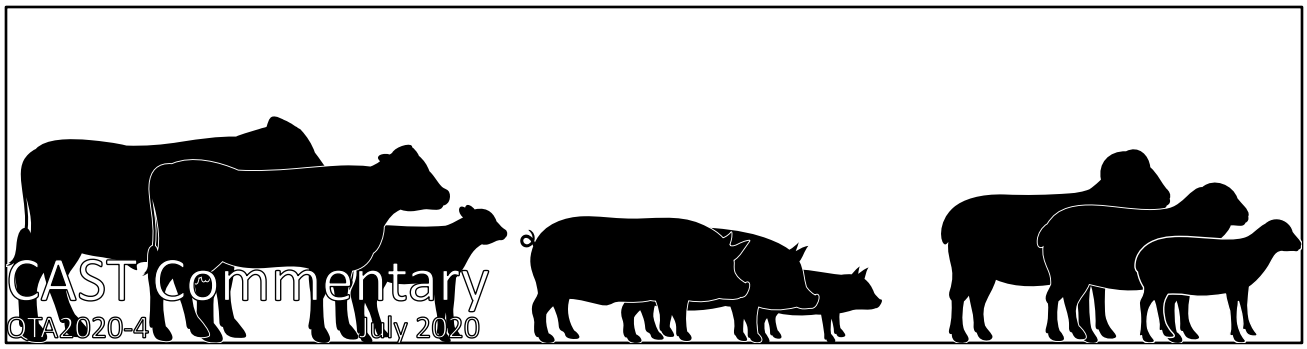

Impacts on Human Health and Safety of Naturally Occurring and Supplemental Hormones in Food Animals

Authors: **Robert J. Collier (Chair)**
University of Idaho
Moscow, Idaho

Laura L. Hernandez
University of Wisconsin-Madison
Madison, Wisconsin

Jimena Laporta
University of Florida
Gainesville, Florida

Jim Lauderdale
Upjohn/Pharmacia (Retired)
Augusta, MI

Zachary K. Smith
South Dakota State University
Brookings, South Dakota

John L. Vicini
Bayer Crop Science
St. Louis, Missouri

Reviewers: Dale Bauman
Cornell University
Ithaca, New York

Garth Boyd
The Context Network
Vail, Colorado

Geoffrey Dahl
University of Florida
Gainesville, Florida

CAST Liaison: **Juan Tricarico**
Innovation Center for U.S. Dairy
Rosemont, Illinois

Introduction

Since the end of the Second World War, the United States has pursued a national policy of an abundant and inexpensive food supply. This policy has been hugely successful at meeting these goals. The average share of disposable personal income spent on total food by consumers in the U.S. from 1960 to 2018 fell from 16.8% to 9.7%, driven by a declining share of income spent on food at home (Figure 1). Concomitantly, increased animal productivity has improved efficiencies of animal production and reduced the carbon footprint for production of meat, milk, and eggs (Capper 2011; Capper and Cady 2020; Pelletier, Ibarburu, and Xin 2014; Putman et al. 2017; Thornton 2010).

Some components of the technologies employed by animal producers to improve efficiency of animal production include improved nutrition and reproduction; advances in genetics; and health and management practices; as well as feed additives, hormonal treatments, and growth enhancing technologies (GETs). These technologies have reduced cost of food production and reduced impacts of animal production on the environment (Avery and Avery 2007).

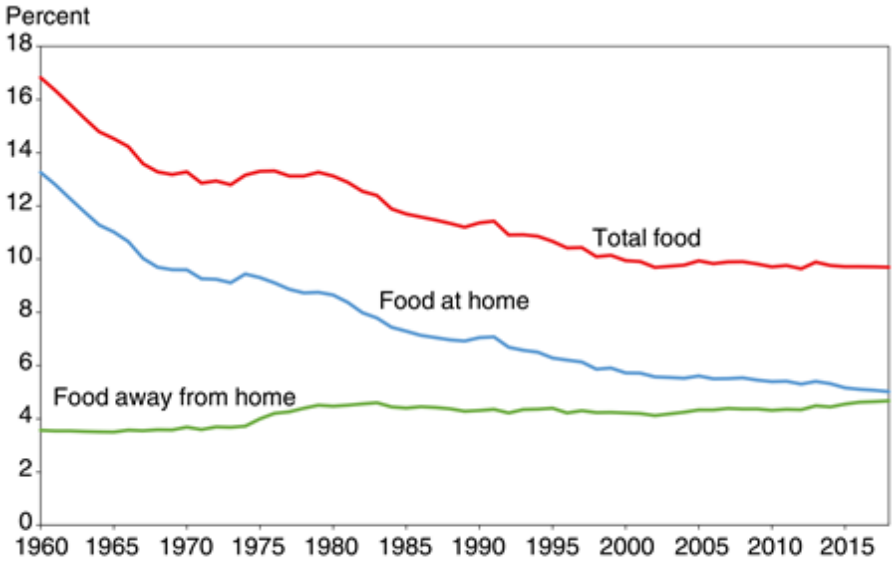

Figure 1. Share of disposable personal income spent on food in the United States, 1960-2018.

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Originally, food was simply perceived as a means to obtain the necessary nutrients and energy for the body. As the evolution of nutrition science progressed food began to be seen as a means of supporting adequate growth and development of the body. Currently food is also perceived as a key factor influencing the prevention of some diet-related diseases (Pogorzelska-Nowicka et al. 2010). Thus, a substantial effort in the food production industry goes towards the improvement of food healthiness (Decker and Park 2010). In addition, there is the requirement to establish that residues of naturally occurring and synthetic compounds used to improve animal efficiency do not pose a human health risk.

The use of growth enhancing technologies (GETs) has improved the quality of meat products by repartitioning fat into muscle mass and reducing fat content of meat products .

The use of GETs has improved the quality of meat products by repartitioning fat into muscle mass and reducing fat content of meat products (Bauman and Currie 1980). Currently, there are six GETs approved by the Food and Drug Administration (FDA) in the United States and 30 other countries for use in beef animals. Three of these are naturally occurring (testosterone, estrogen, and progesterone) and three are synthetic—melengestrol acetate (MGA), trenbolone acetate (TBA), and zeranol. In addition, bovine somatotropin (bST) is approved for use in lactating dairy cows to increase yield of milk. Oxytocin, gonadotropin hormone releasing hormone (GnRH), prostaglandins, and gonadotropins (luteinizing hormone and follicle stimulating hormone) are approved for use in improving reproductive performance of domestic animals. All of these compounds have undergone rigorous testing for human and animal safety under guidance of the FDA Center for Veterinary Medicine (CVM). The safety of meat and dairy products from use of these compounds has been established by multiple regulatory organization worldwide including the FDA and the World Health Organization (WHO).

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Food Safety Oversight

In the United States, the oversight of food safety at the national level presently involves at least twelve agencies, of which four predominate: the U.S. Department of Agriculture (USDA), the FDA, the Environmental Protection Agency (EPA), and the National Marine Fisheries Service.

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The FDA has jurisdiction over domestic and imported foods that are marketed in interstate commerce, except for meat and poultry products. The FDA's Center for Food Safety and Applied Nutrition (CFSAN) seeks to ensure that these foods are safe, sanitary, nutritious, wholesome, and honestly and adequately labeled. The CFSAN exercises jurisdiction over food processing plants and has responsibility for approval and surveillance of food-animal drugs, feed additives and of all food additives (including coloring agents, preservatives, food packaging, sanitizers, and boiler water additives) that can become part of food. The CFSAN enforces legal limits (tolerances) for pesticide residues that are set by the EPA and shares with USDA Food Safety and Inspection Service (USDA-FSIS) responsibilities for egg products. NOAA Fisheries is responsible for the stewardship of the nation's ocean resources and their habitat. They provide oversight for productive and sustainable fisheries, safe sources of seafood, the recovery and conservation of protected resources, and healthy ecosystems—all based on sound science and an ecosystem-based approach to management.

What are hormones?

Hormones are chemical messengers produced in one type of cell or tissue of an organism and transported in tissue fluids to regulate a specific set of cells or tissues in another part of the organism. Hormones are classified into different groups based on their chemical structures and resulting physiological functions. Hormones are able to exert their actions on specific cells types or tissues through interaction with a receptor that is specific to an individual hormone, similar to a lock and key system. The following section will describe the various hormone classification used in animal agriculture to improve production efficiency based on their chemical makeup.

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Steroids

Steroid hormones as a group are lipids with a structure that contains four fused rings. Besides the commonly known reproductive steroids (testosterone, estrogens, progestogens), cholesterol, Vitamin D, and bile salts are also steroids. Because they are lipid soluble, steroid hormones can cross cell membranes which are composed of a phospho-lipid bilayer. Steroid hormones can easily cross cell membranes of the gut and are generally impervious to digestion so they are orally active. Steroid hormones exert their actions via receptors inside the cell (cytoplasmic, nuclear). Once bound to a specific receptor, a hormone is capable of inducing gene expression which results in activation of various pathways inside the cell resulting in specific activities such as growth, reproduction, and lactation.

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Peptides/Proteins

There are 20 major amino acids and these comprise the more than 20,000 different proteins in the body, including the protein hormones.

There are 20 major amino acids and these comprise the more than 20,000 different proteins in the body, including the protein hormones. The amino acids are analogous to letters in the alphabet and each different protein has a specific sequence of amino acids that determine its three-dimensional structure and function. As stated previously, protein hormones are composed of varying numbers of amino acids and are usually water soluble and are not usually lipid soluble. Therefore, protein hormones cannot cross the cell membrane and require receptors on the cell surface which bind and then internalize the protein hormone. The cell surface receptor that a peptide/protein binds to is very specific to the hormone itself, and the hormone will bind to the receptor with very high affinity (Michael et al. 2006). The binding of the hormone to its receptor initiates an intracellular pathway that is responsible for stimulating the hormone's action. These hormones regulate a large variety of physiological actions such as energy metabolism, ionic balance, reproduction, appetite control, growth and development, and secretion of other hormones (Michael et al. 2006). Insulin, for example is a protein hormone that regulates uptake of glucose in cells. Insulin is often needed by diabetics to help regulate their blood sugar but because it is a protein it cannot be given orally and must be injected. Examples of proteins or peptides which are used in animal agriculture include growth hormone (GH)), also called somatotropin (ST), which is involved in regulation of growth and lactation, and GnRH, which is involved in regulation of reproduction.

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Prostaglandins

Prostaglandins perform a variety of hormone-like actions such as controlling blood pressure and smooth muscle contraction.

Prostaglandins are modified fatty acids produced in animals and humans that are formed chiefly by the action of the enzyme cyclooxygenase on arachidonic acid. Since they are fatty acid derivatives, they can also cross cell membranes and are orally active. Prostaglandins perform a variety of hormone-like actions such as controlling blood pressure and smooth muscle contraction. In animal agriculture prostaglandins are used to improve reproductive efficiency by regulating the reproductive cycles of domestic animals.

