Quebracho Tannin Influences Lipolytic Activity in Mature Porcine Adipocytes

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Abstract

Fat deposition in pork enhances flavor of meat; however, too much fat is an undesirable commodity in a health-conscious society. Therefore, manipulating the nutritional components of a swine diet to aid in the deliberate deposition of fat for the purpose of flavor while avoiding overconditioning is an aim in production. Nutrient additives, such as condensed polyphenolic tannins, inhibit pre-adipocyte maturation, but the role on lipid metabolism in mature adipocytes (MA) remains unclear. Therefore, it is hypothesized that quebracho tannin will alter lipid metabolism in porcine MA. Subcutaneous adipose tissue was collected from 5 ± 0 month old (n=3) barrows weighing 37.7 ± 1.84 kg. Tissue was enzymatically dispersed (collagenase type II) to isolate lipid filled adipocytes. After enzymatic separation the cells were rinsed and divided into 2 groups for separate incubation periods plus tannin treatment: 1) 2 hr incubation time with/without tannin (Quebracho *Schinopsis lorentzii;* 0 mg, 0.1 mg, 0.5 mg, and 1 mg) or 2) 24 hr incubation time with/without tannin (0 mg, 0.1 mg, 0.5 mg, and 1 mg). Approximately 4.0x10^5 cells/well were cultured in triplicate/treatment dose at 37°C with 5% CO_2 in atmosphere. Upon termination of the culture period, media was processed for analysis of glycerol content to determine lipolytic activity using an enzymatic colorimetric assay. The MIXED procedure of SAS for factorial treatment design was utilized to determine the effect of time and tannin treatment on lipolytic activity in cultured MA. Glycerol content was significantly higher (P≤0.001) in tannin treated cultures. Time tended (P=0.1) to influence the magnitude of lipolytic activity. Hence, quebracho tannin appears to augment lipolytic activity in cultured porcine MA. Determining the effect of tannin on lipolytic regulators will support the supposition that tannins influence MA lipid metabolism.

Introduction

Overconditioning livestock in food production is an issue that producers aim to regulate. While fat deposition in pork does in fact enhance the flavor of the meat product, the nutritional perception of overconditioning in a health-conscious society can be a dilemma for producers. To avoid overconditioning the nutritional components of the swine diet can be manipulated by the addition of nutrient additives. Nutrient additives such as tannins can inhibit preadipocyte maturation, which prevents the formation of new lipid-filled fat cells. Tannins are natural plant derived polyphenolic compounds that are classified as hydrolysable or condensed [1,2]. Condensed polyphenolic tannins have been shown to increase enzymatic protein expression and lipolytic activity in goat adipose tissue [3]. Ruminant animals are more tolerant to tannins and can use them with relative efficiency due to microbial activity in the rumen[4,5]. Monogastic animals exhibit a lower tolerance to tannins; however, in measured quantities tannins may have beneficial properties to cellular utilization of nutrients similar to ruminants. Therefore, the study reported herein was conducted to determine the affect of condensed tannins on porcine MA to investigate a potential use of tannins in the regulation of adipocyte metabolism.

Materials & Methods

- **Animals**
  - Three crossbred (Yorkshire x Hereford x Hampshire) barrow piglets, 5 ± 0 months of age weighing 37.7 ± 1.84 kg.
- **Tissue Collection**
  - Piglets were anesthetized and subcutaneous adipose tissue (3 grams) was harvested from the cervical vertebral region.
  - Tissue Transport: Harvested adipose tissue were placed in pre-warmed (37°C) Hanks solution (HyClone; Logan, UT) for transportation. Adipose tissue was enzymatically digested for cell culture.
- **Cell Preparation**
  - **Cell Isolation:**
    - Tissue was digested in phenol-free Dulbecco's Modified Eagle's Medium Nutrient Mixture F12 Ham (DMEM; Sigma Aldrich; St. Louis, MO) with Type II Collagenase (1.5 mg/ml; Sigma) in a vigorously shaking water bath at 37°C for 90 min.
- **Cell Culture**
  - **Treatment Culture:**
    - Mature adipocytes (MA; 100% cell viability via trypan blue test) (~4.0x10^5 cells/well) were cultured in triplicate in standard non-attachment media [phenol-free DMEM, 0.1 mg/ml streptomycin, 100 U/ml penicillin, 2% CFBS, 1.5% bovine serum albumin, 2.5 mM L-glutamine] with/without treatment of quebracho tannin (0 mg, 0.1 mg, 0.5 mg, and 1 mg) at 37 °C in an atmosphere of 5% CO_2 in air with 95% humidity for 2 or 24 hr.
      - Quebracho (*Schinopsis lorentzii)* tannin is a condensed form of tannin from a species of tree indigenous to arid climates.

