

Implant Programs for Long-fed Holstein Steers

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Introduction

In recent years Holstein feeder steers have become of great importance in the beef production feed yards, particularly in the southwestern U.S. Their uniformity, live performance, and carcass value outweigh their potential deficits in carcass characteristics and increased maintenance costs. In Arizona alone approximately 225,000 Holstein steers are raised in beef production facilities. This represents approximately 75% of the cattle fed for beef in Arizona with their value exceeding \$200 million.

With such a large economic impact it is clear to see the how the Holstein breed has affected the beef industry. The Holstein induction into beef production came directly from there availability. There are 9,141,000 (Hoard's West, 2003) total dairy cows in production in the United States. The Holstein Association estimates that 90% of those are Holstein. This leaves 8,226,900 Holstein cows in production. Assuming 50% of calves born are male, an estimated 4,113,450 bull calves are available. Removing those lost to retention, the veal market, and death loss there are over 3 million Holstein bull calves available for purchase. The west specifically has over 40% (Hoard's West, 2003) of all dairy production and Arizona has over 146,000 milking cows on its 132 (Hoard's West, 2003) dairies. The results are a highly available, fairly inexpensive market for Holstein bull calves and beef producers in Arizona have taken advantage of this market. Holstein feeder steers represent a single breed with a relatively narrow genetic base. The dairy industry relies heavily on artificial insemination resulting in over 85% of all births being of AI conception (<http://www.holsteinusa.com>, 09/30/03). This coupled with long-term utilization of only a few sires resulted in a very narrow genetic background. These factors result in Holsteins having a predictable, uniform performance.

Upon entry to the feedlot Holstein have another advantage over traditional beef cattle. Most beef cattle experience weaning by abrupt separation, marketed through sale barns, and transportation to the feed yard. Once in the feed yard they are exposed for the first time to feed bunks, water troughs, human contact, concentrate diets, and a multitude of pathogens. On the contrary, Holstein steers are raised in confinement settings where they gain exposure to disease causing agents and are weaned shortly after birth. Upon arrival to the feed yard they experience less overall stress since they have knowledge of feed bunks and water troughs and were raised on a concentrate diet. Most will enter the feed yard consuming 75 to 80% concentrate diet, which decreases that amount of time necessary to work up to a full 85 to 90% concentrate diet. Furthermore, having previously encountered most stressing factors (disease pathogens, commingling, climatic conditions, etc.), Holsteins have a lower morbidity and mortality rate compared to conventional beef breeds.

Post-slaughter Holsteins have benefits and drawbacks concerning their carcass value. Hide and trim values increase profitability while deficits in carcass performance hinder prices. Hide value is increased since Holstein hides are larger, thinner, and tend to be non-branded. This creates a high quality, suppler hide that is more valuable to tanners. Holstein hides tend to command a higher price on the open market than do the established beef breeds. The trim generated from fabrication tends to be leaner and sells at a higher price (Siemens, 1996).

Upon finishing, Holsteins tend to be a leaner, heavier animal compared to most beef breeds. Holsteins tend to have less than three-tenths of an inch back fat. However, Holsteins have greater amounts of kidney, pelvic, and heart fat. Sixty to 80% of animals will grade choice and there are no taste differences in the meat they produce (Siemens, 1996).

Holsteins do have a comparatively larger rumen and digestive tract and will have a larger offal drop. Having a leaner animal at harvest will lower the muscle to bone ratio and this coupled with the larger offal drop will lower the dressing percentage. Holstein carcasses will also produce a lower yield of boneless sub-primal cuts and have different muscle shapes. This produces an altered appearance to the trained eye. Comparing rib eye areas (REA) of a Holstein and a beef breeds the REA will appear longer, narrower and smaller in the Holstein. This is not a primary concern to packers or feeders since the change in appearance is relatively small and in the end the consumer probably cannot tell the difference.

In summary Holsteins are highly available for feedlot entry, are very uniform than traditional beef breeds. They are leaner animals at finish with a thinner hide, and less external fat. Their hide and trim command higher prices on the world market. Holsteins also have increased NE_m and are less environmentally hardy for colder climates, but do well in the arid southwest. They have a lower dressing percentage, greater offal drop, greater kidney, pelvic and heart fat, and altered muscle shapes. Therefore, research to optimize management options to increase muscle size and potentially decrease kidney, pelvic and heart fat is needed.

