RISK ASSESSMENT OF GROWTH HORMONES AND ANTIBIOTICS RESIDED IN MEAT

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Abstract—**Risk assessment plays a key role for the security of food safety. The process of risk assessment goes following hazard identification, hazard characterization, exposure assessment and risk characterization, which provides scientific background in the decision of risk management options for the protection of public health. Exposure to hazardous compounds through food consumption should not exceed the acceptable daily intake (ADI) that is drawn by risk assessment. Growth promoters including hormonal substances and antibiotics are used legally or illegally for growth promotion of livestock animals. The hormonal substances still under debate about human health impact are estradiol-17ß, progesterone, testosterone, zeranol, trenbolone and melengestrol acetate (MGA). Those are approved for use in various countries excepting European countries. Lots of the results of risk assessment on human health impacts of natural steroid hormones presented negligible impacts when they are used under the good veterinary practice. For the synthetic hormone-like substances, ADI and MRLs have been established for food safety. Small amount of antibiotics added into feedstuff present growth promotion effects via prevention of infectious diseases at a dose lower than therapeutic dose. Induction of resistant bacteria to antimicrobials critically important in human disease control and disruption of human normal intestinal flora are the major concerns by the use of antimicrobial growth promoters in food-producing animals. Regulatory guidance such as ADI and MRLs reflects fully the impact of the drugs on human gastrointestinal microflora. However, risk assessment on the antimicrobial resistance needs large scale of evidence for the relationship between antimicrobial use in food-producing animals and the occurrence of antimicrobial resistance in human pathogens before deciding any risk management options. In this article, the risk profiles of two kinds of growth promoters, hormonal or antibacterial one, are provided based on the recent information of the toxicity and human exposure and recommendations for the risk management to prevent any human health impact by the use of growth promoters are also presented.**

Index Terms — **risk assessment, growth promoters, growth hormones, antibiotic feed additives, human health impact**

I. INTRODUCTION

Veterinary drugs including antibiotics, growth hormones, antihelminths, and etc. are used for disease control and growth promotion of livestock animals. However, concerns on the safety of livestock products and prevalence of antimicrobial resistance have been increased according to the increased use of veterinary drugs.

Risk assessment is an integrative strategy to assume the probability of human illness caused by ingestion of livestock products containing residual veterinary drugs. Both antimicrobials and growth hormones used for growth promotion in food-producing animals have provoked lots of debate upon the safety of livestock products for human consumption. Many studies have been performed to estimate the real provability of human health impact. We need to understand the human health risk of antibiotics and growth hormones under the basis of framework composed of assessment of exposure amount, dose-response relationship and consequence of human illness.

The major goal of this article is to illustrate the method for risk assessment of growth hormones and antibiotics and to provide the result of quantitative risk assessment based on scientific knowledge and available data.

II. PRINCIPLE AND METHODS OF RISK ASSESSMENT FOR VETERINARY DRUGS USED IN FOOD ANIMALS

Veterinary drugs are a kind of chemical hazards in food of animal-originated. Many of those substances are evaluated for the potency of human health impacts in case they remain in food. The approval of use of veterinary drugs in food-producing animals can be made after systemic evaluation of the efficacy, target animal safety, human health risk and environmental impacts. In the viewpoint of risk management, the maximum residue limits (MRLs) are regarded as tools of monitor for the compliance with those approved conditions of use and the ADI level that is a decision point for the human health impact.

Risk assessment for veterinary drugs consists of assessing the toxicological and microbiological impact and identifying the acceptable consumption levels that the compounds should not exceed. Toxicological impact means any of biological adverse effects caused by direct intake of veterinary drugs such as body weight change, immune suppression and various disorders in normal body functions. For microbiological impact, the targets of ingested veterinary drugs are human intestinal microflora rather than human body. Human intestinal microflora play important roles for the maintenance of human health. Major functions of gut microbiota for human health are; metabolic fermentation of non-digestible dietary components and endogenous mucus, controlling proliferation and differentiation of intestinal epithelial cells, development and homoeostasis of the immune system and protection against exogenous pathogens (Shenderov, 1998). Microbiological impact needs to be evaluated when the ingested residue compound is anti-microbiologically active, not transformed irreversibly to inactive metabolites and enters lower intestine by any administration route (Jeong et al., 2009; JECFA, 2000a). Many of veterinary antimicrobials are allotted into a class requiring microbiological risk assessment.

Wide range of scientific data is required to make sure that a veterinary drug can be used in a manner that does not have an adverse effect on public health. Where appropriate, the data should be generated in accordance with national or international Good Laboratory Practices (GLP) guidelines. The data should cover acute toxicity, short-term toxicity, long-term toxicity, reproductive toxicity, carcinogenicity, genotoxicity, specific organ toxicity including immunotoxicity, neurotoxicity, endocrine disruption effects and impact on human intestinal microflora, metabolism and depletion in target animals and environmental fate.

Risk assessment of veterinary drugs resided in foods is performed following to the integrative steps of hazard identification, hazard characterization, exposure assessment and risk characterization (WHO, 2006). At the step of hazard identification, known or potential adverse health effects in humans are identified, which are induced by a veterinary drug or its metabolites that may be present in a particular food. Toxicological evaluation, toxicokinetics assessment, cancer/non-cancer evaluation are mainly performed for hazard identification. At hazard characterization step, the characteristics of the adverse effects associated with a veterinary drug or its metabolites present in food are demonstrated. In addition, the levels that clearly do not cause any adverse effect on human health are evaluated according to the dose-response relationship. NOAEL (no-observed-adverse-effect-level), ADI, benchmark dose at lower confidence limit (BMDL), uncertainty factors, and threshold level for toxicological concerns are drawn from the step of hazard characterization. ADI is calculated by dividing NOAEL with uncertainty factor. When a veterinary drug or its metabolites are microbiologically active and can enter lower intestine without inactivation, microbiological ADI is also assessed as well as toxicological ADI. Lower value of ADI is finally selected as its ADI (Table 1). In case the chemical is evaluated as a complete carcinogen, which means a genotoxic carcinogen, it is recommended to operate a policy of prohibition and control levels of "as low as reasonably practicable" (FAO/WHO, 2004).