Amines

The primary class of amines used in animal agriculture are the catecholamines, a group of chemically related compounds which affect nerve transmission and smooth muscles compounds.

Amine hormones are derived from the amino acids tryptophan (serotonin and melatonin) or tyrosine (thyroid hormones, and the catecholamines epinephrine and norepinephrine) and are secreted from the pineal, thyroid, and adrenal gland. The primary class of amines used in animal agriculture are the catecholamines, a group of chemically related compounds which affect nerve transmission and smooth muscles compounds. Catecholamines are also involved in regulating energy metabolism which is the primary reason they are used in animal agriculture to improve growth efficiency.

Sources of Hormones in Foods

Produced by Animals

Hormones produced by animals that are involved in regulation of growth, reproduction, and other biological functions are present throughout the body and are found naturally in meat, milk, and eggs. The hormones in milk are

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perhaps the most extensively studied and the presence of hormones in milk and their physiological significance was suggested during the early 1900s. Milk contains numerous protein hormones, steroid hormones, thyroid hormones, peptide growth factors, and prostaglandins (Grosvenor, Picciano, and Baumrucker 1993). The milk of all mammals, including humans, provides not only essential nutrients, but also numerous bioactive factors (e.g., cytokines, nucleotides, enzymes, vitamins, growth factors, and hormones) that modulate development and confer immune protection to the young. The first documented presence of steroid hormones in cows' milk was in 1929 by Yaida. Since then, several naturally occurring bioactive peptides and hormones have been identified and detected in bovine milk and dairy products (Mills et al. 2011; Park and Nam 2015; Richardson and Mattarella 1977; Schams and Karg 1986; Vargas-Bello-Pérez, Márquez-Hernández, and Hernández-Castellano 2019).

Naturally occurring hormones either originate from maternal blood and are secreted into milk through an active transport or are synthesized by the mammary gland and excreted into milk.

Naturally occurring hormones either originate from maternal blood and are secreted into milk through an active transport or are synthesized by the mammary gland and excreted into milk. They serve messenger and signaling roles in the regulation of mammary function and enhancement of immune development and gastrointestinal tract maturation (Hamosh 2001). The various ranges of concentrations of hormones in bovine milk according to their origin are summarized by Jouan and colleagues (2006) in Table 1.

Hormone Type	Example	Concentration
Gonadal		
Adrenal	glucocorticoids	0–50 ng/ml
Pituitary		
Hypothalamic	somatostatin	10–30 ng/ml
Parathyroid hormone-related protein		40–100 ng/ml
Insulin		5–40 ng/ml
Calcitonin		700 ng/ml
Melatonin		5–25 pg/ml

Table 1. Examples and concentrations of selected hormones present in bovine milk (Jouan et al. 2006).

Steroid hormones, including estrogens, progesterone, and testosterone, are present in milk, but most attention by consumers is given to estrogens because of concerns about estrogen-dependent cancers.

Steroid hormones, including estrogens, progesterone, and testosterone, are present in milk, but most attention by consumers is given to estrogens because of concerns about estrogen-dependent cancers. Estrone (E1) and estradiol (E2) are detectable in milk and a major factor that affects their concentration in milk is the amount of milk fat (Pape-Zambito, Magliaro, and Kensinger 2008; Pape-Zambito, Roberts, and Kensinger 2010). For instance, for skim milk and whole milk, respectively, E1 concentrations were 2.9 and 7.9 pg/ml and E2 concentrations were 0.4 and 0.9 pg/ml. Concentrations in high-fat ice cream, which tend to be premium brands were even greater. In a survey of retail milk across the United States (Vicini et al. 2008), whole organic milk had significantly more E2 than whole conventional milk. Likewise, progesterone was also greater in organic milk, and this is not because of the fat content of these samples, which were not affected by the production method claim on the label. Pape-Zambito and colleagues (2010) also examined organic compared to con-

ventional production methods for retail milk purchased in Pennsylvania. They tested various milk fat percentages (skim, 1%, 2%, and whole) and saw a similar increase in E2 concentration with increasing milk fat for both organic and conventional milk. They also had a statistical interaction as a result of a greater increase in E2 as milk fat increased for organic milk compared to conventional. Although organic milk had more E2 in both studies (Pape-Zambito, Roberts, and Kensinger 2010; Vicini et al. 2008), the greater E2 concentration because of organic production is minor and should not be a reason to avoid consuming milk from either production method. E1 and E2 in milk were greater in pregnant cows in the last trimester of pregnancy (Pape-Zambito, Magliaro, and Kensinger 2008). Other studies have found that progesterone in milk is greater for pregnant than for non-pregnant cows (Chenault et al. 2003), and this is during a stage of lactation that normally contributes a lower portion of milk to a farm's milk tank.

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Certain protein hormones are naturally in milk and of these, somatotropin and insulin-like growth factor 1 (IGF-1) have received the most attention in the last 25 years because of commercialization of recombinant bovine somatotropin (rbST). In general, the concentrations of bST in unpasteurized milk are at or near the limits of detection of highly sensitive analytical assays. Somatotropin does not bind to the human receptor for growth hormone and rbST is denatured by pasteurization (JECFA 2014). Conversely, bovine IGF-1 is detectable in milk, is not affected by pasteurization, and does have biological activity in humans. Amounts of IGF-1 in samples of milk from individual cows vary considerably from 1 to 13 ng/ml (JECFA 2014) and retail milk contains approximately 3 ng/ml, with organic retail milk having slightly less (Vicini et al. 2008). In general, circulating concentrations of IGF-1 are greater for cows with positive nutrient balances (energy and protein) and are reduced with negative nutrient balances (McGuire et al. 1992; Vicini et al. 1991); however, the concentrations of IGF-1 are greater in milk from cows in early lactation, when cows tend to be in lower nutrient balances.

In general, the concentrations of bST in unpasteurized milk are at or near the limits of detection of highly sensitive analytical assays. Somatotropin does not bind to the human receptor for growth hormone and rbST is denatured by pasteurization.

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In general, the amounts of naturally occurring hormones in milk and dairy products are significantly lower than production of the same hormones by humans (JECFA 2000a, b). In some cases, the biological actions of the hormones are species-specific, and most times they do not reach systemic circulation because they are naturally degraded in the small intestine. The FDA guidelines state that no physiologic effects could be expected when consumption is ≤1% of the endogenous quantities produced by the segment of the population with the lowest daily production (FDA 2018). An illustrative example was performed by Macrina and colleagues (2012), who tested the potential ingestion of estrogens by consumption of retail milk and dairy products. Production rates of E1 plus E2 in humans range from 54,000 to 630,000 ng/day. These authors estimated total E1 intake from three servings of whole milk was 68 ng/day, which represents 0.01% to 0.1% of the daily production rate in humans. These levels are far below the current guidelines for safe consumption and should rule out concerns regarding disorders or hormone imbalances based on the consumption of dairy products.

Other animal products, such as eggs, beef, poultry, pork and fish also contain hormones, of which steroid hormones have been characterized. Consumption of steroids in the diet are considerably lower than steroids produced in humans including children (Hartmann et al. 1998).

No hormonal products are or have been approved or used for poultry production, therefore no exogenous hormonal residues exist in eggs. Eggs contain the natural hormones E2 (less than 0.03 to 0.22 µg/kg), progesterone (12.5 to 43.6 µg/kg), and testosterone (0.04 to 0.49 µg/kg) (Doyle 2000).

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For endogenous sex steroid hormones, also known as gonadal steroids, the FDA Center for Veterinary Medicine (FDA-CVM) establishes incremental increases beyond exposure because of endogenous exposures rather than an acceptable daily intake (ADI). Therefore, because of the existence of these naturally occurring hormones in humans and food-producing animals, the human consumer is exposed throughout his/her lifetime to rather large quantities of these steroid hormones through his/her own daily production and, to much lesser quantities, from untreated food-producing animals. Daily steroid production values in pre-pubertal boys and girls, the most sensitive segment of the population, are presented in Table 2.

Sex status	Estradiol	Progesterone	Testosterone
Female-menstrual cycle	270 to 445	418 to 19,580	240
Female-gestation	2,000 to 37,800	94,000	320
Female-postmenopausal	8	326	140
Female-ovariectomized	n/a	239	n/a
Female- prepubertal	31	253	32
Male-adult	48	416	6,480
Male-prepubertal	6	150	65

Table 2. Steroid production in humans during 24 hours (µg/24 hours) (Farber and Arcos 1983).

The FDA-CVM concluded that no physiological effect could be expected in consumers eating animal products containing additional amount of the hormone that is less than or equal to 1% of the amount produced by the human daily. An additional safety factor is present in that only about 10% of the ingested steroid would be absorbed. The FDA-CVM calculated a permitted increased exposure above the amount naturally present in untreated target animals (1% of the daily production values; Table 3).

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Hormone/Tissue	Muscle	Liver	Kidney	Fat
Estradiol	0.12 µg/kg	0.24 µg/kg	0.36 µg/kg	0.48 µg/kg
Progesterone	5 µg/kg	15 µg/kg	30 µg/kg	30 µg/kg
Testosterone	0.64 µg/kg	1.3 µg/kg	1.9 µg/kg	2.6 µg/kg

Table 3. Incremental increases allowed for the natural steroid hormones (Code of Federal Regulations 2019).

Products containing these natural steroids are approved (label) for use in beef cattle only, therefore the tissues for human consumption are muscle, liver, kidney, and fat. No residues resulting from the use of these steroids is permitted

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in excess of the increments above the concentrations of the steroid naturally present in untreated animals.

Produced in plants or by molds

Although not the scope of this paper, it should also be noted that animal products are not the only source of hormones in the human diet. There are plant-derived estrogens, known as phytoestrogens and some can be present at levels in feeds to cause a negative effect on reproductive performance, milk yield, or health of animals (Adams 1995). The most common phytoestrogens in the human diet are isoflavones that are found in soy products and are consumed in large amounts in some populations. Mycotoxins, such as zearalenone, can also have weak estrogenic activity and be found in foods. Because they have weak estrogenic activity, it is possible that they can block estrogen receptors and decrease estrogen-induced maladies, such as breast cancer, or they could reduce the effectiveness of hormone replacement therapy (Cornwell, Cohick, and Raskin 2004; Ehling and Reddy 2015). However, this effect might be dependent on the estrogen background, which could account for the equivocal results from health studies (Lee et al. 2009).

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Hormones used in animal production-reproductive management

Beef, and dairy cows and heifers

During the 1950s frozen bovine semen was developed and artificial insemination (AI) with progeny-tested bulls became recognized as an effective way to make rapid genetic progress for milk yield and beef production. A major deterrent to AI in cattle is the requirement for daily estrus detection throughout the year for dairy cattle and daily for 60 to 90 days or more for beef cattle. Therefore, research over the past 60 plus years led to identification of effective estrus and breeding management protocols (estrus synchronization) to allow for AI during one pre-determined day or over a few day interval (Lauderdale 2009). Products were identified and granted FDA-CVM approval for use in cattle for estrus and breeding management (Code of Federal Regulations 2019) according to label directions. The protocols can use steroids, GnRH, and Prostaglandin F2-Alpha (PGF2α) to achieve single day timed breeding (use of GnRH plus PGF2α or GnRH plus PGF2α plus steroid) or several day pre-determined breeding (PGF2α or steroid plus PGF2α). Each product has regulatory authority approval for their labeled indicated use (Freedom of Information for each New Animal Drug Application [NADA] or generic [ANADA] as cited for each product below).

