AMERICAN WAGYU ASSOCIATION

STUDY

NEWSLETTER

June | 2016

A LETTER FROM - MARTIN ANDERSEN

Dear AWA Member,
The American Wagyu Association is pleased to announce we have reached a point where we can conduct the Lipid Study. This is an important study that will provide valuable evidence of the beef quality and health benefits of Wagyu beef as compared to Angus beef, as well as for grass-fed vs. grain-fed. We are excited to be conducting this study and we need your help to make it a reality. To complete the Study we need registered steers born between January 1st and April 30th 2016 for delivery to the feeding facility after weaning. The delivery date will be announced later. Texas A&M will provide the Angus cattle.

We will need:

- 20 Black Fullblood Wagyu
- 20 Red Fullblood Wagyu
- 20 F1 Wagyu (Angus type dams sired by black Wagyu bulls)

You will retain ownership of your steers and receive all of the processed meat other than one steak that will be used for testing for the study. The AWA will provide transportation to the feed lot for the animals selected. The committee will announce the selected animals around July 1st.

Feed and per diem cost estimates for the study, which you will be billed for monthly are:

- Grass fed black Wagyu F1: $1.00/day for 540 days.
- Grain fed black Wagyu F1: $2.50/day for 540 days
- Grass fed red Wagyu full blood: $1.00/day for 660 days.
- Grain fed red Wagyu full blood: $2.50/day for 660 days.
- Grass fed black Wagyu full blood: $1.00/day for 660 days.
- Grain fed black Wagyu full blood: $2.50/day for 660 days.

We have contacted some Wagyu meat companies about purchasing the carcasses if you do not need some or all of your harvested animals and we expect to have this as an option prior to final commitments of the steers to the study.

Please contact Martin Andersen at trrwagyu@yahoo.com or 406-858-2284 if you have any questions. For members who would like to participate in this study please complete the attached nomination form and submit it by email office@wagyu.org or by mail to American Wagyu Association, PO Box 3235, Coeur d Alene, ID 83816.

Best regards,
Lipid Study Committee

Lipid Study Submission Form
2016 Spring Born Steers

Name		**AWA Membership #**	
Address		**Contact Person (s)**	
City		**Address**	
State		**Phone**	
Zip Code		**Email**	

	Steer Information				Category			Parents	
#	Animal IDENT or Tattoo/Tag	Animal Date of Birth mm/dd/yy	Name or Suffix (If Applicable)	Fullblood Registration # (If Applicable)	Fullblood Red Wagyu	Fullblood Black Wagyu	F1	Sire Registration # or Information	Dam Registration # or Information
Ex	Z 123	04/01/16	XYZ Yasu Doi	FB0003		√		FB0001	FB0002
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Print Full Name _____ Signature _____ Date _____

Once you have completed form/s they can be faxed to (208) 292-2670, emailed to office@wagyu.org or mailed to:

Amercian Wagyu Association
PO Box 3235,
Coeur d'Alene, ID 83816

LIPID QUESTIONS AND ANSWERS

Q: How can I participate in the study?
A: Simply fill out the form that is part of this Newsletter.

Q: How many animals can I submit?
A: As many as you'd like, as long as they meet the criteria outlined on the form.

Q: If more animals are offered than needed how will the eventual participants be determined?
A: The Lipid study committee will establish a series of criteria that will insure a good balance of genetics and then it will be first come first serve.

Q: Who will feed the animals?
A: Texas A & M University will establish the feeding protocol and provide all care for the subject animals in College Station Texas.

Q: Will I retain ownership of my animal(s)?
A: Yes you will and you can pick up the carcass at the conclusion of the test.

Q: How is the ration established?
A: The University will determine the ration and will vary it as needed to maintain an efficient feeding program.

Q: Who will pay for the cost of feed?
A: You the participants will pay for your animals ration.

Q: How much will it cost?
A: It is difficult to give an exact cost at this time, however it will be comparable to feeding costs in other feedlots around the country. An estimate is about $1.00 per day for grass fed and $2.50 per day for grain fed for each animal.

Q: Who's going to supply the angus steers?
A: Texas A & M University will supply the Angus Steers.

Q: How will I pick up the carcass when the study is completed?
A: Each individual will be responsible for pickup of their individual carcass (s). Fabrication of that carcass may be arranged with the University.