During exposure assessment, the likely intake amount of a veterinary drug or its metabolites pertaining toxicological concerns, as well as exposures from other sources where relevant is estimated. Estimated Daily Intake (EDI) amount is calculated using the nationally or internationally accepted approach. The EDI value of veterinary drugs is drawn by the sum of all values that calculated by multiplying the median residue levels in food components with estimates of dietary intake. When groups of high risk consumers or sensitive population responding to a specific veterinary drug are identified, the exposure amount is assessed more carefully. The approval of the usage of veterinary drugs is determined by the comparison of EDI with ADI. If EDI is higher than ADI, the approval may not be possible or withdrawn.

Estimation of the severity and occurrence of known potential adverse health effects in a given population, based on hazard identification, hazard characterization, and exposure assessment is performed at the step of risk characterization. The evaluation of the risk caused by the consumption in highly sensitive population groups can be performed when required in the viewpoint of public health. The margin of exposure (MOE), margin of safety (MOS), level of protection (LOP) and MRLs are determined at this step. A large margin of safety is generally posed, which lead to a level well below that cause any toxic effects in animal studies. The actual intake of approved substance is fully lower than estimated level of human health concerns because it is extremely unlikely that each and every item of food consumed over an individual's lifetime will have been treated with a particular compound.

For the risk management, It is ADI not MRL that refers the human health-related risk by a certain amount of consumption of veterinary drug via food intake. The data of toxicity and residue have been assessed and conditions are placed on their registration to ensure that the MRLs are not exceeded before the approval of veterinary drugs.

Inspection and surveillance for residues of veterinary drugs is very important regulation tools to secure food safety. However, it is not practical to monitor all items of suspected compounds in food. The risk scoring for setting priorities in a monitoring of residues is recommended as an efficient strategy for risk management that is, more intensified monitoring is applied for the components having higher score of risk potential. Risk is scored according to their toxicological thresholds, estimated exposure amount, occurrence of antimicrobial resistance, violation frequency and etc. Newly developed approaches including BMDL and MOE evaluation, EDIs calculation, mode of action (MOA) assessment and etc provide more scientific and practical ways for risk assessment. The endeavor to make more reasonable and sound directions in the authorization of chemicals, establishment of ADI and/or MRLs and scoring of risk priority may guarantee food safety via state-of-the-art health risk assessment and risk management advice, optimally attuned to the consumer' needs.

Table 1. Comparison of toxicological and microbiological risk assessment

Item and procedure	Toxicological risk assessment	Microbiological risk assessment
Target	• Human (usually extrapolated from animal)	Human intestinal flora
Evaluation	• General oral toxicity (acute, short-term, and long-term toxicity) • Genotoxicity • Carcinogenicity • Reproductive toxicity • Developmental toxicity • Immunotoxicity • Other specific toxicities • Observation in humans	• Emergence of antimicrobial resistance in human intestinal normal microflora • Disruption of barrier composed with human intestinal normal microflora • Change of metabolic microbiological activity
Endpoint	No-observed-adverse-effect-level (NOAEL) of the most sensitive toxic effect	No-observed-adverse-effect-concentration (NOAEC) of the most sensitive human gut flora
Uncertainty factor	• Inter-species differences: 10 • Intra-species differences: 10 • Nature of toxic effects, data quality: 2～10	• Data quality: 2～10 • Kind of the most sensitive human flora
Calculation of ADI	NOAEL(㎍/kg bw/day)/uncertainty factor	{MIC50 (㎍/g) × Mass of colonic content (g)}/{Fraction of bioavailable dose × Uncertainty factor × Body weight (kg)}

III. Potential human health impacts of growth hormones used in food animals

The hormonal substances used for growth promotion in cattle are the naturally occurring steroids, estradiol-17ß, progesterone, and testosterone, as well as the synthetic compounds zeranol, which has high affinity for estrogen receptors, trenbolone acetate, which has affinity to androgen receptors, and melengestrol acetate, which has similar activity with progestins.

1. Estradiol

Estradiol-17ß, alone or in combination with other hormonally active substances, is administered to cattle by subcutaneous implant, usually in the ear, to improve the rate of weight gain and feed efficiency (Wagner, 1983). The amount of treatment per animal of estradiol benzoate is 10~28 mg, which is equivalent to 8~24 mg of 17ß-estradiol. The release rate from one type of commercial implant is approximately 60 ㎍ per animal daily (JECFA, 2000b).

Estradiol exerts its biological effects largely by binding with intracellular receptors and induces growth and development of the reproductive tract and breast and the appearance of secondary sex characteristics after binding with receptors in females.

In general, orally administered estradiol is inactive because it is metabolized and conjugated in the gastrointestinal tract and liver (Moore et al., 1982). Fine-particle formulations of estradiol given orally for contraception or hormone replacement therapy for menopausal women show bioavailability of 5% of that of a dose administered intravenously (Kuhnz et al., 1993). Estrogen does not exert a teratogenic effect in a human study for approximately 7,700 infants whose mothers took oral contraceptives while pregnant with them (Rothman & Louik, 1978). Estradiol has genotoxic potential that induces micronuclei, aneuploidy and, cell transformation *in vitro*, and oxidative damage to DNA and DNA single-strand breakage *in vivo* (IARC, 1979, 1999). In long-term studies of carcinogenicity in mice or in rats, increased incidences of tumors were found in mammary and pituitary glands, uterine cervical, vaginal, testicles, lymphoid organs and bone (IARC, 1979). Malignant kidney tumours occurred in intact and castrated male hamsters and in ovariectomized female hamsters. IARC (1987) also concluded that estradiol-17ß is a Group I human carcinogen that has sufficient evidences for carcinogenicity to humans. The carcinogenicity of estradiol is found to be a result of its interaction with hormonal receptors because the tumors largely occur in tissues pertaining high levels of hormone receptors. Overall, estradiol is evaluated as a genotoxic carcinogen, however, it is need to be considered that estradiol is a natural hormone synthesized in human body and used as a human medicine.