There are currently approximately 24 implants available for use in finishing cattle and thus, the combinations of implants for use in finishing cattle is enormous. No one implant or implant program is universally used by feedyards. Personal preference and company value-added benefits are the driving forces for feedyards in selecting implant programs. Therefore, continued research with implants and implant programs is imperative for providing scientific information to help feedyard producers and nutritionists select implant programs that will both enhance weight gain and efficiency of weight gain and also produce a desirable product for the consumer.

It is well recognized that implant programs improve average daily gain, feed efficiency and protein deposition (Montgomery et al., 2001). In addition, use of implants has increased carcass weight (Hermesmeyer et al., 2000) and longissimus muscle area (Roeber et al., 2000). However, there is a general concern that eating quality maybe sacrificed through the use of anabolic implants (Montgomery et al., 2001). A number of studies have evaluated the effects of implants on performance and carcass characteristics, this review will concentrate on the specific objectives set forth in our research program.

Implant Programs on Performance and Carcass Characteristics

Approximately 98% of feedyards implant cattle at least once (USDA-APHIS, 2000). Frequency of implanting varies with weight and age of the animals entering the feedyard. For heavy weight cattle (318 kg or greater at placement), 66.8% of feedyards implanted once, 30.0% implanted twice, and only 0.4% implanted three or more times during the finishing period (USDA-APHIS, 2000). Duckett et al. (1997) reported that a single implant increased average daily gain by 26.4%, and that combination implants (trenbolone acetate and estrogen based; e.g. Revalor S or Synovex Plus) produced the greatest response. These combination implant programs were also most efficacious in improving feed efficiency (10.9%). Although studies evaluating effects of multiple implant programs, results suggest that the most improvement in performance and feed efficiency results from the use of an estrogen (e.g. Synovex S) followed by a combination of strong estrogen and strong androgen (e.g. Revalor S or Synovex Plus; Duckett et al., 1997).

For light weight steers (less than 318 kg when placed on feed), 18.1% are implanted once, 74.0% implanted twice, and 6% implanted more than three times during the finishing period during 1999 (USDA-APHIS, 2000). The number of calf-fed (greater than 180 days on feed; Galyean, 1996) animals has increased recently, because of early-weaning of beef calves and more importantly, because of an increased supply of dairy calves fed for beef. These cattle typically enter the feedyard at less than 140 kg of body weight and for Holstein calves, are on feed for approximately 270 to 330 days.

The effects of implant regimen and aggressive implant treatment of long-fed Holstein steers on the overall feedlot performance and carcass characteristics of these animals have not been carefully studied. Six implant regimens involving estrogen and trenbolone acetate (androgen)-based implants increased ADG (12.2%), DMI (4.6%) and improved feed efficiency (6.7%), suggesting that implants may be efficacious in these long-fed cattle (Zinn et al., 1999). Some studies, however, have reported that implanting during the pre-weaning phase decreased feed intake during the finishing phase (Mader et al., 1994), although an earlier study by the same group (Mader et al., 1985) found that pre-weaning implants increased post-weaning feed intakes. Hence, the effects of aggressive implant programs in long-fed steers on overall performance and, more importantly, on carcass characteristics deserve additional attention. Implanting trenbolone acetate along with 20 mg estradiol benzoate and 200 mg of progesterone in Holstein steers on day 0, 56, 112, and 168 significantly reduced the percentage of carcasses grading USDA Choice (Apple et al., 1991).

Although the use of the current grading system may be open for debate, it is still the standard used, and many producers market on a carcass basis. Therefore, it is imperative to evaluate management programs that affect carcass characteristics. Consumers continue to associate quality grade (Choice, Select, etc.) with eating characteristics of beef and merchandisers have stated that insufficient marbling is the greatest quality challenges facing the beef industry (Roerber et al., 2002). Of the carcass characteristics commonly measured, it appears that implants have their greatest influence on carcass weight, marbling (and its associated influence on quality grade), ribeye area, and yield grade. We will discuss marbling because of its influence on quality grade.