Q: Can I sell my carcass on the rail?
A: Absolutely, in fact several members have agreed to buy the carcasses of any of the participants who wish to sell at the end of the study. The price cannot be yet established, but it will be at a premium over the market at the conclusion of the test.

Q: Can I pick the study ie. Grass or Grain fed?
A: Absolutely not. This is a blind study and no attempt will be made to channel the animals in any particular direction.

Q: How big and how old should the calves submitted be?
A: The study is to kick off with the reception of the cattle in September or October of this year. An exact date has not yet been determined. It is anticipated that calves will then be in the six to seven month old range and weigh in the five hundred plus pound range. They should be spring 2016 calves.

Q: Who will be responsible for getting my animal(s) to the test site?
A: The Committee will arrange for pickup. The number of animals being donated by one site, and the distance will be a part of the selection criteria.

WAGYU SHORT TERM STUDY OUTLINED

As many of you know your American Wagyu Association is undertaking academic studies to determine, if you will, the place Wagyu fills in the health conscience diet of modern day America. For years Wagyu advocates have touted Wagyu as the healthy breed, indicating that Wagyu fat composition has a higher ratio of monounsaturated fat to saturated fat and that it is high in oleic acid. Two studies have been created to validate these claims. Both to be conducted under the leader ship of Dr. Stephin Smith of Texas A&M /University.

The short term or first of these studies has actually been completed, and was outlined by Dr. Smith at our annual General Meeting in Ocala and once again at the recent Texas Wagyu Association meeting in Salaso as well as the Australian Wagyu Association meeting in Hunters Valley NSW.

To conduct the short term study, samples from ten full-blood Wagyu grain-fed animals were received from two producers Free range natural chickens were purchased from local grocery stores, and ten samples of Pacific Northwest wild caught salmon were provided.

The results of this study are outlined below, but in essence it does show we're on the right track for the proposed long term study.

Martin Anderson the chairman of the Lipid Study Group has introduced the requirements of the long term study and we have provided a detailed outlook of the study. The key to this Newsletter however is the sign up form. Think about it. This is an opportunity to get some data on your animals at minimum cost. Build your EPD information, with the basic cost of feed only and freezer full of Wagyu meat in the end. Heck of a deal.

WAGYU SHORT TERM STUDY - RESULTS

I. Investigators

Stephen B. Smith (contact PI), Professor, Department of Animal Science, Texas A&M University, College Station, TX 77843. Phone: 979-845-3939, E-mail: sbsmith@tamu.edu Sherwin Siff, M.D., Clinical Professor, Baylor College of Medicine and UTMB, Galveston, TX Jimmy Horner, Ph.D., CEO, Protocol Technologies, Bridgeport, TX

Project title: Comparing differences in lipid composition among Wagyu beef, poultry, and salmon

II. Objectives

Document and compare total lipids, fatty acid composition and lipid melting points of Full Blood Black Wagyu cattle, free range chicken breasts, and wild salmon

III. Rationale and Approach

Free range chicken breasts and wild salmon are felt to be much healthier then any type of beef. This is thought to be certain by physicians, nutritionists, dietitians and many chefs as well as a large portion of our population. Is this true with beef from Full Blood Black Wagyu cattle, and if so, how much difference exists?

IV. Study Design - Source of Materials

Rib steaks were obtained from two sources of Black Wagyu Full Blood steers, Lone Mountain Cattle Company and Muddy Flats Cattle Company. Additionally, chicken breasts from 10 free range chicken breasts were purchased from three retail outlets in the Bryan/College Station area, and filets from 10 fresh caught salmon were shipped by Ralph Valdez.

Table 1. Study Design

Group	Meat Source	Number of Samples
1	Full Blood Black Wagyu steers, grain-fed	10
2	Chicken breast, free range	10
3	Salmon, fresh-caught	10

V. Methods

Samples and shipping. Beef rib steak, chicken breast, and salmon filets were frozen and shipped on dry ice to the Department of Animal Science, Texas A&M University.

Fats and moistures. Representative portions of each muscle sample (approx. 100 g of each muscle) were pulverized under liquid nitrogen. Five grams of each pulverized samples were analyzed for total fat and moisture on a CEM rapid analyzer as per the manufacturer's specifications.