JECFA (2000b) determined the NOAEL (No-observed-adverse-effect level) of estradiol-17ß as 5 μg/kg bw/day based on human epidemiological data rather than animal toxicity data. The value was calculated from 0.3 mg/day of

estradiol administered orally to women, which did not relieve any symptoms of the menopause and no changes in the serum concentrations of corticosteroid-binding globulin (CBG). The ADI of 0-50 ng/kg bw/day was determined by dividing the NOAEL of 5 µg/kg bw/day with an uncertainty factor 100.

Estradiol-17ß occurs naturally in all mammals. The background levels vary with the age and sex of each animal species. The highest natural levels are found in pregnant animals. The normal daily production of 17ß-estradiol is 6.5 µg in prepubertal boys, 48 µg in men and 37.8 mg in pregnant women (Angsusingha et al., 1974). The use of estradiol as a growth promoter in cattle may produce twofold to several ten fold increases in the levels reaching peak in the liver and fat of steers and calves (Paris, et al., 2006). The amount of estradiol in muscle tissue in veal calves, heifers or steers treated was 11-280 ng/kg, whereas 3-35 ng/kg were detected in non-treatment groups. The intake amount of estradiol via meat of treated animals (0.0045-0.180 µg per 500g portion of meat) is approximately forty times to thousands times lower than the amount of human daily production of the hormone (Table 2). In addition, estradiol turns to be inactivated when administered orally due to gastrointestinal and/or hepatic metabolic functions. JECFA (2000b) concluded that the amount of exogenous 17ß-estradiol ingested via meat from treated cattle would be incapable of exerting any hormonal effects in human beings because bioavailability is very low in case estradiol administered orally and even absorbed into circulating system, the circulating estradiol is inactive form mainly bound to sex hormone-binding globulin (Fotherby, 1996). JECFA recommended that establishment of MRLs is unnecessary for it is structurally identical to that produced endogenously in human beings showing great variation in levels according to age and sex (Table 3).

2. Progesterone

Progesterone is administered to cattle in combination with estradiol benzoate at ten to one ratio (progesterone 100～200 mg with estradiol 10～20 mg) as an ear implant to increase the rate of weight gain and feed efficiency. Progesterone is also used to synchronize estrus in lactating and non-lactating dairy cows and goats via an intravaginal sponge. Exogenously administered progesterone is structurally identical to the progesterone produced in animals and humans. Progesterone is poorly absorbed by oral ingestion and inactivated in the gastrointestinal tract and/or in the liver, which makes the bioavailability of progesterone as less than 10% after oral administration (Simon et al., 1993). Orally administered micronized progesterone for hormone replacement therapy in women reached peak in plasma concentration within 4 h and returned to baseline by 6 h (Nahoul et al., 1993; Sitruk-Ware et al., 1987).

The main function of progesterone is to regulate the female reproductive cycle and preparation and maintenance of pregnancy in association with estrogens (JECFA, 2000b). Progesterone also induces increase of plasma cholesterol and low-density lipoprotein and decrease of high-density lipoprotein, and sodium excretion. The major metabolites found in plasma are pregnanediol 3a-glucuronide, 17-hydroprogesterone, and 20a-dihydroprogesterone. Most of progesterone in blood are bound with CBG or albumin, approximately 17% of serum progesterone is bound to CBG and 80% to albumin, and 2.5% is in the free form. A receptor for CBG exists on the surface of target cell membranes can activate adenyl cyclase when bound to steroid (Ribinson et al., 1985)

Production amount of progesterone in human body is various according to the physiological status such as at 418 µg/day in premenopausal women in the follicular phase of the reproductive cycle and at 94,000 µg/day in late pregnancy; in post-pubertal men at 416 µg/day (Table 2) (Galbraith, 2002). The therapeutic dose of fine-particle progesterone is 400 mg/day for 10 days in women and a dose of 300 mg/day for 10 days per month is well tolerable (JECFA, 2000b). In a study with female BALB/cfC3H/Crgl mice, progesterone 100 µg administered subcutaneously alone for five days beginning 36 h after birth caused ovary-dependent, persistent vaginal cornification and hyperplasia in the vaginal and cervical epithelium and significantly higher incidence of mammary tumours in mammary tumour-virus bearing mice (Jones & Bern, 1977). Progesterone increased the incidences of ovarian, uterine, and mammary tumours in mice and mammary gland tumours in dogs (IARC, 1979) and these effects were regarded as hormone activity related. The IARC concluded that there is limited evidence for the carcinogenicity of progesterone in experimental animals and no evaluation of the carcinogenicity of progesterone to humans can be made in the absence of epidemiological data (IARC, 1979; 1987). Progesterone showed no evidence in genotoxicity. It did not induce dominant lethal mutations in mice or chromosomal aberrations in rats treated *in vivo*. It did not induce chromosomal aberrations or sister chromatid exchanges in cultured human cells, nor chromosomal aberrations or DNA strand breaks in rodent cells. Progesterone was not mutagenic in bacterial systems (IARC, 1987). In vitro DNA adducts formation assay also showed negative results (Seraj et al., 1996). Also, progesterone did not induce any adverse effects on fertility and development in rats and rhesus monkeys (Wharton & Scott, 1964).

In humans, progestogens are mainly used for contraception and for hormone replacement therapy. In a human study to explore the effects of oral micronized progesterone on endometrial maturation, twelve healthy post menopausal women were given orally 300 mg micronized progesterone daily or twice daily for 14 days after estrogen priming for 30 days. The group receiving 300 mg/day showed incomplete conversion of the uterus to full secretory activity, while the group receiving 600 mg/day showed full secretory conversion of the uterus with suppression of mitotic activity. Glandular glycogen was significantly increased by 124% at 300 mg/day and 291% at 600 mg/day. The nuclear estrogen

receptor content in the stroma of endometrium was decreased by both doses of progesterone but the group given 300 mg/day did not reach significance (Kim et al., 1996). In other human studies, post menopausal women were given 200 or 300 mg/day of progesterone orally for the last 14 days of percutaneous estradiol treatment of 1.5 or 3 mg/day for 21 of 28 days for one or five years. There was no evidence of endometrial hyperplasia or carcinoma after five years of treatment with estradiol and progesterone (Moyer et al., 1993). Treatment of oral fine-particle progesterone 200 or 300 mg/day to sixty women with oligomenorrhea or amenorrhoea showed effects of withdrawal bleeding with unchanged lipid concentrations (Shangold et al., 1991). There were no adverse effects in women receiving fine-particle progesterone orally for hormone replacement therapy, which induced minor changes in plasma lipoprotein profile in some but not all and no change in haemostatic parameters (Sitruk-Ware et al., 1987).