Research over the past 60 plus years led to identification of effective estrus and breeding management protocols (estrus synchronization) to allow for AI during one pre-determined day or over a few day interval.

Products were identified and granted FDA-CVM approval for use in cattle for estrus and breeding management for use according to label directions.

1. Steroids: Steroid products are a progestogen (an analog of progesterone), melengesterol acetate (NADA 034-254; 039-402), and the natural steroid, progesterone, delivered with an intravaginal device called a continuous intravaginal drug releasing device (CIDR) (NADA141-200). Progesterone and progestogens block estrus; if delivered for sufficient time, removal of the progestogen block will allow cattle to return to estrus in a pre-determined synchronized interval.

2. GnRH: GnRH products that are salts of the natural GnRH, gonadorelin, are Cystorelin® (NADA 098-379), Factrel® (NADA 139-237), Fertagyl® (ANADA of Cystorelin, 200-134, OvaCyst® (ANADA of Cystorelin 200-069), and GONABreed® (ANADA of Cystorelin, 200-069). GnRH induces the release of an ovulatory surge of luteinizing hormone, resulting in ovulation of a dominant follicle or initiation of a new follicular wave.

3. Gonadotropins, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH): are natural products but not used since their releasing hormones (GnRH, LHRH, FSH-RH) are available through regulatory authority approval and are less expensive.

4. Prostaglandins: PGF products are salts of either the natural PGF—dinoprost—and are Lutalyse®, Lutalyse®HighCon (NADA 108-901), ProstaMate® (ANADA of Lutalyse 200-253, and In-Synch® (ANADA of Lutalyse 108-901) or a PGF analog—cloprostanol—and or Estrumate® (NADA 113-645, estroPLAN® (ANADA 200-310), and SYNCHSURE™ (ANADA 200-310). $PGF_2\alpha$ and $PGF_2\alpha$ analogs regress the corpus luteum (CL) when administered during days 6 through 18 of the estrus cycle, resulting in cattle returning to estrus on about days 2 through 6 post-injection.

Sows

1. Steroid: The steroid product is the progestogen altrenogest (Matrix®; NADA 131-310). This progestogen is labeled and used for estrus synchronization in gilts and sows.

2. Gonadotropins, LH, and FSH, the natural gonadotropins, rather than GnRH products, have a label for use in sows and gilts. PG600 (NADA 140-856) is a combination of FSH and LH and is used to stimulate estrus in non-estrous cycling gilts and sows to facilitate breeding.

3. GnRH: A GnRH product that is an analog of the natural GnRH is triptorelin, used to induce ovulation in postpartum sows.

4. Prostaglandins (PGF): PGF products are Estrumate®, Lutalyse®, and ProstaMate®. These products are used to induce farrowing or terminate unwanted pregnancy at an early stage.

5. Oxytocin (NADAs 046-788; 109-305; ANADA 200-328), a peptide is used to stimulate uterine activity to assist with farrowing and to stimulate milk ejection to assist with piglet nursing.

Ewes

Ewes are seasonal breeders, therefore, some producers desire to produce lambs out-of-season to meet market demands. Progesterone delivered with a CIDR, along with ram introduction, is the program of choice. For in-season breeding, usually in small flocks, a CIDR and a PGF are used for estrus synchronization, similar to cattle.

There is an inherent interval of days to months between use of the hormonal product and harvest for human food. For milk, there is no inherent interval between use of the products and milk consumption.

Impact of hormones used for reproductive management on residues in meat and milk

Hormones used for reproductive management are administered during the time of breeding or at the end of gestation. Since the objective of the animal production enterprise is to produce animals safely, humanely, and profitably, there is an inherent interval of days to months between use of the hormonal product and harvest for human food. For milk, there is no inherent interval between use of the products and milk consumption. Each product identified has been granted FDA-CVM approval for its label use. In order to receive FDA-CVM approval, adequate studies were completed to satisfy FDA-CVM requirements:

(1) For products using natural hormones, humans are exposed to their own production of these hormones (Tables 2 and 3). In addition, the same hormones are present in animal products consumed by humans as a result of internal production of these hormones by the animal. These products are approved by regulatory authorities based on studies documenting that the amount of additional natural hormone following product use is below a level deemed to be consistent with human food safety, therefore, humans are not at risk from eating food from animals supplemented with these hormones.

(2) For analogs of the natural hormones, the FDA-CVM required information and laboratory animal toxicological testing to determine safe levels in the animal products that we eat. Furthermore, the FDA required that the manufacturers demonstrate that the amount of hormone left in each edible tissue after treatment is below the appropriate safe level. A safe level is a level which would be expected to have no harmful effect in humans as codified by FDA-CVM.

Hormones used in animal production-growth management

In the United States, livestock producers have used various types of GETs (Table 4) such as steroidal implants since the 1950s and β-AA since the early 2000s to improve carcass leanness, increase average daily gain (ADG), alter dry matter intake (DMI), and produce heavier weight and leaner animals when harvested at equal duration of days on feed. Also, the use of GETs enhances live weight gain per unit of feed intake and this is referred to as feed efficiency (FE). The most widely used compounds, however, are steroidal implants and beta-adrenergic agonists (β-AA). Steroidal implants and β-AA improve animal growth performance by way of differing biological processes. Steroidal implants increase frame growth (i.e., long-bone growth) resulting in a heavier market weight when the beef animal reaches an industry acceptable level for fatness. Steroidal implants can be used multiple times throughout the life of a beef animal; it is not uncommon for a beef animal to be administered a suckling-, growing-, and finishing-phase growth promoting implant. The USDA-APHIS (2011) reported that greater than 90% of all feedlot cattle in the United States receive a steroidal implant during the feedlot phase of production. Steers administered a single finishing phase implant 143 days before harvest have increased carcass weight by 30 kg over non-implanted steers (Smith et al. 2018). β-AA are typically only fed during the last 21 to 42 days on feed in pigs and

In the United States, livestock producers have used various types of GETs such as steroidal implants and β-AA since the early 2000s to improve carcass leanness, increase average daily gain (ADG), alter dry matter intake (DMI), and produce heavier weight and leaner animals when harvested at equal duration of days on feed.

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cattle and 7 to 14 days on feed in turkeys. Pigs and cattle fed ractopamine hydrochloride (HCl) have increased daily gains and decreased carcass fatness. Samuelson and colleagues (2016) conducted a survey of 22 consulting nutritionists that managed approximately 14 million head of cattle annually (representing more than half of all feedlot cattle in the United States) and reported that approximately 85% of their clients used ractopamine HCl for approximately 31 days prior to harvest.

The most common and widely used type of GET are steroidal implants with anabolic activity that are used for beef cattle. More than 30 commercially available implants are marketed in the United States to beef cattle producers.

Steroidal implants with anabolic activity

The most common and widely used type of GET are steroidal implants with anabolic activity that are used for beef cattle. More than 30 commercially available implants are marketed in the United States to beef cattle producers. These are classified from low to high potency (Johnson and Beckett 2014). Implants are classified into these groups based on the differing amounts and ratios of anabolic compounds contained in the implant pellets. The active ingredients contained in steroidal implants belong to one of three major categories of hormones: androgens (e.g., trenbolone acetate or testosterone propionate), estrogens (e.g., estradiol-17β, estradiol benzoate, or zeranol), and progestins (e.g., progesterone). Steroidal implants are administered primarily using small pellets that are placed under the skin on the back of the animal's ear; this location ensures that no pellets will enter the food chain since ears are removed from the animal early in the harvest process and are not consumed by humans. Steroidal implants with anabolic activity have been proven safe over multiple years of study and have a zero-day withdrawal prior to harvest, because research shows that by harvest time, no residue remains that would be concerning to human health (Table 3).

Steroidal implants with anabolic activity have been proven safe over multiple years of study and have a zero-day withdrawal prior to harvest, because research shows that by harvest time, no residue remains that would be concerning to human health.

Human health concerns related to residues associated with GETs were largely linked to the use of Diethelstilbesterol (DES). The use of orally administered DES for cattle was approved by the U.S. Food and Drug Administration in 1954, and the use in of implants in growing-finishing cattle rations was approved in 1956. These products were rapidly adopted by the beef cattle industry (Raun and Preston 2002). The discovery of a low incidence of DES residues in the livers of cattle associated with misuse was later discovered and reported. These residues, along with the report of cervical cancer in daughters of mothers treated with prescription DES during pregnancy, led the Food and Drug Administration to remove oral DES for cattle from the market in 1972 and implants the following year (Table 4). The removal of DES from the market led to the development of a number of other growth stimulation products for cattle (Raun and Preston 2002).

Beta adrenergic agonist

Beta-adrenergic agonists are GETs approved by the FDA that is delivered through the animals' feed. These compounds are approved as growth regulators in meat animals including cattle, swine, and turkey, and are fed during the last 7 to 42 days prior to harvest depending upon the species. These growth regulators are absorbed from the digestive tract then enter the bloodstream where they bind with receptors on both fat and muscle tissue (Mersmann 1998). When bound to fat, it triggers increased fat breakdown and impedes lipogenesis (formation of new fat) (Hosford et al. 2015; Parr et al. 2014). When bound to muscle, it results in an increase in muscle accumulation and a de-

Beta-adrenergic agonists are a GET approved by the FDA that is delivered through the animals' feed. These compounds are approved as growth regulators in meat animals including cattle, swine, and turkey, and are fed during the last 7 to 42 days prior to harvest depending upon the species.

Growth Regulator	Year of FDA Approval	Applicable species
Oral diethylstilbestrol (DES)	1954	Cattle
DES implant	1956	Cattle
Estradiol benzoate / progesterone implants	1956	Castrated male cattle
Estradiol benzoate / testosterone propionate implants	1958	Intact and ovariectomized female cattle
Oral melengestrol acetate	1968	Intact female cattle
Zeranol (36 mg) implants	1969	Cattle
Oral DES removed from market	1972	-
DES implants removed from market	1973	-
Silastic estradiol implant	1982	Cattle
Estradiol benzoate / progesterone	1984	Cattle
Trenbolone acetate (TBA) implants	1987	Cattle
Estradiol (17-β) / TBA implants	1991	Castrated male cattle
Bovine somatotropin	1993	Lactating dairy cows
Estradiol (17-β) / TBA implants	1994	Intact and ovariectomized female cattle
Zeranol (72 mg) implants	1995	Cattle
Estradiol (17-β) / TBA implants	1996	Grazing cattle not fed in confinement
Ractopamine hydrochloride [2]	2000	Pigs
Ractopamine hydrochloride	2003	Cattle
Zilpaterol hydrochloride	2006	Cattle
Initial and delayed release: Estradiol (17-β) / TBA implants [2]	2007	Castrated male cattle
Ractopamine hydrochloride [2]	2008	Turkeys
Gonadotropin Releasing Factor – Diphtheria Toxoid conjugate [2]	2011	Pigs
Extended release: Estradiol benzoate / TBA implants[2]	2014	Cattle
Initial and delayed release: Estradiol (17-β) / TBA implants [2]	2017	Intact and ovariectomized female cattle
Delayed release: Estradiol (17-β) / TBA implants [2]	2017	Cattle

[1] Adapted from Johnson et al. 2013.
[2] Smith et. al. 2018

Table 4. Chronological sequence of FDA approval of growth regulators used in the U.S. animal production industry.