Hermesmeyer et al. (2000) reported an interaction between level of feed intake and implant regimen for marbling score such that marbling scores were lower for steers implanted with Synovex Plus and given a restricted dietary intake (90% of ad libitum intake) and implanted with Revalor S and fed for ad libitum intake than for other treatments evaluated. In addition, more control steers (steers not implanted) graded USDA Choice than steers implanted with Synovex Plus (Hermesmeyer et al., 2000). Herschler et al. (1995) reported decreased marbling score with implants containing 1:5 or 1:10 estradiol:trenbolone acetate. Foutz et al. (1997) reported that implants containing 140 mg trenbolone acetate (Revalor S) decreased quality grades, and these authors implied that the additional implanting with trenbolone acetate during finishing may not be profitable if quality grade is reduced. In addition, Samber et al. (1996) reported that use of three successive implants resulted in significantly lower percentages of Choice and Prime carcasses compared to a non-implanted control group. On the other hand, it has been reported that more aggressive implant programs do not appreciably impact marbling or quality grade (Schoonmaker et al., 2001). Differences among studies are difficult to explain. Part of the discrepancies may be related to the type of cattle used. Schoonmaker used Angus crossed calves, Hermesmeyer reported using crossbred calves (no breeds mentioned), Foutz used Limousin x British, Herscher reported outcomes from Angus/Hereford, Charolais/Limousin and Brahman x Anus/Hereford and Samber reported using English x Continental crossbred steer calves. Few studies have evaluated the effects of aggressive implant programs using Holstein steers fed for beef.

Effects of Implants on Tenderness

In order for the beef industry to remain in competition with other sources of protein (e.g. fish, poultry and pork) a consistent product with desired tenderness must be produced. As mentioned previously, use of implants can decrease marbling and thereby decrease quality grades. Palatability of beef products is related to marbling because of its effects on flavor and juiciness. In addition, it has long been thought that increased marbling is directly correlated with increased beef tenderness.

Beef tenderness is generally measured in one of two ways (Nichols et al., 2002): 1) Warner Bratzler shear force and 2) use of taste panel evaluation. Recent studies (D. E. Goll, personal communication) have indicated that increased net muscle calpain activity, usually due to decreased calpastatin activity results in increased tenderness. Some limited studies have been conducted evaluating endogenous proteases with implant regimens.

Studies evaluating implant regimens on Warner-Bratzler shear force have produced conflicting outcomes. Samber et al. (1996) reported that loin steaks from steers that had been implanted with a combination trenbolone acetate/estradiol as the initial and final implant, or as the initial, intermediate and final implant had higher Warner-Bratzler shear force values than steaks from steers not implanted. However, other implant treatments used in the study (a total of seven different implant treatments in the study) did not affect Warner-Bratzler shear force. Likewise, Roeber et al. (2000) reported that steaks from control cattle had lower (indicative of more tender) Warner-Bratzler shear force values than steaks from steers given a combination trenbolone acetate/estradiol implant. Again, a total of eight implant regimens were used in this

study. Apple et al. (1991) reported that implants did not affect Warner-Bratzler shear force for steaks from Holstein steers. Other studies have concluded that implants did not affect Warner-Bratzler shear force or even had a positive response in improving tenderness in bulls (Hunt et al., 1991).

Like Warner-Bratzler shear force, conflicting results have been reported for beef tenderness as measured by taste panels. Roeber et al. (2000) reported that consumer rating for tenderness like and tenderness level were influenced by implant strategy (a total of 8 implant treatments used). Consumers rated steaks from unimplanted steers as more tender than steaks from all treatment groups except one (a progesterone/estradiol implant; Component T-S). Likewise, Apple et al. (1991) suggested that overall tenderness scores were lower for steaks from Holstein cattle implanted with progesterone/estradiol and zeranol plus trenbolone acetate than control steers and steers implanted with zeranol. In contrast to these studies, Cranwell et al. (1996) reported that implanting with trenbolone acetate only, a combination trenbolone acetate/estradiol, or estradiol only improved tenderness in cull cows compared with nonimplanted controls.

Given the conflicting results with both of the common measures for beef tenderness, more basic research needs to be conducted to elucidate mechanisms responsible for tenderness. Morgan (1997), in a review, reported that steaks were tougher for aggressively implanted than nonimplanted or conservatively implanted cattle, regardless of postmortem aging time. However, it was further reported that after 21 d of postmortem aging, Warner-Bratzler shear force values of steaks from cattle intermediately or aggressively implanted were similar to that of nonimplanted control steaks aged 7 d. Morgan (1997) suggested that steaks from the more aggressive implant strategies responded to postmortem aging, but the time required to become as tender as nonimplanted or conservatively implanted cattle was much longer.