In comparison study for the concentration of progesterone in edible tissues from non-treated and treated veal calves, heifers or steers, the ranges of progesterone were not different between each group except the amount in adipose tissue from treated animals (3.20~8.66 ㎍/kg) was higher several times than control animals (0.87~1.60 ㎍/kg) (Table 2) (Paris et al., 2006). This increased amount is about thousands times lower than daily production amount in adult men and women in normal status.

JECFA established the ADI of progesterone as 0-30 μg/kg bw based on the LOAEL of 200 mg/day (equivalent to 3.3 mg/kg bw/day) for change in the human uterus. 100 as an uncertainty factor were allotted as 10 for extrapolation from LOAEL to NOAEL and 10 for individual variations (JECFA, 2000b) (Table 3).

3. Testosterone

Testosterone propionate (200 mg) in combination with estradiol benzoate (20 mg) is administered to cattle as an ear implant for growth promotion. Orally administered testosterone is mainly inactivated during digestion and hepatic metabolism. The bioavailability of orally treated testosterone is approximately 3.6% of the administered dose. The plasma half-life was 10 min after intravenous administration and about 90% of the administered dose is excreted into the urine (Tauber et al., 1986).

Testosterone is synthesized in testicular Leydig cells, ovarian thecal cells, and adrenal cortex and it exerts activity via binding with the androgen receptor. Testosterone is a precursor of other steroid hormones. The active metabolite of testosterone is dihydrotestosterone (DHT) that is metabolized to androsterone, androstanedione, and 3 α- and 3β-androstanediol (Miyamoto et al., 1998). The major functions of testosterone are pubertal development for spermatogenesis, regulation of the differentiation of the prostate, stimulation of erythropoietin production in the kidney and stem cells in the haematopoietic system and acceleration of growth during puberty in conjunction with growth hormone.

In a human study, 400 mg of fine-particle testosterone administered orally for 21 days was well tolerated without any significant side effects in healthy male volunteers (Johnsen et al., 1974; 1976). Increase of prostate glands in weight and volume and the amount of serum testosterone, DHT, androstenedione and estradiol were found by the intramuscular injections of 200 mg testosterone enanthate (equivalent to 8 mg/kg bw) for 28 weeks in adult male baboons (Karr et al., 1984). Many of studies on genotoxicity showed that testosterone alone has no genotoxic potential (Han et al., 1995; Ho & Roy, 1994; Lasne et al., 1990; Seraj et al., 1996; Tsutsui et al., 1995). Testosterone 10 mg induced resorption of embryos in female SD rats when it was treated subcutaneously on day 10 of gestation (Sarkar et al., 1986). For carcinogenic potential of testosterone, IARC (1979) evaluated that it is reasonable, for practical purposes, to regard testosterone as if it presented a carcinogenic risk to humans in the absence of adequate data in humans but it has sufficient evidence for the carcinogenicity in experimental animals.

In human medicine, testosterone is used to treat a deficient testicular function in men and to replace hormones in postmenopausal women in combination with estrogen (Sands & Studd, 1995). Orally administered testosterone undecanotate induced progression of virility, testicular growth, and acceleration of growth associated with puberty in delayed boys at 40 mg per day for 15-21 months without any side-effects (Butler et al., 1992). In a human study with eunuchs, 25 and 100 mg testosterone administered orally did not exert any effects but 400 mg exerted effects such as sexual desire, erection, ejaculation, and general well-being (Johnsen et al., 1974). In another study, oral administration of testosterone at 100 mg/day restored sexual function slightly (Foss & Camb, 1939).

JECFA (2000b) established the ADI of testosterone as 0-2 μg/kg bw on the basis of the NOAEL of 100 mg/day (equivalent to 1.7 mg/kg bw/day) in the study of eunuchs and an uncertainty factor of 1000. When comparing the ADI value, the amount of testosterone via intake of beef from hormone-treated animals is thousands times lower than ADI (Table 3).

Table 2. Comparison of the amount of steroid hormones produced daily in human body and ingested via diet from hormone-treated animals

Hormones	Total daily production (㎍/day) (JECFA, 2000b; EFSA, 2007)	Ingested amount via muscle intake* (㎍/day)	Muscle residues (㎍/kg) (Paris et al., 2006)
Estradiol	< 14 (prepubertal boys) 10~24 (prepubertal girls) 27~68 (adult men) 30~ 470 (adult women)	0.0033~0.084	0.011~0.28
Progesterone	150~250 (prepubertal children) 416~750 (adult men, premenopausal women)	0.069~0.231	0.23~ 0.77
Testosterone	30~100 (prepubertal children) 210~480 (adult female) 2100~6900 (adult male)	0.0093~0.108	0.031~ 0.360