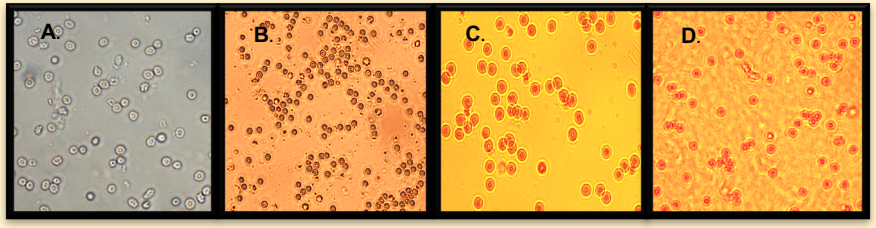

Figure 1. Integrity of cells with/without treatment. A. MA in 0 mg treatment, B. MA in 0.1 mg treatment, C. MA in 0.5 mg treatment, and D. MA in 1 mg treatment.

- **Glycerol Assay Analysis**
  - **Enzymatic Colorimetric Assay:**
    - Free glycerol content was measured using an enzymatic colorimetric assay glycerol kit from Sigma Aldrich for the cultured cells with/without treatment of quebracho tannin (0 mg, 0.1 mg, 0.5 mg, and 1 mg) for 2 or 24 hr incubation periods.
- **Statistical Analysis**
  - The MIXED procedure of SAS for factorial treatment design was utilized to determine the effect of time and tannin treatment on lipolytic activity in cultured MA. The PDIFF option was conducted to identify significant differences among treatment means.
  - All means are reported as LS MEANS±SEM.

Results

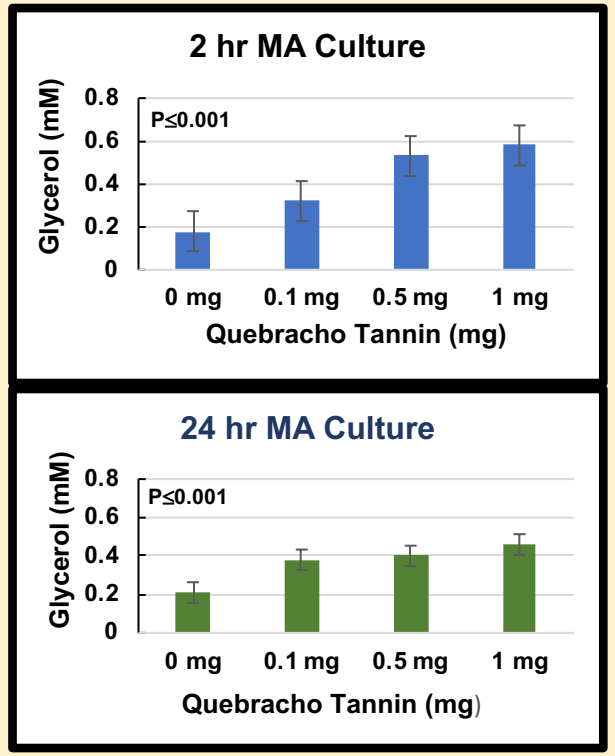

Figure 2. Glycerol media content after MA cultured with/without the addition of quebracho tannin (0 mg, 0.1 mg, 0.5 mg, and 1 mg) for 2 or 24 hr. Addition of quebracho tannin increased glycerol content in both the 2 and 24 hr cultures. Time tended (P=0.1) to influence availability of free glycerol in the 24 hr culture.

Conclusion

- Quebracho tannin appears to augment lipolytic activity in porcine MA adipocytes
- Time tended to stabilize the influence of tannin on lipolytic activity.
- Investigating the mechanism of quebracho tannin on lipolytic activity in porcine MA will support a potential use for the molecule in the whole animal.

Acknowledgements

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