Item	ADI, µg/kg of BW	Liver, µg/kg	Muscle, µg/kg
Clenbuterol	0-0.004	0.6	0.2
Ractopamine HCl	0.1	40	10
Zilpaterol HCl	0-0.04	3.5	0.5

Table 5. Maximum residue levels of beta-adrenergic agonists. From Joint FAO and WHO Expert Committee on Food Additives 2014.

crease in the rate of muscle tissue breakdown (Hosford et al. 2015; Mersmann 1998; Parr et al. 2014).

There are two β-AA that have been approved by the FDA for use in meat animal production in the United States.

There are two β-AA that have been approved by the FDA for use in meat animal production in the United States. One compound is identified as β1-AA and is approved for use in cattle, swine and turkey—ractopamine HCl and the other is identified as β2-AA which is approved for use in cattle—zilpaterol HCl (Table 5). Ractopamine HCl is fed with a zero-day withdrawal prior to harvest in cattle, swine, and turkey; while cattle fed zilpaterol HCl are subjected to a 72-hour withdrawal period prior to harvest.

Melengestrol acetate (Heifermax or MGA) is a synthetic progestin which is orally active. It is used in finishing heifers as a means to combat estrous cyclicity and is also used in female breeding synchronization programs .

Melengestrol acetate

Melengestrol acetate (MGA) (e.g., HeifermaX®) is a synthetic progestin which is orally active. It is used in finishing heifers as a means to combat estrous cyclicity and is also used in female breeding synchronization programs (Perry et al. 2005; Sides et al. 2009). Although progesterone is not generally thought of as anabolic in nature, this compound increases endogenous estradiol secretions which have been shown to increase gains by 6 to 9% in treated heifers compared to non-treated heifers (Bloss et al. 1966). When MGA is fed to breeding animals the risk for environmental exposure through meat is even less because it is likely that these females will be bred, become pregnant, deliver a calf, and raise that calf to weaning prior to the cow entering the beef supply chain.

Gonadotropin releasing hormone antagonist is used in beef and pork production around the world as an alternative to castration.

Gonadotropin releasing hormone antagonist

Gonadotropin releasing hormone antagonist (e.g., Improvest®) is used in beef and pork production around the world as an alternative to castration. Animals might receive injections of the antagonist throughout the course of production and this results in reduced endogenous testosterone production which is a major causative agent of off flavors and odors associated with pork harvested from intact males (Bilskis et al. 2012).

Produced in animals by transgenes.

There is one FDA-approved transgenic animal for food use, AquAdvantage™ salmon. By using this genetic modification, the salmon can grow year-round as compared to seasonal growth of conventional salmon.

There is one FDA-approved transgenic animal for food use, AquAdvantage™ salmon. All salmon produce GH, and this Atlantic salmon has a transgene with a regulatory element derived from ocean pout and a DNA sequence for growth hormone from Coho salmon. By using this genetic modification, the salmon can grow year-round as compared to seasonal growth of conventional salmon. Regulatory studies were conducted to determine residues of GH and other hormones related to the GH axis in salmon tissues (FDA-CVM 2010). The study examined tissues from farmed salmon from Canada (n=10), farmed salmon from the study sponsor (n=33), and transgenic salmon from the sponsor (n=30). Tissue GH was below the assay's limit of quantitation (LOQ) for all 73 samples and there were no significant differences between groups for concentrations of estradiol, testosterone, 17-ketotestosterone, T3, or T4. IGF-1 was not detectable for 100%, 67% and 73% of the farm control, sponsor control and transgenic salmon, respectively. FDA concluded that the difference in IGF-1 was insignificant compared to normal levels of exposure to salmon in the diet.

Hormones used in animals-lactation management

Perhaps the most recognized hormone used in dairy management is bST, which is naturally produced by the pituitary gland to regulate growth and lactation.

Bovine Somatotropin

Perhaps the most recognized hormone used in dairy management is bST, which is naturally produced by the pituitary gland to regulate growth and lactation. Its physiological function is exerted upon binding to receptors in responsive tissues, like any other protein hormone. It has been known since the 1930s that this hormone has the ability to increase milk production in cows. The mechanism of action of endogenous bST to promote galactopoiesis is well understood. Briefly, it involves changes in the metabolism and mobilization of nutrients from tissues (e.g., muscle, liver, and adipose) to support increased milk production. In other words, it makes nutrients available for the synthesis of milk by the mammary gland (Etherton and Bauman 1998). Advances in biotechnology and engineering allowed the production of bST by recombinant DNA technology in the 1970s. Recombinant bST (rbST) is a synthetic version of the natural hormone that is commercially produced by the same process used to make human insulin for diabetics: purified from recombinant bacteria.

Clinical studies demonstrated that bST elicited no biological actions of somatotropin in humans even if injected or orally administered to humans.

Differences in the ability of somatotropin from one species to elicit biological effects in other species have been extensively reported. This term was defined as “species limited” (Bauman 1992). Clinical studies demonstrated that bST elicited no biological actions of somatotropin in humans even if injected or orally administered to humans (Collier and Bauman 2014). The sequence of bST differs by about 35% from human somatotropin, which makes bST unable to bind to the human somatotropin receptors in body tissues (Juskevich and Guyer 1990). The digestive tract secretes enzymes that break all ingested proteins down to amino acids that are absorbed. Therefore, both bST and rbST are not hormonally active in humans and, if ingested, they are rapidly digested to small peptides and amino acids because they are protein hormones.

The FDA reported that there is no legal basis requiring the labeling of milk from cows that were supplemented with rbST since the milk is indistinguishable from milk from cows not supplemented with bST.

Composition of milk (fat, protein, lactose, cholesterol, minerals, and vitamins) and manufacturing characteristics are not substantially altered with bST use (Bauman 1992; Lynch et al. 1992). For example, bST treatments do not impact milk minerals and enzymes, freezing point, pH value, milk flavor, distribution of whey casein and protein, cheese coagulation time, standard curd firmness, or cheese yields (Laurent et al. 1992; Van Den Berg 1991). A recent meta-analysis by Saint Pierre and colleagues (2014) summarizing 26 studies published in peer-reviewed journals or reviewed by a regulatory agency concluded that despite increased milk and component yields in cows treated with rbST, body condition score was not altered. The FDA reported that there is no legal basis requiring the labeling of milk from cows that were supplemented with rbST since the milk is indistinguishable from milk from cows not supplemented with bST. “Indeed, milk labeled as rbST-free and organic or unlabeled conventional milk do not differ in composition (O’Donnell et al. 2010; Vicini et al. 2008), and there is no validated test that can distinguish among these milk sources.”

IGF-1 is not inactivated by pasteurization and therefore is present in retail cows’ milk. 00 the additional amount of IGF-1 that might be absorbed represents 0.09% of the normal daily production of IGF-1 in adults.

Concentration of IGF-1 in milk from cows treated with rbST is within the range of IGF-1 concentrations in milk from untreated cows (Collier et al. 1991; Prosser, Fleet, and Corps 1989). It is known that some biological actions of bST are in part mediated by IGF-1. Unlike other milk proteins, IGF-1 is not denatured (inactivated) by pasteurization and therefore is present in retail cows’

milk. However, IGF-1 makes up only 0.00003% of total milk proteins, and the additional amount of IGF-1 that might be absorbed by humans drinking milk from rbST cows (assuming no degradation and complete absorption) represents 0.09% of the normal daily production of IGF-1 in adults. Many body fluids including gastrointestinal secretions of humans contain IGF-1. The amount of IGF-I in 1.5. Liters of milk from rbST-treated cows as compared with milk from untreated cows is only about 0.8% of gastrointestinal secretion of IGF-1 (WHO 1998). The levels of IGF-1 found in the milk of rbST-treated cows are still within the physiological range typically observed in early lactation of untreated cows and less than that found in human milk or saliva (Collier et al. 1991; Collier and Bauman 2014). Furthermore, similar to bST results, studies with laboratory animal models have demonstrated that IGF-1 has no biological activity even if administered orally at high doses (Juskevich and Guyer 1990). Given that proteins are digested if consumed orally, the possible absorption of orally consumed IGF-I has been directly examined in humans, specifically premature neonates and young adults; results are convincing and provide no evidence that orally consumed IGF-I is absorbed in humans (Corpeleijn et al. 2008; Mero et al. 2002).

Similar to bST results, studies with laboratory animal models have demonstrated that IGF-1 has no biological activity even if administered orally at high doses.

Some studies have reported a potential relationship between blood levels of IGF-1 and the risk of prostate (Harrison et al. 2017) and breast cancer (Hankinson 1998). However, recent studies have failed to confirm that milk consumption is associated with increased cancer risk (López-Plaza et al. 2019; Preble et al. 2019).

The FDA, WHO, and National Institutes of Health have independently stated that dairy products from rbST-treated cows are safe for human consumption. The most recent review of the human safety issues was performed in 2014 by the 78th meeting of the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives and this committee reaffirmed the human safety of rbST.

The FDA, WHO, and National Institutes of Health have independently stated that dairy products from rbST-treated cows are safe for human consumption.

A recent assessment on the use of rbST by Collier and Bauman (2014) summarized that (1) consuming milk from dairy cows treated with bST poses no risk for human health and (2) the welfare does not differ between bST treated cows and untreated high-producing dairy cows. Rigorous sequential safety assessments of rbST have made it the most highly tested and exhaustively studied recombinant product for use in dairy ruminants. Although the use of rbST is still approved in the United States, the demand for the product has decreased in recent years. Many large grocery store chains no longer carry milk from cows treated with this recombinant hormone.