*: calculated considering a person intakes 300g muscle per day

4. Zeranol, melengestrol and trenbolone

Zeranol, melengestrol and trenbolone are all synthetic xenobiotic growth promoters. Zeranol is a non-steroidal anabolic agent administered subcutaneously as an ear implant to cattle and shows estrogenic activity (Katzenellenbogen *et al.,* 1979). Zeranol is metabolized to zearalenone and taleranol and the tissue residue level of zeranol are ranged 0.01～1.21 ㎍/kg witk peak level in the liver tissue (Paris et al., 2006). Orally administered zeranol showed weak estrogenic effect in long-term toixicity studies in rats, dogs and monkeys occurring changes in mammary glands and reproductive organs (Davis et al., 1977; Everett et al., 1987; Revuelta et al., 1997; WHO, 2000b). Zeranol and its metabolites, zearalenone and taleranol were negative in a number of *in vitro* and *in vivo* genotoxicity assays (Bartholomew & Ryan,1980; Ingerowski, et al., 1981; Scheutwinkel et al., 1986; Williams, 1984). In carcinogenicity studies in rats and mice, only mice showed a higher incidence of tumors of anterior lobe of the pituitary gland than control group, but this effects was regarded to be owing to estrogenic properties of zeranol (Everett, *et al.,* 1987; Gardner, 1941; JECFA, 1988). In the uterotropic assay using sexually immature rats, orally administered zeranol, zearalanone and taleranol presented estrogenic potency 1/150, 1/400 and 1/350 that of estradiol-17ß, respectively (Everett *et al.,* 1987). In ovariectomized female cynomolgus monkeys, zeranol dosed orally for 13 weeks induced maturation of vaginal epithelial cells at 0.5 and 5 mg/kg bw/day and the NOAEL was evaluated as 0.05 mg/kg bw/day based on the estrogenic effects of zeranol (JECFA, 1988). JECFA recommended the ADI as 0～0.5 ㎍/kg bw/day applying uncertainty factor 100 for interspecies and individual differences (JECFA, 1988) (Table 3).

Melengestrol is a synthetic progestogen administered orally as a feed additive to improve feed efficiency. The approved feeding doses are ranged 0.25～0.50 mg/heifer per day during the period of fattening and finishing period (Neidert et al., 1990). Its activity is revealed via high affinity for the progesterone receptor as well as increases in prolactin secretion and activation of estrogen receptor (Perry et al., 2005). Melengestrol acetate (MGA) is metabolized to 2ß,15ß-dihydroxy methyl MGA, 6-hydroxy methyl-MGA, 15ß-hydroxy-MGA and 2ß-hydroxy MGA in vitro system prepared from cattle and the most active metabolite among them is 2ß-hydroxy MGA showing 9-times less potent than MGA (WHO, 2004). The residue level found in Canadian beef heifers treated with MGA at a rate of 0.40 mg/animal per day during 1982-1984 were 2.8 pg/kg as mean value (ranging <2 to 28.7 pg/kg), and 4.6% of all samples had residues of MGA more than 10.0 pg/kg of fat (Neidert et al., 1990).

Melengestrol acetate is low acute toxic chemical in rodents after oral administration. Melengestrol acetate is not a genotoxic chemical in full range of *in vitro* and *in vivo* assays including bacterial and mammalian cellular gene mutation assays, unscheduled DNA synthesis assay and micronuclei test in mice. In a tumor study, a higher incidence of mammary tumors was found in C3Han/f mice but the reason was caused by the increased release of prolactin rather than direct action of MGA (JECFA, 2000c). Orally administered MGA induced reproductive toxicity as impaired pregnant and parturition and greater pup loss in beagle dogs and the NOAEL for reproductive toxicity was 2 µg/kg bw/day (JECFA, 2000c; Lauderdale, 1977). MGA exerted embryotoxicity, fetotoxicity and teratogenicity including resorption, dead fetuses, visceral malformation and incomplete skeletal ossification in rabbits, which NOAEL was 0.4 mg/kg bw/day (JECFA, 2000c). The most appropriate end-point for MGA is the progestational effect such as changed menstrual cycle found in female cynomolgus monkeys with the NOAEL of 5 µg/kg bw/day (JECFA, 2000c). The ADI of 0～0.03 µg/kg bw/day was established by applying an uncertainty factor 200 to the NOAEL. The MRL recommended by JECFA is 1, 10, 2 and 18 ㎍/kg for cattle muscle, liver, kidney and fat, respectively (JECFA, 2006c) (Table 3).

Trenbolone acetate (TBA) is a synthetic anabolic steroid administered to cattle as a subcutaneous implant in the ear of cattle to increase feed efficiency alone or in combination with estradiol-17ß or zeranol (Metzler and Pfeiffer, 2001;

Pottier et al., 1973). TBA exerts its anabolic effects via binding to androgen and glucocorticoid receptors (Sillence and Rodway, 1990). The approved dose is 200 mg/implant per heifer or steer 60-90 days before the slaughter (Heitzman and Hardwood, 1977). Major metabolites of TBA are stereoisomers 17α- and 17β-trenbolone (Hoffman et al., 1984; Pottier et al., 1973). 17ß-trenbolone is mainly found in muscle tissue, whereas the 17α-trenbolone is mainly occurring in liver and bile excreta (JECFA, 1988). The binding affinity to the androgen receptor is similar to that of dihydrotestosterone but stronger affinity to the progesterone receptor than that of progesterone (Hoffman et al., 1984). 17α-trenbolone and the other metabolites of TBA have lower binding affinity to androgen and progesterone receptors (Bauer et al., 2000). When TBA is co-administered with estradiol-17ß, TBA makes delay of the estradiol excretion (Heitzman, 1983). TBA is a weak toxic chemical with oral LD50 is 1,000～1,500 mg/kg bw. The genotoxicity of TBA, 17α-trenbolone and 17ß-trenbolone were negative in various assays of *in vitro* and *in vivo* (Ingerowski et al., 1981; Lutz et al., 1988; Schiffman et al., 1988). In carcinogenicity studies, TBA given via feeding induced liver hyperplasia in mice at 0.9-9 mg/kg bw/day and islet-cell tumours of the pancreas in rats at 1.85 mg/kg bw/day as a consequence of the hormonal activity of TBA (Schiffman et al., 1985; 1988). TBA induced hormonal effects involving decreased testosterone levels in serum of male pigs, reductions in weights of testes, ovaries, and uteri, atrophy of testicular interstitial cells, suppression of cyclic ovarian activity, absence of glandular development of the uterine endometrium, and lack of alveolar development and secretion in the mammary glands at higher level of 2 μg/kg bw/day in pigs (van Leeuwen, 2004; JECFA, 1988). Orally given ß-trenboloneBOH induced the antigonadotropic activity in castrated male rhesus macaque monkeys aged 8 - 17 years as maintenance of seminal vesicle morphology and serum levels of testosterone and estradiol. The no-hormonal-effect level was evaluated as 2 μg/kg bw/day in this study (Wilson et al., 2002). JECFA (1988) recommended the ADI of TBA as 0～0.02 ㎍/kg bw/day according to the no-hormonal-effect-level of 2 μg/kg bw/day based on the hormonal effects observed in pig and castrated monkey and an uncertainty factor 100. The MRL of TBA is 2 ㎍/kg of ß-trenbolone for cattle muscle and 10 ㎍/kg of α-trenbolone for cattle muscle (Table 3).

