A Dissertation

for the Degree of Doctor of Philosophy

Effects of ambient temperature and rumen-protected fat or glycerol supplementation on growth performance, rumen characteristics, and blood metabolites in Korean cattle steers

외기온도 및 사료 내 반추위 보호 지방과 글리세롤 첨가가 거세한우의 성장, 반추위 성상 및 혈액 성상에 미치는 영향

January, 2019

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Overall summary

Extremely high or low ambient temperature has a negative effect on cattle, such as decreased productivities due to altered physiological homeostasis. The main physiological changes in heat or cold stress condition are depressed ruminal capacity and glucose and energy metabolism. Glycerol can be used as glucogenic precursor, and rumen protected fat (RPF) can be energy supplier (mainly lipid form) without affecting the rumen environment. Therefore, both of the RPF and glycerol supplementation may have a positive effect on cattle under hot or cold condition. However, little information of effects of RPF or glycerol on Korean cattle steers under cold or hot condition is available. Thus, this study was conducted to evaluate the effects of 1) hot or cold condition and, 2) dietary glycerol or RPF supplementation on growth performance, rumen characteristics, and blood metabolites in Korean cattle steer in several growth stages. Total 5 feedlot trials were conducted under cold or hot condition [Study 1: cold condition, RPF (0.5%); study 2, cold condition, RPF (0.8%); study 3, cold condition, glycerol-absorbed wheat bran (6%) as a feedstuff; study 4, hot condition, RPF (0.8%); study 5, hot condition, glycerol (3%)]. As a result, neither cold nor hot condition deteriorate growth performances. Circulating glucose was increased during colder periods, whereas major ruminal VFAs were not changed. Under the hot condition, decreased serum cholesterol was observed, and rumen VFAs were tended to be lowered. RPF supplementation (0.5 or 0.8 %) did not improve growth performances, and did not affect rumen environment, although the blood HDL and cholesterol were increased by RPF supplementation. Glycerol supplementation improved growth performances, without affecting blood glucose concentration, but glycerol supplementation decreased the ruminal C2 concentration and C2:

C3 ratio and increased C3 concentration. In conclusion, the cold or hot condition of these studies did not affect growth performance, although some blood and ruminal parameters were changed. Glycerol supplementation (3.0% in the concentrate diet) improved growth performances with major ruminal VFAs change. RPF supplementation did not affect growth performances, with no effect on rumen environment and increased blood lipid metabolites.

Keywords: Ambient temperature, blood metabolites, glycerol, growth, Korean cattle steer, rumen characteristics, rumen-protected fat

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List of Abbreviations

ADG: average daily gain

ADF: acid detergent fiber

BW: body weight

C2: acetate

C3: propionate

C4: butyrate

C5: valerate

CS: cold stress

DDGS: dried distillers grains with solubles

DMI: dry matter intake

G:F ratio: gain to feed ratio

HDL: high density lipoprotein

HS: heat stress

IMF: intramuscular fat

ME: metabolizable energy

NDF: neutral detergent fiber

NE: net energy

NEFA: non esterified fatty acid

TDN: total digestible nutrition

VFA: volatile fatty acid

Unit and Marks

°C: degree Celsius

mM: millimole per litter

mEq: milliequivalent

IU: international units

mg: milligram

kg: kilogram

dL: deciliter

CHAPTER ONE

Introduction

Cold or hot environment reduce cattle productivity, such as decrease of growth performances, dry matter intake, reproductive efficiency, and/or milk performance. These decreased productivities result from altered physiological homeostasis, including hormonal status, metabolic rate, lipid, fat, or carbohydrate metabolism, and rumen capacity. However, cattle resistance to heat- or cold-temperature stress varies from species to species, which is due to genetic differences. Heat or cold temperature stresses on dairy cows and beef cattle are well studied, however, limited information is available in physiological responses to heat or cold stress in Korean cattle. Nutritional methods for alleviation of heat or cold stress have been attempted in response to altered rumen abilities, carbohydrate metabolism (especially glucose), fat metabolism, and increased energy requirements under heat or cold stress condition. The ruminal activity is altered by heat or cold condition, therefore, the add ition of unprotected forms of fat may decrease rumen fermentation and may adversel y affect rumen motility. Therefore, in this studies, we used dietary rumen–protected fat (RPF) as a supplementary source of fat in concentrate diet, which may increase energy absorption in the small intestine, while minimizing fermentation of fat in the rumen, and effectively compensating for the increased maintenance energy. Glycerol, the 3 carbon backbone in triglyceride, is also known as substrate for gluconeogenesis in the liver and kidney, which can provide energy for cellular metabolism, and mainly fermented into C3 in

the rumen by specific microbes. As described above, in the cold or hot condition, glucose and NEFA were more consumed. In this situation, it would be possible to reduce cold or heat stress by supplementation of glycerol, with glucogenic effect and fermentation characteristic. To determine RPF or glycerol supplementation level under cold condition, shrink body weight (SBW) and ambient temperature were used. In this study, the BW of each study is different, thus, BW was needed as a factor to estimate the changed NEm. According to NRC (2014), required net energy for maintenance (NEm) of beef cattle adapted to the thermal environment is related to the ambient temperature and SBW as the following equation.

$$NEm = [0.0007(20\ ^{o}C - \text{ambient temperature}) + 0.077]\ SBW^{0.75}$$

This equation indicates that the NEm requirement of beef cattle changes by 0.0007 Mcal/kg $SBW^{0.75}$ for each degree that ambient temperature differed from 20°C. Under the hot condition, increased NEm was presented as the follow equation (NRC, 2014).

