performance when fed to poultry, especially in broiler chickens (Kumla et al. 2020).

This work aimed to compare the results obtained for some growth and physicochemical parameters of meat quality of broiler chickens (*Gallus Gallus*), fed with a diet supplemented with 3% of pleurotin against those obtained when using a traditional diet.

## MATERIALS AND METHODS

**Pleurotin.** Pleurotin was obtained by cultivating *Pleurotus ostreatus* on 1 kg bags of apple pomace, for a period of 50 days. This period was divided into an initial phase without light, during the first 15 days, in which the primary *Pleurotus* inoculum colonizes the medium. Subsequently, once a total coverage of the mycelium is observed over the entire pomace mass, the bags were subjected to a daily photoperiod of 12 hours, an average temperature of  $22\pm 2$  °C, and a relative humidity of  $85\pm 5\%$ . During the luminous phase, the fruiting bodies of the fungus emerged through the bag, and after a few days they were harvested, leaving the apple pomace and the mycelia of the fungus as a homogeneous mass which was subsequently used for food supplementation of the chickens.

**Broilers' Management and Treatments.** The study was conducted at Hospital Escuela de Medicina Veterinaria (HEMEVE) de la Universidad Nacional de Río Negro, Choele Choel, Argentina, in March-May 2019.

Forty-eight 2-day-old non-sexed broilers were randomly assigned to two treatments: the control diet (CD) treatment, in which a conventional diet was used with balanced meals for initiation and completion, and the supplemented diet (SD) treatment, which replaced 3% of the constituents of the conventional diet with pleurotin.

The experience lasted for 48 days and in the first eight days, the birds were divided into two groups of 24 individuals, one group for each treatment (CD and SD), in two identical cages, with a total area of 30 m<sup>2</sup>. The birds were fed *ad libitum* and the temperature was controlled at 25 °C, using air conditioning equipment and infrared lights. In this initial period, they consumed the same type of commercial starter, as well as water consumption.

From the ninth day, supplementation began in the group corresponding to the supplemented diet (SD), in which 3% m/m of the commercial food was replaced by pleurotin. On day 12, each initial group was subdivided into four experimental units (replicates) of 6 chickens each, which were assigned an area of 2 m<sup>2</sup>. The change from starter to finisher feed was made on day 33 and day 40 for ground corn.

Oxytetracycline was given at a concentration of 1 mg L<sup>-1</sup> during the first seven days of stay through drinking water. They were also given piperazine 1.5 g L<sup>-1</sup> of water (antiparasitic) for one day.

After slaughtering, the cloaca of each bird was swabbed to discover the flora, focusing on *Salmonella*, *Shigella*, and lactic acid bacteria (LAB). Selective and differential culture medium *Salmonella Shigella* (S-S) Agar (Britania®) was used for isolation from *Salmonella* spp. and *Shigella* spp. starting of swabs from the cloaca. Man Rogosa Sharpe (MRS) Agar (Merck®) was used for isolation from LAB. GRAM stains were also made, with microscopic observations to characterize the enteric flora.

Birds were starved for 12 hours but were allowed access to fresh water. All birds were slaughtered according to animal welfare regulations, bled appropriately, plucked, and eviscerated (SENASA 2015).

**Growth parameters.** The individual weight of day-old birds was recorded. Weighing thereafter was done at non-regular time intervals (between two and five days) to assess the weight evolution as affected by the treatments. The weights were measured using a weighing scale with a  $\pm 1$  g accuracy. The accumulated weight increase per interval was recorded.

The average daily gain (ADG) in weight during the whole period (48 d) of the experiment was determined.

$$ADG = \frac{\text{Total weight gained}}{\text{number of days}}$$

Final live weight (FLW) and carcass weight (CW) were measured before and after the slaughter and cleaning process, respectively.

Feed conversion ratio (FCR) was computed with feed intake and the live weight data during the total period as follows:

$$FCR = \frac{\text{Total amount of feed consumed}}{\text{live weight produced}}$$

Mortality was recorded, and after slaughtering and cleaning, carcasses were stored in a freezer ( $-5$  °C) in hermetically sealed bags until further analysis.

The beak -the distance between the top of the upper mandible and its corners- (BL), and the tarsus -the distance between the calcaneus and ankle- (TL) of each bird was measured using a gauge.