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Oxytocin

Oxytocin is a small peptide hormone made up of a sequence of nine amino acids. The main physiological function of oxytocin is to cause contraction of smooth muscle cells. In females, oxytocin is best known for its ability to stimulate labor, by promoting uterine contractions, and milk ejection, by contracting the myoepithelial cells surrounding the milk alveoli in the mammary gland (Crowley and Armstrong 1992). This hormone has also been linked to emotional and behavioral processes, including maternal behavior and mother-offspring bonding (Caldwell et al. 2017; Hashimoto, Uezono, and Ueta 2012). The role of oxytocin in the milk-let down process is well understood. Basal

The main physiological function of oxytocin is to cause contraction of smooth muscle cells. In females, oxytocin is best known for its ability to stimulate labor, and milk ejection.

circulating levels of oxytocin are low (1–5 pg/ml), but oxytocin is naturally released into blood circulation after 0.5–3 min of tactile stimulation of the teat or nipple (e.g., suckling of the offspring or pre-stimulation during milking) in quantities ranging from 10 to 100 pg/ml of plasma. A minimum of 10 pg/ml is enough to elicit a milk-ejection response (Schams and Karg 1986). Within the mammary gland, oxytocin binding to its receptor causes myoepithelial cells surrounding the alveoli to contract and consequently, the milk stored in the alveoli travels into the mammary ducts, the gland cistern and finally out of the gland (Akers and Lefcourt 1984). Oxytocin has a short half-life in the blood (0.55–3.6 min), meaning that it is rapidly broken down and cleared from circulation. Therefore, the active removal of milk must be closely timed with stimulation of the teat or nipple. More than 80% of the milk is stored in the alveolar fraction of the gland and will only be available after milk ejection is induced by the release of oxytocin (Bruckmaier and Blum 1998).

Oxytocin was approved by the FDA in 1972 for therapeutic use in several animal species, specifically for inducing uterine contractions and contractions of smooth muscle cells of the mammary gland for milk letdown.

Oxytocin was approved by the FDA in 1972 for therapeutic use in several animal species, specifically for inducing uterine contractions and contractions of smooth muscle cells of the mammary gland for milk letdown. In humans, oxytocin injection (brand name Pitocin®) is used for its ability to stimulate labor and milk ejection. The initial dose of Pitocin® is set at 0.5–1 milliunits/min (mU/min). Afterwards, the dose can be gradually increased in increments of 1–2 mU/min until the desired effect is established. Exogenous administration of this hormone has been widely used in veterinary and clinical medicine, including companion animals. In dairy cows, complete removal of the alveolar milk at each milking is crucial to remove inhibitors of lactation built up in milk and maintain milk synthesis and secretion throughout a lactation. To achieve this, a calm routine is crucial for successful milking. Disturbed milk ejection can be caused by unfamiliar surroundings, lack of routine, and a multitude of different stressors, associated with a reduced release or complete absence of oxytocin from the pituitary (central inhibition) which impairs milk ejection (Bruckmaier, Schams, and Blum 1993). This not only causes milk losses, primarily in primiparous and late lactation cows, but also is associated with an increased risk of mastitis. To overcome this issue, a synthetic form of oxytocin can be exogenously administered intramuscularly to dairy cows before each milking to aid with milk ejection-reflex (Beloand Bruckmaier 2010). An alternative to oxytocin injections is vaginal or cervical stimulation to promote oxytocin release and trigger a milk ejection response, however this practice is time-consuming, laborious, and not practical in most dairy farms' milking parlors or robotic milking settings.

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Current FDA regulations allow the use of injected (intravenous, subcutaneous, or intramuscular) oxytocin in livestock.

Current FDA regulations allow the use of injected (intravenous, subcutaneous, or intramuscular) oxytocin in livestock. Each milliliter of injection formulation contains 20 U.S.P. units of oxytocin. The recommended dosage for obstetrical use is 5 ml in cows and horses and 1.5–2.5 ml in ewes and sows, however it is most commonly used to assist in milk letdown in cows (0.5–1.0 ml) and sows (0.25–1.0 ml). The plasma concentrations of oxytocin achieved by injecting the recommended doses are higher than plasma concentrations of un-injected controls at milking. Oxytocin is also used in dairy cows for mastitis therapy (Knight et al. 2000) or chronically to increase milk production (Nostrand et al. 1991), even though oxytocin is listed only for use in "post-parturition therapeutic applications" which presumably does not include prolonged use to increase dairy milk production. In fact, oxytocin is not currently

Oxytocin is not currently approved for widespread use in U.S. dairies, but is permitted for cows with milk difficulty under veterinary supervision.

Oxytocin is currently included on the National List of Allowed and Prohibited Substances as a synthetic substance allowed for use in organic livestock production.

approved for widespread use in U.S. dairies, but is permitted for cows with milk difficulty under veterinary supervision. The chronic use of oxytocin reduces the responsivity of the mammary gland to oxytocin. Consequently, withdrawal can be detrimental for milk ejection, complete udder emptiness, intramammary infections, and milk synthesis (Macuhová, Tancin, and Bruckmaier 2004). Oxytocin is currently included on the National List of Allowed and Prohibited Substances as a synthetic substance allowed for use in organic livestock production.

After exogenous administration of high doses of oxytocin, milk and plasma oxytocin levels were comparable to those of untreated cows during milk ejection.

Public concerns over the use of this hormone in dairy livestock not only to aid milk ejection but to routinely boost milk output resulted in studies to address oxytocin levels in the milk, potential milk alterations, or transfer to human consumers. After exogenous administration of high doses of oxytocin, milk and plasma oxytocin levels were comparable to those of untreated cows during milk ejection (Prakash et al. 2009). Even though these authors reported that oxytocin is stable at different pasteurization temperatures, the milk is considered safe for human consumption. Although a few short-term experiments using oxytocin report slight changes in milk fat, chronic use of oxytocin at milking does not alter milk fat content and protein percentages. Moreover, chronic use of this hormone in dairy cows at milking causes an overall greater milk yield between 3 and 10%, and consequently total quantity of milk fat and milk protein, with no apparent effect on health (Ballou et al.1993; Nostrand et al. 1991). It is important to note that regardless of the source, whether secreted endogenously or administered exogenously, oxytocin physiological effects are observed within minutes and it is metabolized rapidly, leading to inactive products which are the same amino acids used to make all proteins. Because this drug metabolizes rapidly there is no apparent risk of residues occurring in milk.

Use of hormones to improve cow health

Granulocyte colony stimulating factor

Maintaining good management practices as well as stimulating immunocompetence and disease resistance of cows by proper use of immunomodulators can help to reduce antibiotic use in dairy farms.

The dynamic, metabolic, and physiologic changes occurring as a cow transitions from pregnancy into lactation predispose the cow to increased mastitis susceptibility alongside other metabolic problems. Mastitis is the most common disease in dairy cattle characterized by various degrees of severity. Although mastitis can occur from physical injuries, it is primarily caused by biological agents such as bacteria introduced either during the milking process or from environmental contact. Lactating dairy cows with mastitis produce lower milk quantity and quality relative to healthy cows (Wilson et al. 2004), leading to significant economic losses (Hogeveen, Huijps, and Lam 2011; Rollin, Dhuyvetter, and Overton 2015). Currently, antimicrobial treatment is indispensable to maintain bovine udder health, animal welfare, and dairy farm economics. Emergence and spread of antimicrobial resistance is an urgent matter of public interest, and consequently, antimicrobial usage in production livestock is a critically discussed subject (Krömker and Leimbach 2017). Maintaining good management practices as well as stimulating immunocompetence and disease resistance of cows by proper use of immunomodulators can help to reduce antibiotic use in dairy farms (Trevisi et al. 2014).

Human granulocyte colony stimulating factor (rhG-CSF) was administered to dairy cows with the goal of activating the function of peripheral blood granulocytes and protecting the mammary gland from an experimental *Staphylococcus aureus* challenge.

Another immune system activator in mammals is a protein (cytokine) known as granulocyte colony-stimulating factor (G-CSF) which is naturally produced by white blood cells (immune cells). This protein stimulates the precursors of immune cells such as neutrophils, macrophages, monocytes, eosinophils, and basophils in a process called myeloid cell proliferation. Humans taking G-CSF supplements have increased levels of circulating immune cells (Karawajczyk et al. 1997), and it has been used to aid in recovering depleted immune cells following chemo/radiation therapy and hematopoietic stem cell transplantation (reviewed by Elfenbein and Sackstein 2004). Human granulocyte colony stimulating factor (rhG-CSF) was administered to dairy cows with the goal of activating the function of peripheral blood granulocytes and protecting the mammary gland from an experimental *Staphylococcus aureus* challenge (Nickerson et al. 1989). These authors observed a 46.7% reduction in new infections in quarters of treated cows compared with controls. More recently, a polyethylene glycol-conjugated bovine granulocyte colony-stimulating factor (PEG-bG-CSF) treatment, was the first product of its kind to be approved by the FDA for the reduction in the incidence of clinical mastitis in the first 30 days of lactation in periparturient dairy cows.

Since the bioavailability of PEG-bG-CSF after oral exposure is negligible, the FDA concluded that because of the negligible oral bioavailability of PEG-bG-CSF there is no need to establish an ADI. Furthermore, since the oral bioavailability of PEG-bG-CSF is negligible the ingestion of potential residues of PEG-bG-CSF in animal tissues or products does not pose a risk to consumers (Rhodes 2018).

Hormone and hormone-like products used for livestock production are regulated in the United States by the FDA.

ADI, MRL and safe levels for hormones in foods

Risk Estimates of Residues in Food

Both the FDA and the JECFA establish residue levels tolerances or Maximum Residue Limits (MRL).

Risk assessment is an integrative strategy to assume the probability of human illness caused by the ingestion of livestock products containing residual veterinary drugs. Risk assessments for use of antimicrobials in livestock production have largely involved the risk for development of antibiotic resistance of microbes while risk assessment for hormones have largely assessed potential risks for impact on human growth, development, and disease. Hormone and hormone-like products used for livestock production are regulated in the United States by the FDA. Internationally, the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) is a committee of the Food and Agriculture Organization (FAO) of the United Nations and the WHO. One of JECFA's roles is to perform risk assessments on residues of veterinary drugs in food. Both the FDA and the JECFA establish residue levels tolerances or Maximum Residue Limits (MRL). From the viewpoint of risk management, MRL are regarded as a monitoring tool for compliance to the approved conditions of use, and the ADI level is a decision point for human health impacts (Jeong et al. 2010).

Endogenous steroid hormones are produced mainly by the ovaries (estrogen, progesterone) and testes (testosterone), throughout the lifetime of humans and mammals.

Human exposure to hormone residues

Endogenous steroid hormones are produced mainly by the ovaries (estrogen, progesterone) and testes (testosterone), throughout the lifetime of humans and mammals. These hormones are required for proper physiological functions,

such as growth, function of the reproductive organs, and development of secondary sex characteristics. The existence of these naturally occurring hormones in humans and food-producing animals documents the human consumer is exposed throughout his/her lifetime to rather large quantities of these steroid hormones through his/her own daily production and, too much lesser quantities, from untreated food-producing animals.

Products requiring an interval between use and reduction of residues to a safe concentration must have a practical method for determining the quantity of the product in edible animal tissue ingested.