If implanting cattle, particularly with aggressive implant programs, leads to decreased tenderness and/or increased aging time to produce acceptable tenderness, measurement of endogenous proteases may be used to evaluate potential effects on beef tenderness. In particular, degradation of key myofibrillar and associated proteins by μ -calpain (a calcium-dependent protease) has been implicated as a major cause of postmortem tenderization (Koochmaraie, 1996). However, Boehm et al. (1998) first questioned whether μ -calpain is responsible for a major proportion of postmortem tenderization or whether m-calpain is involved in this process. Calpastatin is an inhibitor of calpain that is present in skeletal muscles of all animals. Geesink and Koochmaraie (1999) implied that inhibition of μ -calpain activity by calpastatin limits the rate and extent of postmortem proteolysis and thereby prevents meat tenderization. Therefore, high concentrations of calpastatin is indicative of “tough” meat.

Gerkin et al. (1995) reported that neither activity of m- or μ -calpain activity was influenced by implant treatments (Synovex S [estrogen based], Finaplix –S [trenbolone acetate based], or Revaolor-S [combination estrogen/trenbolone acetate based] relative to nonimplanted controls in genetically identical clone steers (Brangus). However, muscles from steers implanted with Revalor S or Synovex S had higher calpastatin activity than muscles from nonimplanted steers. These authors suggested that the differences in calpastatin activity were not of sufficient magnitude to affect either of the calpain post-mortem activities and these changes were probably

not associated with differences in beef tenderness. In contrast, in a non-refereed publication (Kerth et al., 1995; as cited by Nichols et al., 2002), calpastatin activities were higher in nonimplanted steers than muscles from steers implanted with a combination implant. Given the conflicting results, further research should be conducted to evaluate the effects of implants and in particular, aggressive implant programs, on calpain and calpastatin activities in muscle.

Effects of Implants on Muscle

One mechanism of action of steroids may be the result of increased muscle protein synthesis (Nichols et al., 2002). Using 120 mg of trenbolone acetate (Revalor S), Johnson et al. (1996) reported that carcasses contained more protein on d 40 compared to nonimplanted steers. Moreover, implanted steers had an 82% increase in daily carcass protein accretion during the first 40 d of feeding. Hayden et al. (1992) also reported deposition of skeletal muscle protein was markedly increased within the first 40 d after implanting. This increase in protein deposition appears to be an additive effect (Hutcheson et al., 1997), with combination implants having greater daily protein accretion than either estrogenic or androgenic implants alone.

The mechanism of action of steroid implants on protein accretion has not been completely elucidated. Nichols et al. (2002) suggested that the anabolic response of ruminants to steroids may be due to indirect actions such as alterations in blood concentrations of growth factors (e.g. growth hormone or somatomedins). Some evidence suggests that increased production of insulin-like growth factor- 1 (IGF-1) and higher number of actively proliferating muscle satellite cells might be involved in anabolic steroid-induced growth (Johnson et al., 1998a,b). Johnson et al. (1996) reported that circulating concentrations of IGF-1 and steady-state hepatic IGF-I mRNA concentrations were significantly higher in steers implanted with a combination of trenbolone acetate and estradiol compared with nonimplanted steers. In addition, steady-state IGF-1 mRNA concentration in the longissimus dorsi of implanted steers were higher than in the corresponding muscle of nonimplanted steers. Thus muscle tissue might produce higher concentrations of IGF-1 in trenbolone acetate-estradiol-implanted treated steers.

Effects of Implants on of Adipose Tissue

Just as with muscle tissue, there have been conflicting reports on the effects of steroid implants on adipose tissue accretion. Some studies found that fat content increased in Angus and in Simmental crossbred cattle implanted with trenbolone acetate (Perry et al. 1991), whereas another study found that trenbolone acetate implantation had no effect on carcass fat accretion during the finishing period of crossbred (no breeds specified) cattle (Johnson et al., 1996). It has been suggested that nonimplanted steers reach physiological maturity more rapidly than implanted steers, and because physiological maturity is associated with an increased rate of fat deposition, such differences in maturity may account for the differences in fat deposition between implanted and nonimplanted steers (Nichols et al., 2002). Another study using three different implant regimens: 1) 28 mg estradiol benzoate plus 200 mg trenbolone acetate on d 0; 2) 28 mg estradiol benzoate plus 200 mg trenbolone acetate on d 0 and 61; and 3) 20 mg estradiol benzoate plus 200 mg progesterone on d 0 followed by 28 mg of estradiol benzoate plus 200 mg of trenbolone acetate on d 61 of a 127-d feeding period decreased total fatty acid content of the LM of steers (no breed specified) from a commercial research feedlot in Kansas (Duckett

et al., 1999). When expressed on a per mg weight of tissue, however, the fatty acid concentration was the same in the implanted and nonimplanted steers because of the larger muscle size of the implanted steers (Duckett et al., 1999).