Fatty acid analysis. Fatty acid composition of lipids was measured by standard laboratory techniques (Chung et al., 2006; Turk and Smith, 2009). Approximately 5 g of each pulverized muscle sample was homogenized with 5.0 mL of chloroform:methanol (2:1, vol/vol) and held with shaking at room temperature (approximately 20°C) for 48 h to extract total lipids. Approximately 100 mg of each lipid extract was converted to its fatty acid methyl esters, which were analyzed with a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA), equipped with a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Identities of FAME were established by comparison to authentic standards (GLC 96; Nu-Chek Prep, Inc, Elysian, MN, U.S.A.).

Slip points. Melting points of the lipids were approximated by determining slip points by techniques established by this laboratory (Smith et al., 1998). After heating to approximately 45°C, lipids were drawn 1 cm into glass capillary tubes. Duplicate tubes were collected for each sample and frozen at –20°C. After freezing, the capillary tubes were suspended vertically in a chilled water bath with the portion of the tube containing lipid submerged in the water. The water bath was heated at 2°C/min with constant stirring, and temperature of the water was monitored with a Type K thermocouple (model KTSS-HH, Omega Engineering, Inc., Stamford, CT, U.S.A.) attached to a digital thermometer (model 91100-50, Cole-Parmer Instrument Co., Vernon Hills, IL, U.S.A.). Slip point is defined as the temperature at which the lipid moved up the capillary tube.

Cholesterol concentrations. Cholesterol concentrations of the muscle samples will be analyzed using gas chromatography (Chung et al., 2006). Briefly, 100 g of each muscle was freeze-dried and homogenized in a home-style electric grinder. Cholesterol was extracted with 3 mL of ethanol to 100 mg of dried tissue. The lipids was saponified by the addition of 1 mL of 33% (wt/vol) KOH and heating for 60 min in an 80 – 90°C water bath. Cholesterol was isolated on an SPB-1, fused capillary column [30 mm X 0.53mm (i.d.)](Supelco, Bellefonte, PA, U.S.A.). Stigmasterol was used as the internal standard to quantify the total cholesterol.

Statistical analysis. All statistical analyses was performed by using SPSS version 11 (SPSS Inc., Chicago, IL, U.S.A.). Fatty acid composition, total fat and moisture, slip points, and cholesterol concentrations were compared by ANOVA. The $P < 0.05$ probability level was established for statistical significance.

VI. Results and Discussion

Fatty acid percentages. Wagyu rib steaks contained an average of 16.1% total extractable lipid, while chicken breast and salmon filets contained 1.8 and 2.7% total lipid, respectively (Table 1). Oleic acid comprised 45.2% of the total lipid in Wagyu steaks, whereas oleic acid comprised 31.8 and 16.0%, respectively, of chicken breast and salmon filet total lipids. Lipids from salmon filets contained 15.6 and 22.4%, respectively, of the marine fatty acids, eicosapentaenic acid (EPA) and docosahexaenoic acid (DHA), whereas chicken breast and

Wagyu rib steaks contained less than 1% EPA and DHA. Chicken breast contained more linoleic acid (the most abundant omega-6 fatty acid) and α-linolenic acid the most abundant omega-3 fatty acid) than either salmon or Wagyu beef. A portion of the linoleic acid was converted to arachidonic acid, reflected by the fact that chicken breast contained more arachidonic acid that salmon or Wagyu beef. Some portion of the α-linolenic acid was converted to EPA and DHA, which were higher in chicken breast than in beef.

Lipid melting points of chicken breast (29.9°C) and Wagyu rib steaks (27.3°C) did not differ statistically, but both were higher than lipid melting points of salmon filets (2.5°C) (Table 1). The low melting point of the salmon filet lipids was caused by two factors: The low percentage of stearic acid (the primary determinant of lipid melting points of beef; Wood et al., 2004; Turk and Smith, 2009); and the high percentages of EPA and DHA, which individually have melting points less than 20°C.

