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Supplementation of grape pomace on broilers fed a high non-starch polysaccharide diet and its effects on growth performance, gut function, intestinal microbiota, and meat quality Matthew J. Pataki \*, Nikita Agarwal, Melissa Huang, Nikolai Kolba, Peter R. Gracey, Eliot M. Dugan, Chloe B. Giovannoni, Sara E. Stadulis, Qiang Sui, Patrick A. Gibney, Elad Tako

## ABSTRACT

The United States poultry industry has transitioned from the use of antibiotics as growth promoters to no antibiotic ever programs. This might increase the chances of exposure to several factors that can induce inflammatory responses, such as reused litter, intestinal pathogens, poor-quality feed ingredients, high-energy diets, or heat stress, which can produce subclinical and clinical gut inflammation. Simultaneously, approximately 20% of the 73 million tons of grapes produced annually worldwide go unused, resulting in grape pomace (GP), a polyphenol-rich by-product that has the potential to mitigate these negative impacts of inflammation.

**This study aimed** to investigate the potential use of grape pomace and fermented grape pomace as a substitute for antibiotic growth promoters in broiler feed. A total of 126 (29 d old) Cornish cross broilers (Gallus gallus) were divided into six treatment groups: i) standard diet wheat-corn-soybean meal (STD), ii) 30% rice bran or non-starch polysaccharide (NSP), iii) NSP + zinc bacitracin (AGP), iv) NSP + 0.5% GP (GP), v) NSP + 0.5% Lactobacillus casei fermented GP (LAB FGP) and vi) NSP + 0.5% Saccharomyces cerevisiae EC1118 fermented GP (YST FGP).

The results showed that the NSP-fed group exhibited lower body weight and feed intake compared to STD, however, the inclusion of either GP or FGP improved the growth performance, feed intake, and feed conversion ratio (P < 0.05). Dietary inclusion of the GP or FGP improved the breast muscle yield of chickens compared to NSP; however, it was still lower than the STD (P < 0.05) groups. Inclusion of the FGP was able to reduce the cecal bacterial populations (Clostridium, Klebsiella) compared to NSP, except for E. coli, which was reduced by AGP (P < 0.05). Furthermore, GP inclusion led to a significant increase in the expression of duodenal barrier integrity-related proteins, whereas FGP reduced the expression compared to NSP (P < 0.05). The inclusion of GP and LAB FGP improved the villus surface area and reduced the muscularis thickness of the duodenum compared to NSP (P < 0.05). These findings suggest that a 0.5% dietary incorporation of either fermented or non-fermented grape pomace could potentially serve as an alternative to antibiotic growth promoters in broiler production.

# **RESULTS & DISCUSSION**

Figure 2. Results showed that the NSP-fed group had lower body weight and feed intake, but all treatments mitigated this growth reduction.



n=21 per group. In a row, means assigned





### METHOD

**Study Design:** A total of 126 Cornish cross broilers (*Gallus gallus*) were divided into six treatment groups (n = 21):

- standard diet wheat-corn-soybean meal (STD)
- 33% rice bran or non-starch polysaccharide (NSP) ii)
- NSP + zinc bacitracin (AGP) iii)
- NSP + 0.5% GP (GP)iv)
- NSP + 0.5% *Lactobacillus casei* fermented GP (LAB FGP) V)
- NSP + 0.5% Saccharomyces cerevisiae EC1118 fermented GP (YST FGP) vi)





different lowercase superscript letters are significantly different, P < 0.05.

Figure 3. GP inclusion significantly increased (P < 0.05) gut-barrier-integrity-related proteins (AU).

Group	OCLN	ZO-2	Claudin-3	Claudin-4
STD	$1.76\pm0.03$ $^{\rm a}$	$2.11\pm0.07~^{ab}$	$2.57\pm0.04$ a	$1.95\pm0.03$ a
NSP	$1.78\pm0.05$ $^{\rm a}$	$2.2\pm0.07$ a	$2.52\pm0.07~^{ab}$	$1.97\pm0.06~^{ab}$
AGP	$1.71\pm0.05~^{ab}$	$2.18\pm0.05$ a	$2.49\pm0.05$ a	$1.91\pm0.07~^{ab}$
GP	$1.94 \pm 0.07$ a	$2.34 \pm 0.09$ a	$2.66 \pm 0.05$ a	$2.11 \pm 0.04$ a
LAB FGP	$1.52\pm0.03$ bc	$1.75\pm0.02$ bc	2.19 ± 0.01 b	$1.76\pm0.01$ b
YST FGP	1.49 ± 0.01 °	$1.75\pm0.02$ °	$2.2\pm0.02$ b	1.79 ± 0.01 b
Low AU				High AU

Values are mean ± SEM, n = 7, a,b,c within a column, indicating that means without a common letter are significantly different, P < 0.05 as per Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparison.

**Table 1.** Histological analysis showed variations in villus surface area, muscularis thickness, and crypt depth across different groups.

Group	Villus surface area (mm²)	Crypt Depth (μm)	Muscularis thickness (μm)
STD	<b>112.8 ± 3.2</b> <sup>b</sup>	<b>29.1 ± 1.3</b> <sup>ab</sup>	<b>169.6 ± 3.9</b> <sup>bc</sup>
NSP	99.9 ± 3.2°	<b>20.4 ± 0.8</b> <sup>c</sup>	<b>183.5 ± 4.1</b> <sup>b</sup>
AGP	<b>100.0 ± 4.1</b> <sup>c</sup>	<b>27.2</b> ± <b>1.3</b> <sup>b</sup>	<b>170.9 ± 5.2</b> <sup>bc</sup>
GP	107.6 ± 3.9 <sup>bc</sup>	<b>28.7</b> ± <b>1.2</b> <sup>ab</sup>	158.9 ± 4.5°
LAB FGP	143.1 ± 5.7°	<b>31.1</b> ± <b>1.3</b> <sup>ab</sup>	<b>148.1 ± 4.1</b> <sup>d</sup>
YST FGP	97.8 ± 4.0℃	<b>33.0 ± 1.3</b> <sup>a</sup>	<b>201.8 ± 4.7</b> <sup>a</sup>



**Figure 1.** Depicts the feeding trial design and introduction of the inflammation diet and treatments at different time points. All birds were fed on the standard diet from 1 d to 21 d. All but group 1 were switched to 30% rice bran (NSP) diet. The treatments (AGP, GP, FGP LAB, and FGP YST) were introduced on 29 d. On 34 d, birds were weighed, and blood was drawn from all groups. On day 42, 21 randomly selected birds from each treatment group were euthanized and blood, duodenum, cecum, and the right breast muscle were collected for further analyses (gene expression of key proteins, brush border membrane morphology).

The values are given as means  $\pm$  SEM (n = 5). In a row, means assigned different lowercase superscript *letters are significantly different, P < 0.05 as per Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparison.* 

#### CONCLUSION

These findings further demonstrate the nutraceutical benefits of grape pomace and suggest that incorporating 0.5% grape pomace could replace AGPs in broiler production. However, fermenting grape pomace did not provide significant additional benefits. Further studies are needed to investigate the benefits of grape pomace in healthy and compromised intestinal health.

#### REFERENCES

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