Table 3. Toxicological endpoints and regulatory limits of hormonal growth promoters

Compound	Toxicological endpoint	NOAEL (μg/kg bw/day)	ADI (μg/kg bw/day)	MRLs (μg/kg) for cattle tissues	Ref.
17ß-estradiol	No relieve the symptoms of menopause, Changes in the serum concentrations of corticosteroid-binding globulin	5	0～0.05	unnecessary	JECFA, 2000b
Testosterone	Androgenic effects	1,700	0～2	unnecessary	JECFA, 2000b
Progesterone	Change in the human uterus	3,300 (LOAEL)	0～30	unnecessary	JECFA, 2000b
Zeranol	Estrogenic effects	50	0～0. 5	2 (muscle), 10 (liver)	JECFA, 1988
Melengestrol acetate	Changed menstrual cycle	5	0～0.03	1 (muscle), 10 (liver) 2 (kidney), 18 (fat)	JECFA 2006c
Trenbolone acetate	Androgenic effects	2	0～0.02	2(muscle, ß-trenbolone) 10(liver, α-trenbolone)	JECFA, 1988

IV. POTENTIAL HUMAN HEALTH IMPACTS OF ANTIBIOTICS USED IN FOOD ANIMALS

The name of antibiotic growth promoters comes from the growth promoting effects of antibiotics that were first discovered in the 1940s when chickens fed by-products of tetracycline fermentation were found to grow faster than those not fed (Dibner and Richards, 2005). The mode of action of antibiotics inducing growth promoting effect is mainly through an antibacterials activity and via direct metabolic effect (Butaye, et al., 2003). Suppression of specific toxin-producing organisms and sparing of feed nutrients particularly of urea and amino acids by the antibacterial agents are the mode of actions inducing growth promoting effects (Dibner and Richards, 2005). By suppressing disease-causing organisms, including toxin producers, in the animal's environment, antibiotics may reduce the incidence of

clinical and subclinical diseases that hinder animal performance. The nutrient sparing effect of antibiotics comes from the enhancement of the growth of intestinal organisms that synthesize nutrients required by the animals. Such organisms may provide vitamins and amino acids and digest cellulose to end products that are useful to the animals. In addition, depressing growth of organisms that compete with host animal for nutrients and reducing the wall thickness, implying the potential for improved absorption explain the nutrient sparing effects (Corpet, 2000).

However, many scientists, activists, regulators, and politicians have expressed urgent concern on using antibiotics in food animals for it could select resistant strains of bacteria that harm human health. WHO (2002) recommended that use of antimicrobials for the prevention of disease can only be justified where it can be shown that a particular disease is present on the premises or is likely to occur. Meanwhile, control of subclinical diseases and therapeutic intervention for recognized clinical bacterial diseases using antibacterial agents is frequently the only practical option and therapy makes burden in both economic and humane perspectives when disease-prevention measures fail (Phillips et al., 2004; Snary et al., 2004). Many of concerns on the usage of antimicrobial growth promoters are focused on the contamination of food with bacteria that are resistant to antimicrobials. However, there is a continuing debate on the impact of antimicrobial use in animal husbandry and the risk of resistance transmission to human pathogens.

Presi et al (2009) studied on the risk scoring for setting priorities in a monitoring of antimicrobial resistant bacteria in meat of chicken, pork, beef and veal distributed in four different product categories of fresh meat, frozen meat, dried raw meat products and heat-treated meat products. They provided that fresh and frozen chicken meat contributed 6.7% of the overall risk in the highest category and fresh and dried raw pork meat contributed 4.0%. The contribution of beef and veal was only of 0.4% and 0.1%, respectively. Hurd and Malladi (2008) revealed very low risk impact of antimicrobial feed additives used in food-producing animals on human health by quantitative risk assessment. That is, the predicted risk of suboptimal human treatment of infection with *Campylobacter coli* from swine is only 1 in 82 million; with a 95% chance it could be as high as1 in 49 million for macrolides, and risk from *Campylobacter jejuni* in poultry or beef are even less. In case of penicillin, Cox et al. (2009) noted that the true risk could well be zero providing their calculation that "not more than 0.037 to 0.18 excess mortalities per year might be prevented in the whole U.S. population if current use of penicillin drugs in food animals were discontinued and if this successfully reduced the prevalence of antibiotic-resistant *E. faecium* infections among intensive care unit (IUC) patients." For streptogrammins, banning virginiamycin has been estimated to prevent from 0 to less than 0.06 statistical mortalities per year in the entire U.S. population (Cox and Popken, 2004)

For the purpose of risk assessment of antimicrobials used in food-producing animals, categorization is made based on the importance of each drug class to human health: fluoroquinolones, glycopeptides, streptogramines and etc are allocated into category I that means very high importance; aminoglycosides, microlides, lincosamides and etc into category II that means high importance; tetracyclines and sulphonamides are medium important drugs into category III; and bacitracin and inophores are into low important drugs, category IV (Health Canada, 2002). When antimicrobials are proposed for the usage in food-producing animals, it requires lots of data on the relationship between antimicrobial use in animals and occurrence of antimicrobial resistance in human pathogens and antimicrobial resistance in animal pathogens/commensals and human health consequences, proportions of human infections caused by resistant bacteria versus susceptible bacteria, genetic aspects of antimicrobial resistance, host specificity, virulence, and spread in animal and human populations. International bodies such as CODEX, WHO and OIE developed several guidelines for the management of antimicrobials used for food-producing animals.

Antimicrobial resistance is a multi-dimensional public health issue with broad implications. Because it requires an integrated evidence-based approach to risk management, further development of appropriate risk analysis methodologies is crucial to assess the human health impact of antimicrobial use in animals.