Cholesterol and fatty acid contents. Chicken breast and salmon filets contained approx. the same amount of cholesterol (40.9 and 38.6 mg cholesterol/100 grams of meat, respectively) (Table 2). Rib steaks contained 91.7 mg cholesterol/100 grams of beef, due to the higher lipid content of the beef relative to the chicken and salmon. Cholesterol is located both in cellular membranes and in the lipid droplets contained with fat cells. We recently analyzed cholesterol content in Akaushi ground beef, which contained 96 mg cholesterol/100 grams of beef. Because of the low lipid content of chicken breasts and salmon filets, there was less than one gram of the individual fatty acids per 100 grams of meat (Table 2). The salmon filets contained an average of 0.403 grams EPA and 0.489 grams DHA, whereas EPA and DHA were virtually undetectable in Wagyu beef.

The Wagyu rib steaks contained 7.23 grams of oleic acid /100 grams of beef, which was the most abundant fatty acid in the Wagyu beef. The MUFA:SFA ratio was the same in chicken breast (1.20) as in Wagyu rib steaks (1.14), both of which were higher than the MUFA:SFA ratio in salmon filets (0.82). By comparison, we have determined that lipid from Akaushi ground beef has a MUFA:SFA ratio of 1.26, and the MUFA:SFA ratios of conventional and grass-fed ground beef are 0.79 and 0.90, respectively.

In a previous study, we determined that Akaushi ground beef increases HDL cholesterol (the good cholesterol) in women (Gilmore et al., 2013). In that study, the Akaushi ground beef had a MUFA:SFA ratio of 1.43, and the ground beef patties contained 24 grams of fat per patty. In an earlier study, we documented that ground beef from long-fed, corn-fed Angus steers (MUFA:SFA = 1.10) increased HDL cholesterol in men. We conclude that Wagyu beef contains sufficient oleic acid to cause the same health benefits when fed to men and women.

Literature Cited

Chung, K. Y., D. K. Lunt, C. B. Choi., S. H. Chae, R. D. Rhoades, T. L. Adams, B. Booren, and S. B. Smith. 2006. Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. Meat Sci. 73:432-441. Gilmore, L. A., R. L. Walzem, S. F. Crouse, D. R. Smith, T. H. Adams, V. Vaidyananthan, X. Cao, and S. B. Smith. 2011. Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. J. Nutr. 141:1188-1194. Gilmore, L. A., S. F. Crouse, A. Carbuhn, J. Klooster, J. A. E. Calles, T. Meade, and S. B. Smith. 2013. Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein but not high-density lipoprotein 2b cholesterol caused by high-oleic ground beef in women. Nutr. Res. 33:1003-1011. Smith, S. B., A. Yang, T. W. Larsen, R. K. and Tume. 1998. Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. Lipids 33:197-207. Turk, S. N., and S. B. Smith. 2009. Carcass fatty acid mapping. Meat Sci. 81:658-663. Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66:21-32.

Table 1. Fatty acid composition (% of total fatty acids), total lipid, and lipid melting points of lipids extracted from chicken breast, salmon filets, and Wagyu rib steaks

Item	Chicken breast	Salmon filets	Wagyu rib steaks
		Fatty acid, %	
Myristic, 14:0	0.5^{b}	2.8^{a}	3.0^{a}
Myristoleic, 14:1n-5	0.1^{c}	0.2^{b}	1.6^{a}
Palmitic, 16:0	22.4^{b}	17.6^{c}	28.7^{a}
Palmitoleic, 16:1n-7	4.2^{a}	3.6^{ab}	2.8^{b}
Stearic, 18:0	7.7^{b}	4.0^{c}	12.4^{a}
Oleic, 18:1n-9	31.8^{b}	16.0^{c}	45.2^{a}
Linoleic, 18:2n-6	23.5^{a}	1.0^{c}	3.3^{b}
Linolenic, 18:3n-3	1.8^{a}	0.6^{b}	0.01^{c}
Arachidonic, 20:4n-6	2.3^{a}	0.1^{b}	0.1^{b}
Eicosapentaenoic, 20:5n-3	0.2^{b}	15.6^{a}	0.01^{c}
Docosahexaenoic, 22:6n-3	0.6^{b}	22.4^{a}	0.01^{c}
Total lipid, %	1.8^{b}	2.7^{b}	16.1^{a}
Melting point, °C	29.0^{a}	2.5^{b}	27.3^{a}

Table 2. Cholesterol (mg/100 g meat) and fatty acid composition grams/100 grams meat of chicken breast, salmon filets, and Wagyu rib steaks