Up to this point, no research has been published evaluating implants for Holstein steers on lipogenic gene expression of adipose tissues. Our hypothesis is that implant regimens will influence gene expression of adipose tissue. We conducted a study to evaluate implant regimens for Holstein dairy steers fed for meat on expression of acetyl CoA carboxylase (the rate-limiting enzyme for fatty acid synthesis), stearoyl-CoA desaturase (this enzyme influences composition of adipose tissue), uncoupling proteins (responsible for uncoupling mitochondrial respiration for ATP phosphorylation and increasing thermogenesis in mammals; uncoupling protein 2 is linked to obesity and influenced by fat feeding; (Lee et al., 2002), leptin (level is proportional to the level of adiposity), fatty acid transferases, and fatty acid binding protein. These genes were selected based on availability of probes for measurement and these genes are readily accepted genes in Animal Science research for fat metabolism with published data (Lee et al., 2002). Our objective was to evaluate non-implant programs (control) vs. increasingly more aggressive implant programs on weight gain and gain efficiency, carcass characteristics, and gene expression of adipose (fat) tissue.

Research Project Description

Animals. Twenty Holstein steer calves (average initial body weight approximately 400 lb) were purchased from an Arizona feedlot producer. Steers were raised in a manner consistent with approved protocols at the University of Arizona Feedlot.

Treatments included implant regimens used during raising the animals from a calf to harvest. Treatments were 1) no implant (Control), 2) an implant with zeranol (Ralgro; estrogen-based; Schering-Plough Animal Health, Union, NJ) on d 0 followed by a Synovex-S (estrogen-based; Ft. Dodge Animal Health, Ft. Dodge, IA) implant on d 84 and 168, 3) an implant with zeranol on d 0 followed by an implant on d 84 with Synovex-S followed Synovex Plus (estrogen plus trenbolone-acetate-based; Ft. Dodge Animal Health) implants on d 168, and 4) an implant with zeranol on d 0 followed by an implant with Synovex-Plus on d 84 and 168. Implant programs are typical programs used by commercial feedyards and encompass non-aggressive implant program (trt 2), more aggressive implant program (trt 3), and an aggressive implant program (trt 4). Steers were individually fed a diet typical of commercial feedyards and contained (DM basis) 67.54% steam-flaked corn, 14% sorghum sudangrass hay, 6.25% soybean meal, 5% molasses, 4% tallow, 0.75% urea, and 2.5% of a finishing supplement containing vitamins, minerals, Rumensin and Tylan. Steers were housed in (2.5 x 6 m) outdoor pens with soil surface; each pen contained an individual water source.

Adipose and muscle tissue biopsies were performed at d 0, 84, and 170 of the experiment. Adipose tissue biopsies were as described by Houseknecht et al. (1995). A biopsy sample was collected from the subcutaneous adipose depot of the hip region. Steers were tranquilized with xylazine (30 to 40 mg) 15 min. prior to the procedure. The hip region was shaved and aseptically prepared for surgery. A 2% lidocaine HCl (20 mL, s.c.) was administered in a circular pattern 5 min prior to biopsy. A lateral incision (approximately ~5 cm) was made through the skin and

subcutaneous adipose tissue at an area perpendicular to the long axis of the hip, immediately cranial to the pin bone. Subcutaneous adipose tissue (approximately 1 to 2 g) was dissected from the skin and muscle fascia and then cut out with scissors. The incision was then be cleaned with sterile saline and closed with absorbable sutures. Adipose tissue biopsies were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Upon completion of the feeding period, all animals were humanely slaughtered at the University of Arizona Meats Laboratory. Carcasses were evaluated for USDA quality grades (% Prime, % Choice, % Select, % Standard), ribeye area, fat thickness, and marbling score. Fat samples from the tail head region were collected at harvest and processed as described previously. Two steaks 2.5-cm thick were removed from the posterior end of each longissimus muscle section, vacuum-packaged, and frozen for Warner-Bratzler shear force determination.