Regulatory approval of antibiotics applications for growth promotion in livestock has been based on demonstrable target animal safety, residual drug safety, edible tissue clearance and avoidance and environmental safety as well as such measurable growth promoting effects. The establishment of NOAEL and ADI of antimicrobial growth promoters are based on toxicological and microbiological evaluation. The lowest value of NOAEL for the most sensitive adverse impact on human health is selected as a point of departure of risk assessment. Decision tree for the determination of the adverse microbiological effects of residues of antimicrobial drugs in food-producing animals was provided by JECFA (2000a). Emergency of antimicrobial resistance, barrier disruption effects and change in a specific metabolic microbiological activity are evaluated for residues of antimicrobial drugs in food when the antimicrobials including their metabolites have antimicrobial properties and the drugs enter the lower bowel by any route and the ingested residues are not transformed irreversibly to inactive metabolites and the ADI derived from toxicological data is not sufficiently low to protect the intestinal microflora and finally the data from the therapeutic use of the drug class in humans or from *in vitro* or *in vivo* model systems indicate that effects could occur in the gastrointestinal microflora.

Table 4 presents the results of risk assessment for representative feed additives including bacitracin, tetracyclines, penicillins, streptomycin, bambermycin (or flavomycin), tilmicosin, lincomycin, tiamulin, avilamycin, tylosin, colistin and erythromycin with their ADI and MRLs.

As a whole, it is important to develop appropriate risk analysis methodologies for the assessment of the human health impact of antimicrobial use in animals. We need to bear in mind that the discontinuing of any antimicrobials used in

food-producing animals without a full quantitative risk assessment may be wasted and even harmful both to animal and to human health. Good hygiene practices on farms, in abattoirs, during distribution and marketing of foods and in food preparation by consumers should be insisted and efforts concentrating into minimizing the transmission of all food-borne pathogens regardless of their antibiotic susceptibility are very important.

Table 4. Toxicological or microbiological endpoints and regulatory limits of antimicrobial growth promoters

Compound	Toxicological or microbiological endpoint	NOAEL	ADI	MRLs (μg/kg) for edible tissues	Ref.
Bacitracin	Microbiological endpoint: Inhibition of gram positive strains isolated from human gut flora	5.7 (μg/ml)	0～3.9 (μg/kg bw/day)	For rabbits: 150(muscle, fat, liver, kidney) 100(milk)	EMA, 2003
Tetracyclines	Microbiological endpoint: The selection of resistant Enterobacteriaceae of human intestinal microflora	33 (μg/kg bw/day)	0～30 (μg/kg bw/day)	For cattle, pigs, poultry and sheep: 200(muscle), 600(liver), 1200(kidney) 100(milk), 400(eggs)	JECFA, 1998a
Penicillins	Toxicological endpoint: Hypersensitivity reactions in human	30 (μg/kg bw/day)	0～30 (μg/kg bw/day)	For cattle, chicken and pigs 50 (muscle, liver, kidney) 4(milk)	JECFA, 1998b
Streptomycin	Toxicological endpoint: Decrease in body weight gain	5 (mg/kg bw/day)	0～50 (μg/kg bw/day)	For cattle, chicken, pigs, sheep: 600(muscle, liver, fat), 1000(kidney), 200(milk)	JECFA, 1998c
Bambermycin (Flavomycin)	Microbiological endpoint: Inhibition of Fusobacterium of human intestinal microflora	0.25 (μg/ml)	0～1 (μg/kg bw/day)	Not recommended	Jeong, 2009
Tilmicosin	Toxicological endpoint: Decrease of body weight gain and increase of heart rate	4 (mg/kg bw/day)	0～0.04 (mg/kg bw/ day)	For chicken: 150(Muscle), 2400(liver), 600 (kidney), 250 (skin/fat) For turkey: 100(Muscle), 1400(liver), 1200 (kidney), 250(skin/fat) For cattle and sheep: 100(muscle, fat), 1000(liver), 300(kidney) 50(sheep milk) For pigs: 100(muscle, fat), 1500(liver), 1000(kidney)	JECFA 2009a
Lincomycin	Microbiological endpoint: Inhibition of Gram positive bacteria of human gastrointestinal flora	2.5 (mg/kg bw/day)	0～30 (μg/kg bw/day)	For chicken: 200(muscle),500(liver,kidney), 100(fat) For pigs: 200(muscle), 500(liver), 1500(kidney), 100(fat) 150(milk)	JECFA 2000d
Tiamulin	Toxicological endpoint:	3	0～30	For pigs:	EMA

	Change of electrocardiogram and increase of serum alanine aminotransferase and lactate dehydrogenase	(mg/kg bw/day)	(μg/kg bw/day)	100(muscle), 500(liver) For chicken: 100(muscle, skin/fat) 1000(liver) 1000(eggs) For turkey 100(muscle, skin/gat) 300(liver)	2008
Avilamycin	Toxicological endpoint: No significant adverse effect in ling term toxicity study	150 (mg/kg bw/day)	0～2 (mg/kg bw/day)	For pigs, chicken, turkey and rabbits: 200(muscle, kidney, skin/fat), 300 (liver)	JECFA 2009b
Tylosin	Microbiological endpoint: Inhibition of Gram positive bacteria of human gastrointestinal microflora	1.698 (*μg*/kg bw)	0～30 (μg/kg bw/day)	For cattle, pigs and chicken: Muscle, liver, kidney and 100(fat) 100(milk), 300(eggs)	JECFA 2009c
Colistin	Microbiological endpoint: Inhibition of *E.coli* of human gastrointestinal microflora	7 (*μg*/kg bw)	0～7 (μg/kg bw/day)	For cattle, sheep, goat, pig, chicken, turkey and rabbit 150(muscle, liver, fat) 200(kidney) 50(milk), 300(eggs)	JECFA 2006a
Erythromycin	Microbiological endpoint: Inhibition of *Bifidobacterium* of human gastrointestinal microflora	0.7 (*μg*/kg bw)	0～0.7 (μg/kg bw/day)	For chicken and turkey 100(muscle, liver, kidney, fat) 50(eggs)	JECFA 2006b

V. CONCLUSIONS

Use of hormonal growth promoters and antimicrobial growth promoters in food-producing animals has provoked lots of concerns about human health impacts. A better understanding of human health risks posed by the use of the drugs is essential for the making regulatory decision and any programs that support prudent nonhuman use of hormonal drugs and antimicrobials. Risk assessment plays a key role in the security of food safety. Following to hazard identification, hazard characterization, exposure assessment and risk characterization, we can get more scientific background for the decision of risk management options for the protection of public health.