Item	Chicken breast	Salmon filets	Wagyu rib steaks
Cholesterol, mg/100 grams	40.9^{b}	38.6^{b}	91.7^{a}
Fatty acids, grams/100 grams			
Myristic, 14:0	0.01^{c}	0.09^{b}	0.47^{a}
Myristoleic, 14:1n-5	0.001^{c}	0.004^{b}	0.25^{a}
Palmitic, 16:0	0.41^{b}	0.46^{b}	4.45^{a}
Palmitoleic, 16:1n-7	0.07^{b}	0.11^{b}	0.45^{a}
Stearic, 18:0	0.14^{b}	0.11^{b}	1.89^{a}
Oleic, 18:1n-9	0.58^{b}	0.50^{b}	7.23^{a}
Linoleic, 18:2n-6	0.45^{a}	0.03^{b}	0.48^{a}
Linolenic, 18:3n-3	0.035^{a}	0.012^{b}	0.002^{c}
Arachidonic, 20:4n-6	0.042^{a}	0.003^{c}	0.011^{b}
Eicosapentaenoic, 20:5n-3	0.004^{b}	0.403^{a}	0.002^{c}
Docosahexaenoic, 22:6n-3	0.011^{b}	0.489^{a}	0.002^{c}
Total SFA[1]	0.49	0.68	5.36
Total MUFA[1]	0.66	0.64	7.92
Total PUFA[1]	0.54	0.93	0.49
Total omega-3 fatty acids	0.05	0.90	0.005
MUFA:SFA ratio	1.20^{a}	0.82^{b}	1.14^{a}

WAGYU LONG TERM STUDY

We've earlier taken a look at what we've called "Short Term Study" and I think with the results of that study we can go forth touting the real benefits of Wagyu as documented by a University study. This study is indeed a step in the right direction and can be very effective in promoting the breed.

But it is just a step as we now undertake the "Long Term Study". The long term study has been described as "a well controlled study and would be one of the most important studies; if not, the most important study ever in the Wagyu World". This study is designed to compare the differences in carcass quality among full blood registered black and red Wagyu, black F1 Wagyu, and angus grass & grain-fed steers.

The study will document total lipids, fatty acid composition, cholesterol concentrations and lipid melting points of the animals and it will also include the results of free range chickens and wild salmon.

What do we hope to accomplish with this study? First off we hope to answer a question that had been in the public eye for a number of years: Is grass fed or grain fed healthier. This element of the study should give it wide spread interest and aid in spreading the word on Wagyu even if indirectly. Of more direct interest to the members of the Wagyu Association and Wagyu breeders are some of the other questions the study will answer, are black or red fullbloods healthier?, are F-1 healthier? how does angus fit into the equation and how do free range chicken and wild salmon compare? When you think about it the possibilities are endless. This study will have a huge impact on the breed

The study is to be a double blind study conducted by Dr. Stephen Smith of Texas A&M University and Dr. Sherwin Siff of Houston Texas with Dr. Russell Cross of Texas A&M and Dr.Jimmy Hoerner, noted nutritionist assisting. Dr. Smith is a renowned meat scientist with a history of diagnostic work in the area of meat composition and Dr. Siff is a prominent Wagyu breeder with a scientific background providing and overview of the mechanics of the study.

The study will involve ten full blood black steers, ten full blood red steers, and ten F-1 black steers to be fed on a typical grain fed, feed lot ration and the same number of animal fed on a grass ration. Also the test will involve 20 angus steers, ten on grain and ten on grass.

Funding this study is a grant covering transportation of the animals to the Bryan, College Station Texas area and the chemical testing involved. The animal's on test will be managed by a unit of Texas A&M, and the cost of management, health care, feeding etc. will be borne by the owners of the cattle in the test. Added funding for administrative costs will be solicited from the private

sector. The cattle suppliers will retain ownership throughout and the carcass on the rail, minus the single sample cut will be theirs. A plan to have a buyer available for these cattle is currently being formulated.

The test will commence this fall with weaners in the 400-600 pound range arriving in Texas. Complete details of the study and the logistics and administration are available from the Breed Improvement committee and will be posted on the Association Web Site. Committee chairman, Martin Anderson, has included a letter in this newsletter explaining the test in more detail.

AMERICAN WAGYU ASSOCIATION

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