Laboratory Analysis. Adipose RNA will be isolated and gene expression of adipose tissues as influenced by implant regimen. In particular, we will be measuring acetyl CoA carboxylase, stearoyl-CoA desaturase, uncoupling proteins, uncoupling protein 2, leptin, fatty acid transferases, fatty acid binding protein and adiponectin.

Steaks used for tenderness were weighed, and broiled over an open-hearth electric grill to an internal temperature of 70° C. Steaks were weighed after cooking and percent shrinkage calculated for each steak. After cooling to room temperature, six 1.27-cm cores were removed from each steak parallel to the direction of the muscle fibers. Each core was individually sheared using a Warner-Bratzler shear apparatus.

Results

Data for performance and carcass characteristics are reported in Table 1. As expected, implanting Holstein steers increased final BW ($P < 0.01$), ADG ($P < 0.01$) and daily DMI ($P < 0.01$), but did not influence ($P < 0.40$) the feed:gain ratio. In addition, implants increased hot carcass weight ($P < 0.01$), LM area ($P < 0.01$), and color score ($P < 0.08$). No differences ($P > 0.32$) were noted in Warner-Bratzler shear force values ($P > 0.32$) or marbling score ($P > 0.17$).

Implants increased ADG by approximately 23% and daily DMI by 18% in our study. Likewise, Zinn et al. (1999) reported an improvement in ADG and DMI using six implant regimens involving estrogen and trenbolone acetate (androgen)-based implants. In addition, our study resulted in a 15% increase in hot carcass weight and 17% improvement in LM area without affecting marbling score Warner-Bratzler shear force values. Other studies have also demonstrated that Warner-Bratzler shear force values are not influenced by aggressive implant programs (Apple, 1991; Morgan, 1997), particularly when the meat is aged greater than 21 d (Morgan, 1997). Shear force values in our study indicated that all steaks were tender. We conclude that long-fed Holstein steers can be aggressively implanted without adversely affecting carcass characteristics. However, we acknowledge the limited number of animals on this study and are concentrating our efforts on metabolic profiles to elucidate the mechanism of action.

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Table 1. Effects of implant program on live performance and carcass characteristics of long-fed Holstein steers

Item	Treatments ^a				SEM	Contrast ^b		
	CON	RSS	RSP	RPP		1	2	3
Initial BW, lb	412	415	422	421	14.4	0.63	0.73	0.95
Final BW, lb	1122	1290	1287	1269	28.1	0.01	0.71	0.64
ADG, lb/d								
0 to end	2.8	3.5	3.4	3.4	0.11	0.01	0.58	0.66
DMI, lb/d								
0 to end	17.8	21.2	20.6	21.3	0.77	0.01	0.75	0.54
Feed:gain								
0 to end	6.3	6.1	6.0	6.3	0.19	0.40	0.86	0.21
Hot carcass								
wt, lb	645	749	744	737	16.6	0.01	0.66	0.74
Dressing, %	0.58	0.58	0.58	0.58	0.01	0.52	0.96	0.95
Kidney, pelvis, & heart fat, %	5.1	5.1	4.2	4.4	0.39	0.20	0.11	0.73
Back fat	0.26	0.35	0.26	0.28	0.05	0.54	0.19	0.84
LM area, sq in.	10.0	11.7	11.5	11.8	0.47	0.01	0.96	0.60
Marbling score ^c	554	644	540	643	54.0	0.35	0.40	0.18
Yield grade	3.4	3.5	3.2	3.1	0.25	0.46	0.18	0.80
Color	5.0	5.8	5.4	5.5	0.29	0.08	0.30	0.80
Texture	5.4	5.8	5.8	5.5	0.25	0.27	0.60	0.39
Firmness	5.8	5.8	5.6	5.3	0.24	0.34	0.19	0.30
Shear Force, kg	1.98	2.14	2.25	2.52	0.30	0.33	0.48	0.52

^aCON = no implant; RSS = Ralgro on d 0, Synovex-S on d 84 and Synovex S on d 168; RSP= Ralgro on d 0, Synovex-S on d 84 and Synovex Plus on d 168; RPP = Ralgro on d 0, Synovex-Plus on d 84 and Synovex-Plus on d 168.

^bContrast 1 = CON vs implants; 2 = RSS vs. the average of RSP and RPP; and 3 = RSP vs. RPP.

^cMarbling score 500 = small; 600 = modest; 700 = moderate.