Analytical methods for residues

The objective of the total residue and metabolism study is to develop information on the amount, persistence, and chemical nature of the total residues, and the metabolic fate of the new animal drug in the treated target animals. Products requiring an interval between use and reduction of residues to a safe concentration must have a practical method for determining the quantity of the product in edible animal tissue ingested. This practical method is employed to continuously monitor samples by the USDA-FSIS for muscle, kidney, liver, and fat, and by the FDA for milk to ensure that the proposed use of the product does not pose any human health harm.

Data on residues of xenobiotic hormones that occur are used to establish a no observed effect level (NOEL) which is accepted as the no observed adverse effect level (NOAEL).

For analogs (xenobiotics) of the natural hormones, studies are required by the FDA-CVM to adequately address: (1) toxicology including mutagenicity, 90-day feeding, reproductive, teratology, and special as needed per drug studies, (2) metabolism in the target animal and comparative metabolism in rodents, (3) identification of the tissue(s) containing the greatest concentration of residues (target tissue), (4) identification of the residue that would be measured to determine when the residue(s) were below a safe concentration (marker residue), and (5) time post-treatment when the residue(s) are depleted in the tissues.

A safety factor is applied to the NOEL to obtain an allowable daily intake (ADI). A safety factor (SF) of 100 fold is used unless a product is believed to have potential to be a carcinogen, which uses a SF of 1000 or greater.

Data on residues of xenobiotic hormones that occur are used to establish a no observed effect level (NOEL) which is accepted as the no observed adverse effect level (NOAEL). A safety factor is applied to the NOEL to obtain an allowable daily intake (ADI). A safety factor (SF) of 100 fold (10-fold for extrapolating animal data to humans and 10-fold for variability in sensitivity to the toxicity of the product among humans as well as humans of lower body weights) is used unless a product is believed to have potential to be a carcinogen, which uses a SF of 1000 or greater. Consumption Factors (CF; g/day) have been codified worldwide to be: 300 g for muscle, 100 g for liver, 50 g for kidney, 50 g for fat, 1,500 ml for milk, and 100 g for eggs. A standard human body weight has been codified worldwide as 60 kg. A complete accounting of all information and adequate reports of the studies are required by regulatory agencies worldwide. Good Laboratory Procedures (GLP) are required for validated analytical procedures and studies.

ADI= NOAEL divided by the SF.

Safe Concentration (SC)= [ADI X 60 kg] divided by CF.

Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined.

Once a final ADI, safe concentration, target tissue, and marker residue are selected, a tolerance for the new animal drug can be determined. The tolerance is the maximum concentration of a marker residue, or other residue indicated for monitoring, that can legally remain in a specific edible tissue of a treated animal. The FDA-CVM may assign the tolerance by harmonizing with previously

established international MRL. Tolerances and MRL are both numbers that describe the limits of residues. However, tolerances and MRL are not derived in the same manner and are used for different purposes. At times, it may be appropriate to harmonize the tolerance with an already established MRL.

The FDA cannot approve a compound for use as a new animal drug in food-producing animals when the compound or any of its metabolites has been found to cause cancer in animals or humans, unless: (1) the compound will not adversely affect the animals for which it is intended, and (2) no residue of the compound will be found by approved regulatory methods in any edible tissues. For products that have been identified by regulatory agency personnel to not become a component of food at concentrations considered unsafe, a regulatory method is not required.

The FDA cannot approve a compound for use as a new animal drug in food-producing animals when the compound or any of its metabolites has been found to cause cancer in animals or humans, unless: (1) the compound will not adversely affect the animals for which it is intended, and (2) no residue of the compound will be found by approved regulatory methods in any edible tissues.

The JECFA has established MRL for xenobiotic compounds (i.e., trenbolone acetate and zeranol), and has set an average ADI for natural compounds, such as estradiol-17β and testosterone propionate. Ensuring food safety to the public is based upon calculations from the no-hormonal effect level (NHEL). Once the NHEL is established, a safety factor of 100-fold is assigned and the resulting value is the ADI in µg/kg of body weight. The determination of the NHEL, ADI, MRL all occur during the new animal drug application process and prior to use in commercial practice in order to ensure minimal risk for environmental exposure to humans. The reason there is no MRL for natural compounds is because implanted animals rarely have differing residue levels from non-implanted intact animals following harvest.

Ensuring food safety to the public is based upon calculations from the no-hormonal effect level (NHEL). Once the NHEL is established, a safety factor of 100-fold is assigned and the resulting value is the ADI in µg/kg of body weight.

The non-synthetic beef implants, bST, and GH in transgenic salmon do not have tolerances since these hormones are in milk, fish flesh, and beef naturally and exogenous administration does not result in residues that are outside of the normal range. Moreover, GH from salmon and cattle are species limited and when ingested it is digested. The tolerances for synthetic implants are in Table 6.

The non-synthetic beef implants, bST, and GH in transgenic salmon do not have tolerances since these hormones donot result in residues that are outside of the normal range.

Name	FDA (US 21 CFR 556)	JECFA (JECFA 2014) (ug/kg)
Melengestrol acetate	Fat: 25 µg/kg	Muscle: 1 Liver: 10 Kidney: 20 Fat: 18
Trenbolone acetate	Cattle edible tissue (excluding milk): NR[1]	Muscle: 2 Liver: 10
Zeranol	Cattle edible tissue: NR[1]	Muscle: 2 Liver: 10

Table 6. Tolerances for residues of synthetic growth enhancing technologies in animal tissues. [1]MRL is not required.

Use of endocrine regulators to improve efficiency of growth, lactation and reproduction has contributed significantly to providing a safe and low-cost food supply to American consumers.

Adverse health events in humans associated with correct label use of approved products is virtually unknown and when these events are reported in other countries they are always associated with illegal or off-label use of products that are contaminated with other compounds or were given at dosages not allowed in the United States.

Some products such as pharmaceuticals, marketed for use in animals have withdrawal periods established for use of the product. The withdrawal period or the milk discard time is the interval between the time of the last administration of a new animal drug and the time when the animal can be safely harvested for food or the milk can be safely consumed. Withdrawal times are the intervals between the last product administration and when residues are depleted below the concentration deemed to be safe for human consumption. Withdrawal times are conservative statistical estimates to assure the animal products are safe for human consumption.

The ADI may need to be partitioned across edible tissues based on the expected use of the new animal drug and the food consumption values for each tissue. For products not used in lactating dairy cows, the ADI can be allocated to edible muscle, liver, kidney, or fat. For products approved in lactating dairy cows or for products approved in lactating dairy cows and other meat-producing species, a part of the ADI would be reserved for milk. The remaining part of the ADI would be partitioned among edible tissues.

Summary

Use of endocrine regulators to improve efficiency of growth, lactation and reproduction has contributed significantly to providing a safe and low-cost food supply to American consumers. Furthermore, they have contributed substantially to reducing the carbon footprint of animal agriculture by reducing the nutrient requirement for a unit of productive output whether that is meat, milk or eggs. The intense regulatory scrutiny required to approve these products for use in animal agriculture has also contributed to the safety of the world's food supply

Hormones are naturally present in milk, meat and eggs, and the FDA has concluded that no physiological effect could be expected in consumers eating animal products containing additional amounts of the hormone that is less than or equal to 1% of the amount produced by the human daily and would be digested like other proteins and steroids found in milk and meat. None of the approved naturally occurring hormones in use produce residues in animal products that surpass this target level. For synthetic compounds, the use of production enhancing technologies is heavily regulated in the United States and across the world by multiple agencies resulting in an extremely safe food supply. Correct use of supplemental hormone products in food animals according to their labels is safe, having no adverse effects on human health.

References

Adams, N.R. 1995. Detection of the effects of phytoestrogens on sheep and cattle. *J Anim Sci* 73 (5):1509–1515, https://doi.org/10.2527/1995.7351509x.

Akers, R.M. and A. M. Lefcourt. 1984. Effect of presence of calf on milking-induced release of prolactin and oxytocin during early lactation of dairy cows. *J Dairy Sci* (1):115–22.

Apple, J. K., P. J. Rincker, F. K. McKeith, S. N. Carr, T. A. Armstrong, and P. D. Matzat. 2007. Meta-Analysis of the Ractopamine Response in Finishing Swine. *Profess Anim Sci* 3:179–196.

Avery, A. and D. Avery. 2007. *The Environmental Safety and Benefits of Pharmaceutical Technologies in Beef Production*. Center for Global Food Issues, Churchill, Virginia.

Ballou L. U., J. L. Bleck, G. T. Bleck, and R. D. Bremel. 1993. The effects of daily oxytocin injections before and after milking on milk production, milk plasmin, and milk composition. *J Dairy Sci* 76 (6): 1544–9.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J Dairy Sci* 63:1514–1529.

Bauman, D. E. 1992. Bovine Somatotropin: Review of an Emerging Animal Technology. *J Dairy Sci* 75 (12): 3432–3451.

Belo, C. J., and R. M. Bruckmaier. 2010. Suitability of low-dosage oxytocin treatment to induce milk ejection in dairy cows. *J Dairy Sci* 93 (1): 63–9.

Bilskis, R., N. Sutkeviciene, V. Riskeviciene, A. Januskauskas, and H. Zilinskas. 2012. Effect of active immunization against GnRH on testosterone concentration, libido and sperm quality in mature AI boars. Acta Vet Scand 54 (1): 33–33, doi: 10.1186/1751-0147-54-33.

Bloss, R. E., J. I. Northam, L. W. Smith, and R. G. Zimbelman. 1966. Effects of Oral Melengestrol Acetate on the Performance of Feedlot Cattle. *J Anim Sci* 25 (4): 1048–1053, doi: 10.2527/jas1966.2541048x.

Bruckmaier R. M. and J. W. Blum. 1998. Oxytocin release and milk removal in ruminants. *J Dairy Sci* 81:939–949.

Bruckmaier R.M., D. Schams, and J. W. Blum. 1993. Milk removal in familiar and unfamiliar surroundings: Concentrations of oxytocin, prolactin, cortisol and beta-endorphin. *J Dairy Res* 60 (4): 449–56.

Caldwell H. K., E. A. Aulino, A. R. Freeman, T. V. Miller, and S. K. Witchey. 2017. Oxytocin and behavior: Lessons from knockout mice. Dev Neurobiol 77 (2): 190–201.

Capper, J. L. 2011. The environmental impact of beef production in the United States: 1977 compared with 2007. *J Anim Sci* 89:4249-4261. doi: 10.2527/jas.2010-3784

Capper, J. I. and R. A. Cady. 2020. The effects of improved performance in the U.S. dairy cattle industry on environmental impacts between 2007 and 2017. *J Anim Sci* 98:1–14.

Chenault, J. R., R. E. Hornish, Y. C. Anderson, L. F. Krabill, J. F. Boucher, and M. J. Prough. 2003. Concentrations of progesterone in milk of cows administered an intravaginal progesterone insert. *J Dairy Sci* 86:2050–2060, doi: 10.3168/jds.S0022-0302(03)73794-1.

Code of Federal Regulations. 2019. Food and Drugs. Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=522 and 556.