Recent results of risk assessment on the hormonal substances including estradiol-17ß, progesterone, testosterone, zeranol, trenbolone and melengestrol acerate (MGA) indicate that natural steroid hormones have negligible human health impact when they are used under the good veterinary practice and for the synthetic hormone-like substances, ADI and MRLs are provided.

Antimicrobials are used to present growth promotion effects by adding into feedstuff at the dose of lower than therapeutic dose. Induction of resistant bacteria and disruption of human normal intestinal flora are the major concerns on human health by the antimicrobial growth promoters. In many countries, impact on human normal intestinal flora induced by the residual antimicrobials or their metabolites is fully assessed and the microbiological ADI and MRLs are established based on the microbiological impact before the approval of antimicrobials. However, the risk assessment of antimicrobial resistance requires multi-dimensional information including relationship between antimicrobial use in animals and occurrence of antimicrobial resistance in human pathogens, genetic aspects of antimicrobial resistance in animals and human populations, and etc. Given the complexity of the assessment of antimicrobial resistance, developing more appropriate risk assessment methodologies is crucially required for understanding the human health impact of antimicrobial use in animals.

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ABSTRACTS SESSION A : MARKETING AND CONSUMER SCIENCE

A001

COLOR STABILITY AND α-TOCOPHEROL CONTENT IN BEEF *M. LONGISSIMUS DORSI* AFTER DIFFERENT PACKAGING METHODS

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Abstract—**The objective of this study was to investigate how colour stability and α-tocopherol content of beef M. Lonissimus dorsi (LD) were affected by skin vacuum packaging (SVP) compared to vacuum packaing (VP) and modified atmosphere packaging (MAP). The samples were first packed in vacuum for 7 days and then assigned to the different packaging methods for 7 days. Colour was then measured on samples kept in air during 5 days. The redness (a* value) of LD was higher in MAP on day 0 than for all other packaging methods ($p<0.05$) and also higher in MAP on day 5 than in VP ($p<0.05$). The yellowness (b* value) in SVP on day 5 was higher than in VP ($p<0.05$). Higher metmyoglobin and lower oxymyoglobin content was obtained in VP on day 5 compared with MAP and SVP($p<0.05$). The α-tocopherol content decreased with extension of storage time with no difference between packaging methods. In conclusion, the results from our study indicated that packaging method affected colour stability. Beef packed in SVP had better colour stability than in VP. The colour of samples packed in SVP was similar with MAP at the end (day 5) of our colour detection time.**

Index Terms — **beef, colour stability, packaging method, α-tocopherol**

A002

FACTORS IMPACTING ON CONSUMER'S PERCEPTION OF LAMB COLOUR

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Abstract—**Consumers (n=541) were asked to score 10 samples of lamb loin (m. longissimus thoracis et umborum; LL) on an ordinal scale of 1 (very acceptable) to 5 (very unacceptable). A sample was considered acceptable by a consumer if it scored three or less. The samples were used for testing consumer response to colour during extended display of up to 4 days. A Hunter Lab Miniscan was used for measuring meat on display. Respondents were also asked whether or not they considered the level of iron and omega-3 in lamb to be important. Results of the analysis indicated that L* (lightness) and b* (yellowness) were not significant ($p>0.05$) after adjusting for days aged, a* (redness) and wavelength ratio (630/580 nm) and there were no significant ($p>0.05$) interactions between gender, iron, and omega-3. The effects associated with sex, iron and omega-3, after adjusting for sample differences as accounted for by days aged, a* and ratio, were signficant at the $p=0.05$ level. Respondents who indicated that the level of iron is important scored the same piece of lamb lower (more favourable colour) than those that indicated it was not important. The opposite effect was observed for omega-3, with those that indicated the level of omega-3 was not important scoring the same piece of lamb lower (more with those that indicated the level of omega-3 was not important scoring the same piece of lamb lower (more favourable colour). Consumers altered their tolerance to the browning of lamb meat, dependent on the importance they placed on the levels of iron in the meat.**

Index Terms — **consumer, lamb, colour, iron, omega-3**

A003

INFLUENCE OF PROCESSING TIME ON SENSORY CHARACTERISTICS OF COOKED "LACON"

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***Abstract*—The influence of processing time about sensory properties of cooked "lacon" was studied. For this research, two batches of "lacon" were manufactured and after 56 and 84 days of drying-ripening were sensorial analysis for eight panellists selected from Meat Technology Centre of Galicia. Only four attributes (red colour, cured odour, rancid odour, and flavour intensity) were significantly different respect to time of processing. However, all textural traits studied were not influence of processing time.**

***Index Terms* — dry-cured "lacon", sensory evaluation, processing time**

A004

INFLUENCE OF SALT CONTENT ON SENSORY PROPERTIES OF GALICIAN HAM

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***Abstract*—Sensory characteristics of Semimenbranosus muscle from 60 dry-cured hams were assessed. Hams were salted with different salt levels (0.65 and 1 day/weight) and then ripened following traditional process during 12 months. The results showed appearance, odour, flavour and texture were not significantly affected by salt content ($p>0.05$). However, taste was significantly affected by levels of salt. A more intensity taste was observed in hams with a higher salt content. Texture characteristics were not significantly ($p>0.05$) affected by salt level. Semimenbranosus muscles from hams with a higher salt content were harder and more fibrous than muscles from less salted hams. A decrease in salt content produces less salty hams, but the changes in texture traits should be also considered.**

***Index Terms* — Galician ham, sensory evaluation, salt content**