**Physicochemical meat parameters.** The following physicochemical meat (breast and thigh) parameters were determined:

- pH with a puncture electrode pH meter (Testo 205), immediately after slaughtering.

-Water content (WC) (oven at  $T = 105$  °C until constant weight).

-CIE  $L^*$ ,  $a^*$ ,  $b^*$  color parameters using a Minolta CR400 colorimeter,  $45^\circ$  angle, illuminant D65 on 1.6 cm thick samples placed on a white slab.

- Total fat (TF) (ANKOM T10 extractor).

- Fatty acid composition by GLC, performing methylation with NaOH 0.5N in methanol, F3B as a catalyst and internal standard C23:0 (Matreya LLC) and the corresponding chromatographic analysis (GC Clarus 500, PerkinElmer, capillary column CP-Sil 88 (100 m  $\times$  0.25 mm id; Varian).

**Statistics.** The means of the treatments were evaluated through the T-Student independent samples test. The normal distribution of the data and variance homogeneity were previously verified by applying the Shapiro-Wilks' and Levene's tests, respectively (Di Rienzo et al. 2008). In those cases, in which the test assumptions were not met, data transformations were performed to ensure these assumptions. In all cases, the data presented correspond to the untransformed values.

## RESULTS AND DISCUSSION

Table 1 shows mortality and the growth parameters measured in the assay.

**Table 1.** Mortality, mean values, and Standard Deviation of the growth parameters.

	CD	SD
Mortality	1	1
FLW (kg)	3.03 $\pm$ 0.09 <sup>a</sup>	3.26 $\pm$ 0.14 <sup>b</sup>
CW (kg)	2.47 $\pm$ 0.36 <sup>a</sup>	2.47 $\pm$ 0.23 <sup>a</sup>
ADG (g·day <sup>-1</sup> )	63.2 $\pm$ 1.9 <sup>a</sup>	67.9 $\pm$ 2.9 <sup>b</sup>
FCR (kg·kg <sup>-1</sup> )	2.36 $\pm$ 0.2 <sup>a</sup>	1.79 $\pm$ 0.6 <sup>b</sup>
BL (mm)	19 $\pm$ 2 <sup>a</sup>	19 $\pm$ 1 <sup>a</sup>
TL (mm)	88 $\pm$ 7 <sup>a</sup>	94 $\pm$ 7 <sup>a</sup>

CD: control diet, SD: supplemented diet, FLW: final live weight, CW: carcass weight, ADG: total average daily gain in weight, FCR: feed conversion ratio, BL: beak length, TL: tarsus length. Different letters in each row are statistically different (T-Student  $p \leq 0.05$ ).

The mortality rate was identical and low (4%, 1 bird) both in the control and with the supplemented diet, coinciding with what was reported by Daneshmand et al. (2011), by Toghyani et al. (2012) and by Abou-Zeid et al. (2019) who state that the improvement of viability rate may be related to some bioactive compounds such as (polysaccharides, glucan peptides, vitamin, minerals, and polyunsaturated fatty acid) resulted in increasing immunity response of birds fed mushroom containing diets.

The FLW was on average about 8% significantly higher with the supplemented diet than with the control diet, with the chickens reaching an average of 3.26 kg after 48 days of rearing (Table 1). This last value is almost identical to the one mentioned by Kumar et al. (2019). The difference observed in the FLW differs from that found by Daneshmand et al. (2011) who used a control diet supplemented with 2g kg<sup>-1</sup> of *P. ostreatus* residues. Toghyani et al. (2012) report that a diet supplemented with oyster mushroom powder in a proportion of 20 g kg<sup>-1</sup> improved the live weight and feeding efficiency of chickens (up to 28 days). These authors state that this could indicate that the improvement in FLW during this period may have partially been due to improved nutrient digestibility and better nutrient absorption as reflected by improved feed efficiency.