Collier, R. J., M. A. Miller, J. R. Hildebrandt, A. R. Torkelson, T. C. White, K. S. Madsen, J. L. Vicini, P. J. Eppard, and G. M. Lanza. 1991. Factors affecting insulin-like growth factor-I concentration in bovine milk. *J Dairy Sci* 74 (9): 2905–2911, doi: 10.3168/jds.S0022-302(91)78473.

Collier, R. J. and D. E. Bauman. 2014. Update on human health concerns of recombinant bovine somatotropin use in dairy cows. *J Anim Sci* 92 (4): 1800–7.

Cornwell, T., W. Cohick, and I. Raskin. 2004. Dietary phytoestrogens and health. *Phytochemistry* 65:995–1016, doi: 10.1016/j.phytochem.2004.03.005.

Corpeleijn, W. E., I. van Vliet, D.-A. H. de Gast-Bakker, S. R. D. van der Schoor, M. S. Alles, M. Hoijer, D. Tibboel, and J. B. van Goudoever. 2008. Effect of enteral IGF-1 supplementation on feeding tolerance, growth, and gut permeability in enterally fed premature neonates. J. *Pediatr Gastroenterol Nutr* 46:184–190.

Crowley, W.R. and W. E. Armstrong. 1992. Neurochemical regulation of oxytocin secretion in lactation. *Endocr Rev* 13 (1): 33–65.

Decker, E. A. and Y. Park. 2010. Healthier meat products as functional foods. *Meat Sci* 86:49–55.

Dennett, H. P. J., and C. McMartin. 1979. Peptide Hormones and Their Analogues: Distribution, Clearance from the Circulation, and Inactivation in Vivo. *Pharmacol Rev* 30 (3): 247–292.

Dohoo I. R., K. Leslie, L. DesCôteaux, A. Fredeen, P. Dowling, A. Preston, and W. Shewfelt. 2003. A meta-analysis review of the effects of recombinant bovine somatotropin. 1. Methodology and effects on production. *Can J Vet Res* 67 (4): 241–51.

Doyle, E. 2000. *Human Safety of Hormone Implants Used to Promote Growth in Cattle: A Review of the Scientific Literature*. Food Research Institute, Madison, Wisconsin. 1–24.

Ehling, S. and T. M. Reddy. 2015. Analysis of glyphosate and aminomethylphosphonicacid in nutritional ingredients and milk by derivatization with fluorenylmethyloxycarbonyl chloride and liquid chromatography-mass spectrometry. *J Agric Food Chem* 63:10562–10568, doi: 10.1021/acs.jafc.5b04453.

Elfenbein G.J. and R. Sackstein. 2004. Primed marrow for autologous and allogeneic transplantation: a review comparing primed marrow to mobilized blood and steady-state marrow. *Exp Hematol* 32 (4): 327–39.

Etherton T. D. and D. E. Bauman. 1998. Biology of Somatotropin in Growth and Lactation of Domestic Animals. *Physiol Rev* 78 (3): 745–761.

Farber, T. M. and M. Arcos. 1983. A regulatory approach to the use of anabolic agents. In . E. Meissonnier and J. Mitchell-Vigneron (eds.) Anabolics in *Animal Production: Public health aspects, analytical methods and regulations*. OIE Symposium, Paris, France 15–17 February.

Gaucheron, F. 2011. Milk and dairy products: a unique micronutrient combination. *J Am Coll Nutr* 5 (1): 400S–9S.

Grosvenor, C. E., M. F. Picciano, and C. R. Baumrucker. 1993. Hormones and growth factors in milk. *Endocr Rev* 14 (6): 710–728, doi: 10.1210/edrv-14-6-710.

Hamosh, M. 2001. Bioactive factors in human milk. *Pediatr Clin North Am* 48 (1): 69–86.

Hankinson, S., W. C. Willett, G. A. Colditz, D. J. Hunter, D. S. Michaud, B. Deroo, B. Rosner, F. E. Speizer, M. Pollak. 1998. Circulating concentration of insulin-like growth factor-1 and risk of breast cancer. *Lancet* 351 (9113): 1393–1396.

Harrison, S., R. Lennon, J. Holly, J. P. T. Higgins, M. Gardner, C. Perks, T. Gaunt, V. Tan, C. Borwick, P. Emmet, M. Jeffreys, K. Northstone, S. Rinaldi, S. Thomas, S. D. Turner, A. Pease, V. Vilenchick, R. M. Martin, S. J. Lewis. 2017. Does milk intake promote prostate cancer initiation or progression via effects on insulin-like growth factors (IGFs)? A systematic review and meta-analysis. *Cancer Causes Control* 28 (6): 497–528.

Hartmann, S., M. Lacorn, H. Steinhart. 1998. Natural occurrence of steroid hormones in food. *Food Chem* 62:7-20.

Hashimoto, H., Y. Uezono, Y. Ueta. 2012. Pathophysiological function of oxytocin secreted by neuropeptides: A mini review. *Pathophysiology* 19 (4): 283–98.

Hogeveen, H., K. Huijps, and T. J. Lam. 2011. Economic aspects of mastitis: New developments. *NZ Vet J* 59 (1): 16–23.

Hosford, A. D., J. E. Hergenreder, J. K. Kim, J. O. Baggerman, F. R. B. Ribeiro, M. J. Anderson, K. S. Spivey, W. Rounds, and B. J. Johnson. 2015. Effects of supplemental lysine and methionine with zilpaterol hydrochloride on feedlot performance, carcass merit, and skeletal muscle fiber

characteristics in finishing feedlot cattle. *J Anim Sci* 93(9):4532-4544. doi: 10.2527/jas.2015-9047.

Jeong. S. H., D. Kang, M-W. Lim, C. S. Kang, and H. J. Sung. 2010. Risk Assessment of Growth Hormones and Antimicrobial Residues in Meat. *Toxicol Res* 26 (4): 301–313.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2000a. *Procedures for recommending maximum residue limits-residues of veterinary drugs in food*. Pp. 43–51

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 200b Toxicological evaluation of certain veterinary drug residues in food: Estradiol-17β progesterone and testosterone. WHO Food Additives Series 43

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2000c. Toxicological evaluation of certain veterinary drug residues in food: Melengestrol acetate. WHO Food Additives Series 45

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2014. Residue evaluation of certain veterinary drugs. 78th meeting of the Joint FAO/WHO Expert Committee on Food Additives, 5–14 November, Geneva, Switzerland. http://www.fao.org/3/a-i3745e.pdf.

Johnson, B. J. and J. Beckett. 2014. *Application of Growth Enhancing Compounds in Modern Beef Production Executive Summary*. American Meat Science Association, Savoy, Il.

Johnson, B. J., F. R. B. Ribeiro, and J. L. Beckett. 2013. Application of growth technologies in enhancing food security and sustainability. *Anim Front* 3 (3): 8–13. doi: 10.2527/af.2013-0018

Jouan, P-N., Y. Pouliot, S. F. Gauthier, and J-P. Laforest. 2006. Hormones in bovine milk and milk products: A survey. *Int Dairy J* 16 (11): 1408–1414.

Juskevich, J.C. and C. G. Guyer. 1990. Bovine growth hormone: Human food safety evaluation. *Science* 249 (4971): 875–84.

Karawajczyk, M., M. Höglund, J. Ericsson, and P. Venge. 1997. Administration of G-CSF to healthy subjects: The effects on eosinophil counts and mobilization of eosinophil granule proteins. *British J Haematol* 96 (2) :259-265.

Knight C. H., J. L. Fitzpatrick, D. N. Logue, and D. J. Platt. 2000. Efficacy of two non-antibiotic therapies, oxytocin and topical liniment, against bovine staphylococcal mastitis. *Vet Rec* 146 (11): 311–316.

Krömker, V. and S. Leimbach. 2017. Mastitis Treatment-Reduction in antibiotic usage in dairy cows. *Reprod Domest Anim* 52 (3): 21–29.

Laudedale, J.W. 2009. ASAS Centennial Paper: Contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. *J Anim Sci* 87:801-812.

Laurent F, Vignon B, Coomans D, Wilkinson J, Bonnel A. 1992. Influence of bovine somatotropin on the composition and manufacturing properties of milk. *J Dairy Sci* 75(8): 2226–2234.

Lee, S. A., X. O. Shu, H. Li, G. Yang, H. Cai, W. Wen, B. T. Ji, J. Gao, Y. T. Gao, and W. Zheng. 2009. Adolescent and adult soy food intake and breast cancer risk: results from the Shanghai Women's Health Study. *Am J Clin*. *Nutr* 89:1920–1926, doi: 10.3945/ajcn.2008.27361.

López-Plaza B, L. M. Bermejo, C. Santurino, I. Cavero-Redondo, C. Álvarez-Bueno, C. Gómez-Candela. 2019. Milk and Dairy Product Consumption and Prostate Cancer Risk and Mortality: An Overview of Systematic Reviews and Meta-analyses. *Adv Nutr* 10 (2): S212–S223.

Lynch, J. M., D. M. Barbano, D. E. Bauman, G. F. Hartnell, and M. A. Nemeth. 1992. Effect of a prolonged-release formulation of N-methionyl bovine somatotropin (sometribove) on milk fat. *J Dairy Sci* 75 (7): 1794–1809.

Macrina, A. L., T. L. Ott, R. F. Roberts, and R. S. Kensinger. 2012. Estrone and estrone sulfate concentrations in milk and milk fractions. *J Acad Nutr Diet* 112 (7): 1088–1093.

Macuhová, J., V. Tancin, and R. M. Bruckmaier. 2004. Effects of oxytocin administration on oxytocin release and milk ejection. *J Dairy Sci* 87 (5): 1236–44.

McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *J Anim Sci* 70:2901–2910. doi: 10.2527/1992.7092901x.

Mero, A., J. Kahkonen, T. Nykanen, T. Parviainenm, I. Jokinen, T. Takala, T. Nikula, S. Rasi, and J. Leppaluoto. 2002. IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. *J Appl Physiol* 93:732–739.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J Anim Sci* 76(1):160-172. doi: 10.2527/1998.761160x.

Michael, D. J., H. Cai, W. Xiong, J. Ouyang, and R. H. Chow. 2006. Mechanisms of peptide hormone secretion. *Trends Endocrinol Met* 17 (10): 408–415.

Mills, S., R. P. Ross, C. Hill. G. F. Fitzgerald, and C. Stanton. 2011. Milk intelligence: Mining milk for bioactive substances associated with human health. Int Dairy J 21 (6): 377–401

Nickerson S. C., W. E. Owens, and J. L. Watts. 1989. Effects of Recombinant Granulocyte Colony-Stimulating Factor on Staphylococcus aureus Mastitis in Lactating Dairy Cows. *J Dairy Sci* 72 (12): 3286-94.

Nostrand, S.D., D. M. Galton, H. N. Erb, D. E. Bauman. 1991. Effects of daily exogenous oxytocin on lactation milk yield and composition. *J Dairy Sci* 74 (7): 2119-27.