Regarding the CW, no significant differences were found in both treatments, which agrees with what was found by Toghyani et al. (2012) with a supplemented diet

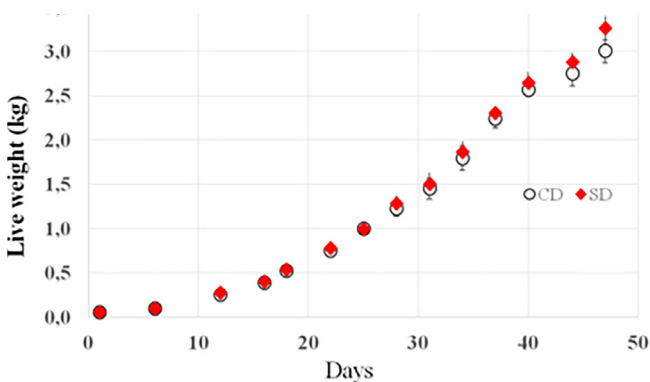
of up to 20 g kg<sup>-1</sup>, by Ekunseitan et al. (2017) with 15 ppm ethanolic extract of *P. ostreatus*, and by Foluke et al. (2014) who measured different carcass cuts.

The FCR was significantly smaller with the SD than with the CD, indicating that pleurotin promotes feed conversion in chicken meat; possibly due to the antibiotic effect of *Pleurotus* remaining in the broiler (Hassan et al. 2020). This result agrees with what was expressed by Akomah et al. (2021) using 1 g kg<sup>-1</sup> of *P. ostreatus* as a supplement in pelleted form, and by Hassan et al. (2020) who used a supplemented diet with 1% and 2% of *P. ostreatus* waste; but differs from what was reported by Foluke et al. (2014) when using a *Pleurotus* spp. compounds or wastes at various levels that detected no significant differences in FCR in broilers.

As stated by Cowieson and Selle (2019), modern-day broilers are capable of converting dietary feed ingredients into chicken meat at ratios approaching 2:1 as was found with the SD in this work and constitute a more ecologically sustainable protein source for human consumption than alternative sources.

Using the SD, an increase of 7.4% was obtained in the total ADG concerning the CD (Table 1). This is a better result compared with that of a not significant 3.6%, obtained by Camay (2016) using 2.5% of mushroom (*P. ostreatus*) waste powder as a supplement to broiler basal feed.

Figure 1 illustrates the mean live weight values of each treatment throughout the rearing time. It can be noted that as of day 28, a slight increase in live weight is observed in broilers fed with the supplemented diet compared to the control. This difference in the last few days can explain the different significant values of the FLW shown in Table 1. The aforementioned increase is more evident from day 40, from which it is inferred that the slaughter carried out from day 30 can provide broilers with a higher live weight at the expense of a supplement amount in the conventional feed.



**Figure 1.** Live weight vs. rearing time for broilers fed with a control diet and supplemented diet.

When evaluating the enteric flora of the cloaca of the birds, no differences related to the dietary treatments were observed.

The lengths of the beaks and tarsi did not differ significantly, depending on the diet used. This could be attributed to the fact that males and females were not differentiated in this work and to the difficulty of accurately measuring these quantities. Adamu et al. (2018) also found no differences between the body linear measurements of broiler chickens fed different wood ash-based diets, except for body length. Table 2 shows the pH values and the CIE color parameters.

**Table 2.** Mean values and Standard Deviation of the physicochemical parameters.

	Breast		Thigh	
	CD	SD	CD	SD
pH	6.05±0.22 <sup>a</sup>	6.32±0.24 <sup>a</sup>	6.14±0.16 <sup>a</sup>	6.13±0.10 <sup>a</sup>
L*	63.80±1.72 <sup>a</sup>	63.89±2.53 <sup>a</sup>	53.62±3.45 <sup>b</sup>	54.05±3.67 <sup>b</sup>
a*	0.37±0.66 <sup>a</sup>	0.24±0.58 <sup>a</sup>	6.65±1.18 <sup>b</sup>	6.51±1.89 <sup>b</sup>
b*	17.16±1.33 <sup>a</sup>	17.16±1.33 <sup>a</sup>	16.28±0.91 <sup>a</sup>	17.50±1.12 <sup>a</sup>

Different letters in each row are statistically different (T-Student  $p \leq 0.05$ ).

The pH values do not differ significantly between diets or types of meat, they are within the range that is considered standard quality for chicken meat (5.8-6.3) as reported by Ristic and Damme (2013). Although the values (6.05-6.32) for the breast and (6.13-6.14) for the thigh are similar to those reported by Lee et al. (2012) with broiler chickens supplemented with *P. eryngii*, these authors found significant differences in the statistical analysis and found a slight increase with the diet supplemented in both types of meat. The pH value directly reflects the muscle acid content and affects the drip loss, water-holding capacity, and color.