O'Donnell A. M., K. P. Spatny, J. L. Vicini, and D. E. Bauman. 2010. Survey of the fatty acid composition of retail milk differing in label claims based on production management practices. *J Dairy Sci* 93:1918–1925.

Pape-Zambito, D. A., A. L. Magliaro, and R. S. Kensinger. 2008. 17Beta-estradiol and estrone concentrations in plasma and milk during bovine pregnancy. *J Dairy Sci* 91:127–135, doi: 10.3168/jds.2007-0481.

Pape-Zambito, D. A., R. F. Roberts, and R. S. Kensinger. 2010. Estrone and 17beta-estradiol concentrations in pasteurized-homogenized milk and commercial dairy products. *J Dairy Sci* 93 (6): 2533–2540, doi: 10.3168/jds.2009-2947.

Park, Y. W. and M. S. Nam. 2015. Bioactive Peptides in Milk and Dairy Products: A Review. Korean *J Food Sci Anim Resour* 35 (6): 831–40.

Parr, S., T. Brown, F. Ribeiro, K. Chung, J. Hutcheson, B. Blackwell, P. Smith, and B. Johnson. 2014. Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride. *J Anim Sci* 92(8):3348-3363.

Pelletier, N., M. Ibarburu, and H. Xin. 2014. Comparison of the environmental footprint of the egg industry in the United States in 1960 and 2010. *Poultry Science* 93:241-255. doi: https://doi.org/10.3382/ps.2013-03390

Perry, G. A., W. V. Welshons, R. C. Bott, and M. F. Smith. 2005. Basis of melengestrol acetate action as a progestin. *Domest Anim Endocrinol* 28(2):147-161. doi: 10.1016/j.domaniend.2004.07.002.

Pogorzelska-Nowicka, E., A. G. Atanasov, J. Horbánczuk, and A. Wierzbicka. 2010. Bioactive compounds in functional meat products. *Molecules* 23:307–326.

Prakash, B. S., V. Paul, H. Kliem, U. Kulozik , and H. H. Meyer. 2009. Determination of oxytocin in milk of cows administered oxytocin. *Anal Chim Acta* 636 (1): 111–115.

Preble, I., Z. Zhang, R. Kopp, M. Garzotto, G. Bobe, J. Shannon, and Y. Takata. 2019. Dairy Product Consumption and Prostate Cancer Risk in the United States. *Nutrients* 11 (7): 1615.

Prosser, C. G., I. R. Fleet, and A. N. Corps. 1989. Increased secretion of insulin-like growth factor I into milk of cows treated with recombinantly derived bovine growth hormone. *J Dairy Res* 56 (1): 17–26.

Putman, B., G. Thoma, J. Burek, and M. Matlock. 2017. A retrospective analysis of the United States poultry industry: 1965 compared with 2010. *Agricultural Systems* 157:107-117. doi: https://doi.org/10.1016/j.agsy.2017.07.008.

Raun, A. P. and R. L. Preston. 2002. History of diethylstilbestrol use in cattle." *J Amer Soc Anim Sci* https://www.asas.org/docs/publications/raunhist.pdf?sfvrsn=0

Rhodes, L. 2018. Changing innovation into a registered product: From concept to regulatory approval. *Theriogenol* 112:75-81.

Richardson, T., and N. Mattarella. 1977. Hormonal Substances in Human Milk, Cow's Milk and Dairy Products. *J Food Prot* 40 (1): 57–64.

Rollin E., K.C. Dhuyvetter, and M.W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine 122: 257–264.

Samuelson, K., M. Hubbert, M. Galyean, and C. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. *J Anim Sci* 94(6):2648-2663.

Schams D, and H. Karg. 1986. Hormones in milk. *Ann N Y Acad Sci* 464:75–86.

Sides, G. E., J. T. Vasconcelos, R. C. Borg, O. A. Turgeon, W. C. Koers, M. S. Davis, K. Vander Pol, D. J. Weigel, and C. M. Tucker. 2009. A Comparison of Melengestrol Acetate Fed at Two Dose Levels to Feedlot Heifers. *The Professional Animal Scientist* 25(6):731-736. doi: 10.15232/S1080-7446(15)30782-8.

Smith, Z. K., A. J. Thompson, J. P. Hutcheson, W. T. Nichols, and B. J. Johnson. 2018. Evaluation of coated steroidal implants containing trenbolone acetate and estradiol-17β on liveperformance, carcass traits, and sera metabolites in finishing steers. *J Anim Sci* 96 (5): 1704–1723, doi: 10.1093/jas/sky095.

St-Pierre, N. R., G. A. Milliken, D. E. Bauman, R. J. Collier, J. S. Hogan, J. K. Shearer, K. L. Smith, W. W. Thatcher. 2014. Meta-analysis of the effects of sometribove zinc suspension on the production and health of lactating dairy cows. *J Am Vet Med Assoc* 245 (5): 550–564. doi: 10.2460/javma.245.5.550.

Thompson, A. J., Z. K. F. Smith, M. J. Corbin, L. B. Harper, and B. J. Johnson. 2016. Ionophore strategy affects growth performance and carcass characteristics in feedlot steers. *J Anim Sci* 94 (12): 5341–5349, doi: 10.2527/jas.2016-0841.

Thornton, P. K. 2010. Livestock production: Recent trends, future prospects. *Phil Trans R Soc Lond B Biol Sci* 365:2853–2867.

Trevisi, E., A. Zecconi, S. Cogrossi, E. Razzuoli, P. Grossi, and M. Amadori. 2014. Strategies for reduced antibiotic usage in dairy cattle farms. *Res Vet Sci* 96 (2): 229–233.

U.S. Food and Drug Administration (FDA) 1989. Freedom of Information Summary. NADA 139-237. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/456.

U.S. Food and Drug Administration (FDA) 1989. Freedom of Information Summary. NADA 046-718.

U.S. Food and Drug Administration (FDA) 1990. Freedom of Information Summary. NADA 131-310. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/396.

U.S. Food and Drug Administration (FDA) 1993. Freedom of Information Summary. NADA 140-856. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/2481.

U.S. Food and Drug Administration (FDA). 1994. Freedom of Information Summary. NADA 034-254. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/297

U.S. Food and Drug Administration (FDA). 1996. Freedom of Information Summary. ANADA 200-124. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/9132.

U.S. Food and Drug Administration (FDA). 1997. Freedom of Information Summary. NADA 039-402. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/304.

U.S. Food and Drug Administration (FDA).1999. Freedom of Information Summary. NADA 200-253. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/977.

U.S. Food and Drug Administration (FDA). 2002. Freedom of Information Summary. ANADA 200-069. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/967.

U.S. Food and Drug Administration (FDA) 2003. Freedom of Information Summary. NADA 141-200. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/714.

U.S. Food and Drug Administration (FDA). 2006. Freedom of Information Summary Original New Animal Drug Application Nada 141-258 Zilmax (zilpaterol hydrochloride) Type A Medicated Article for Cattle Fed in Confinement for Slaughter. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/811.

U.S. Food and Drug Administration (FDA). 2014, Freedom of Information Summary. NADA.108-901. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/363.

U. S. Food and Drug Administration (FDA). 2015. Freedom of Information Summary NADA 141-454. https://www.fda.gov/media/93801/download.

U.S. Food and Drug Administration (FDA). 2018a. Freedom of Information Summary NADA 098-379. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/3740.

U.S. Food and Drug Administration (FDA). 2019. Freedom of Information Summary NADA 113-645. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/7447.

U.S. Food and Drug Administration (FDA). 2020. Freedom of Information Summary ANADA 200-134. https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/9311.

U. S. Food and Drug Administration (FDA). 2018b. General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals Guidance for Industry #3. https://www.fda.gov/animal-veterinary/guidance-industry/guidance-number.

U.S. Food and Drug Administration (FDA). 2019. Steroid Hormone Implants Used for Growth in Food-Producing Animals. https://www.fda.gov/animal-veterinary/product-safety-information/steroid-hormone-implants-used-growth-food-producing-animals (Accessed 22 Jan 2020).

U. S. Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) 2010. VMAC Briefing Packet, AquAdvantage Salmon. https://www.avma.org/sites/default/files/resources/aquadvantage_salmon_briefing_packet.pdf

United States Department of Agriculture- Animal and Plant Health Inspection Service (USDA-APHIS). 2011. *Feedlot 2011 Part IV: Health and health management on U.S. feedlots with a*

capacity of 1,000 or more head. National Animal Health Monitoring System, Fort Collins, Colo.

United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) 2013. *The use of Growth-Promoting Implants in U.S. Feedlots*. https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_is_Implant_1.pdf.

University of Nebraska. 2020. Applied Reproductive Strategies in Beef Cattle (ARSBC). beefrepro.unl.edu.

Van Den Berg G. 1991. A review of quality and processing suitability of milk from cows treated with bovine somatotropin. *J Dairy Sci* 74(2): 2-11.

Vargas-Bello-Pérez, E., R. I. Márquez-Hernández, and L. E. Hernández-Castellano. 2019. Bioactive peptides from milk: animal determinants and their implications in human health. *J Dairy Res* 86 (2): 136-144.

Vicini, J. L., F. C. Buonomo, J. J. Veenhuizen, M. A. Miller, D. R. Clemmons, and R. J. Collier. 1991. Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. *The Journal of Nutrition* 121:1656–1664, doi: 10.1093/jn/121.10.1656.

Vicini, J., T. Etherton P. Kris-Etherton J. Ballam, S. Denham R. Staub D. Goldstein R. Cady, M. McGrath, and M. Lucy. 2008. Survey of retail milk composition as affected by label claims regarding farm-management practices. *J Am Diet Assoc* 108:1198–1203, doi: 10.1016/j.jada.2008.04.021.

Wilson D. J., R. N. González, J. Hertl, H. F. Schulte, G. J. Bennett, Y. H. Schukken, and Y. T Gröhn. 2004. Effect of clinical mastitis on the lactation curve: A mixed model estimation using daily milk weights. *J Dairy Sci* 87:2073–2084.zWorld Health Organization (WHO). 1993. Toxicological evaluation of certain veterinary drug residues in food. In Joint FAO/WHO Expert Committee on Food Additives (JEFCA), Geneva, Switzerland, 9–18, June 1992. World Health Organization., https://apps.who.int/iris/handle/10665/37297.

World Health Organization (WHO). 1998. Toxicological evaluation of certain veterinary drug residues in food. In the 50th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), Rome, Italy. https://apps.who.int/iris/handle/10665/42143

World Health Organization (WHO). 2014. Toxicological evaluation of certain veterinary drug residues in food. In the 78th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), Geneva, Switzerland. https://apps.who.int/iris/handle/10665/128550.

Yaida, N. 1929. Ovarial hormone in blood of pregnant women, of pregnant animals: Ovarial hormone in urine of pregnant women: Ovarial hormone in milk of pregnant animals. *Trans Jpn Pathol Soc* 189:93–101.