The average pH value immediately measured after slaughtering was between 6.05 and 6.32 for the broiler breast meat (which are considered normal values for this meat). The pH is an indicator of meat quality, and a low pH (<5.7) at 24 h *postmortem* is indicative of poor meat quality (Battula et al. 2008).

No significant differences were found in the mean values of L\* (lightness), a\* (redness), and b\* (yellowness) for both breast and thigh with both diets, although they were L\* and a\* were different in the breast than in the thigh. The mean values of dark coloration of the thigh concerning those of the breast are attributed to the higher concentrations of hemoglobin and haem pigments in the thigh than in the breast (Wideman et al. 2016).

According to Qiao et al. (2002) the values of L\* for chopped breasts measured after 24 h are considered normal, however, the values of a\* are lower than those reported by these authors, while those of b\* are higher. Regarding this last parameter, there is a clear increase in the reported values over time. As stated by Mir et al. (2017), it must be considered that factors affecting poultry meat color are the state of the haem pigments, pre-slaughter factors (genetics, feed, handling, stress, heat, and cold stress, gaseous environment), slaughter, chilling and processing conditions.

Table 3 shows the fatty acid composition and water content measured in the assay.

**Table 3.** Mean values and Standard Deviation of the fatty acid composition and water content.

% (DB)	Breast		Thigh	
	CD	SD	CD	SD
WC	2.99±0.28 <sup>a</sup>	2.88±0.12 <sup>a</sup>	2.74±0.21 <sup>a</sup>	2.70±0.19 <sup>a</sup>
TF	14.20±4.95 <sup>a</sup>	9.72±1.20 <sup>a</sup>	26.35±4.02 <sup>b</sup>	30.62±4.09 <sup>b</sup>
FA	6.34±0.65 <sup>a</sup>	6.62±0.54 <sup>a</sup>	22.20±2.09 <sup>b</sup>	25.51±2.75 <sup>b</sup>
SFA	27.75±0.88 <sup>a</sup>	28.17±1.38 <sup>a</sup>	25.18±0.50 <sup>b</sup>	26.49±0.85 <sup>ab</sup>
MUFA	35.75±3.51 <sup>a</sup>	35.80±1.87 <sup>a</sup>	38.86±1.93 <sup>a</sup>	38.72±1.68 <sup>a</sup>
PUFA	29.12±3.86 <sup>a</sup>	28.70±1.54 <sup>a</sup>	32.28±2.93 <sup>a</sup>	29.35±2.41 <sup>a</sup>
FA n-3	2.81±0.42 <sup>a</sup>	2.74±0.16 <sup>a</sup>	3.00±0.31 <sup>a</sup>	2.68±0.25 <sup>a</sup>
FA n-6	26.31±3.45 <sup>a</sup>	25.96±1.38 <sup>a</sup>	29.28±2.62 <sup>a</sup>	26.68±2.16 <sup>a</sup>

CD: control diet, SD: supplemented diet, %DW: % dry weight; WC: water content; TF: total fat; FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; FA n-3: fatty acid n-3; FA n-6: fatty acid n-6. Different letters in each row are statistically different (T-Student  $p \leq 0.05$ ).

## CONCLUSION

The final live weight (FLW), and the total average daily gain (ADG) in weight were significantly higher with the pleurotin-supplemented diet than with the control diet (7.6% and 7.4%, respectively), indicating that pleurotin promotes feed conversion in chicken meat, possibly due to the antibiotic effect. Besides, FCR was 32% smaller with SD than with CD.

It should be emphasized that the use of this supplement was in a very low concentration (3%) and since the productive parameters evaluated, as well as the quality of the meat, were not negatively affected by the interference of pleurotin, it could be considered to incorporate the supplement in a higher percentage.

On the other hand, since pleurotin is obtained from a product that is considered waste in the region's agri-food industry, its incorporation into another food chain is important as a circular bioeconomy mechanism, promoting the sustainability of natural resources.

Meat quality indicators such as pH and color were not different with the control diet than with the one treated with pleurotin. Neither were significant differences found in the composition of fatty acids present in the breast or thigh of the chickens between treatments.

The length of the beaks and tarsi did not show significant differences with both treatments, so it is not a variable to take into account in future trials where broiler chickens are used without differentiating sex and especially due to the uncertainty of the measurement.

Further investigations should be carried out to verify the results found in this work, especially focused on the quality of the meat obtained with this supplement.

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