$$NEmhs = 0.07 \times NEm, \text{ rapid shallow panting}$$

Although other factors, such as lower- or upper- critical temperature (LCT, UCT) of cattle, wind, rainfall, solar radiation, surface area of cattle, internal or tissue insulation, and evaporative losses need more precise NEm calculations. Additionally, LCT and UCT for Korean cattle were not established. In addition, the previous temperature condition of the experimental region was collected to determine ambient climate condition. Thus, the RPF or glycerol supplementation level was determined according to the equations, which using temperature and SBW. As described, the main physiological changes in heat or cold stress condition are depressed ruminal capacity and changes in glucose or lipid metabolism. Thus,

the RPF and glycerol supplementation may positively affect cattle under heat or cold condition. Glycerol can be used to produce propionate as gluconeogenesis precursor, and RPF can be an energy supplier (mainly lipids) without affecting the rumen environment. Little information of effects of RPF and glycerol on Korean cattle steers under the cold or hot condition, both of supplemented ingredient, RPF and glycerol, were used in the both of cold or hot condition. As considering ambient temperature and SBW, the NE of experimental concentrates were determined. Study 1 was conducted to elucidate the effects of 1) cold stress and 2) rumen-protected fat (0.5%) supplementation in concentrate of Korean cattle steers (550.6 kg of BW, 19.7 age of months; NE = 1.22 vs. 1.12 Mcal/kg). Study 2 was conducted to elucidate the effects of 1) cold stress and 2) rumen-protected fat (0.8%) supplementation in concentrate of Korean cattle steers (356.5 kg of BW, 16.8 age of months; NE = 1.31 vs. 1.19 Mcal/kg). Compare to Study 1, a greater amount of RPF was used to make larger dietary NE difference because 0.5 % RPF did not make any significant result in study 1. Study 3 was conducted to elucidate the effects of 1) cold stress and 2) glycerol-absorbed wheat bran (6%) as a feedstuff in diet of Korean cattle steers (217.0 kg of BW, 7.7 age of months; 250g of glycerol/day = 0.5 Mcal/day). Study 4 was conducted to elucidate the effects of 1) heat stress and 2) rumen-protected fat (0.8%) supplementation in concentrate of Korean cattle steers (230.4 kg of BW, 10.7 age of months, NE = 1.22 vs. 1.15 Mcal/kg). Study 5 was conducted to elucidate the effects of 1) heat stress and 2) glycerol (3 %) supplementation in concentrate of Korean cattle steers (466.9 kg of BW, 23.5 age of months, NE = 1.29 vs. 1.18 Mcal/kg).

CHAPTER TWO

Literature Review

1. Effects of heat stress on cattle

Physiological responses to heat stress

Mammals have developed heat-regulating mechanisms that make them to resist or maintain homeostasis under extremely hot environmental conditions, a relatively constant deep-body temperature, independent of the immediate environment (Blackshaw et al., 1994). For the maintenance of body temperature, an animal has to maintain thermal equilibrium with radiation, air temperature, wind, and humidity. To indicate hot condition as numerically, temperature-humidity index (THI) was developed (LCI, 1970; Hahn, 1999). Various physiological changes occur in the digestive system, acid-base balance, and hormonal alteration during hot weather; some in response to decreased dry matter intake (West, 1999). The average body temperature differs for each cattle species (i.e., Holstein, Angus, or Brahman), it is generally known that the dairy cows are less resistant to heat stress than beef cattle, with their higher body temperature. Conversely, Brahman cattle are highly resistant to heat (Gaughan et al., 1999). Even the same species has different resistance to heat stress depending on their genotype (West, 2003). There have been many studies on dairy or beef cattle, but there have been few studies of heat stress on Korean cattle. When the THI approaches enough to affect body temperature, the animal increases its active cooling by

evaporation of water from the respiration or sweating (Lee, 1967). In order to perform this series of heat exchange activities, the energy demand is higher in a heat stress situation (Mader, 2003). In the hot condition, decreased blood glucose concentration is observed, and can be explained by changed hormonal homeostasis, such as insulin, or rapid utilization of blood glucose by increased respiratory rate, result from duration of hyperthermia (O'brien et al., 2010; Hassan et al., 1975). Under hot conditions, increased respiratory rate cause respiratory alkalosis. (Roman-Ponce et al. 1977; Schneider et al., 1988). Increased respiration rate leads to excessive carbon dioxide emissions, decreased saliva buffering capacity, lowered ruminal pH, and rumen capacity, and increased metabolic disease (Nickerson, 2014). The alkalaemia that develops during extended second-phase breathing reduces the dissociation of O2 and haemoglobin, inhibiting O2 delivery to the tissues, including the central nervous system, which make hormonal alteration in hot condition (Hales and Findlay, 1968; Thompson, 1984). Also, increased non-esterified fatty acid (NEFA) concentration was observed under high THI condition, which may result from changed hormonal status (Beede and Collier, 1986), such as catecholeamines and glucocorticoids that typically promote adipocyte lipolysis and NEFA mobilization (O'brien et al., 2010). Under conditions of high heat load, respiration is responsible for only about 15% of heat loss (Finch, 1986). Depressed in rumination, reticulorumen motility, and ruminal activity were observed in hot climate condition which make slow the fractional rate of digesta passage in the gastrointestinal tract and decrease in dry matter intake (DMI) and volatile fatty acid (VFA) production in the rumen (Kadzere et al., 2002; Kelly et al., 1967).

Effects of heat stress on productivity

Feed intake decreases with rising THI, particularly under feedlot conditions (Conrad, 1985). Other reason for decreased feed intake result from liar satiety, which is due to increased water intake for reduction of body water (Pejman et al., 2012). The heat increment for feeding in cattle is high (35-70% of metabolisable energy), depending on the balance of nutrients (Blackshaw et al., 1994). Reduction of fiber intake, particularly with a minimum fall in metabolizable energy, may lower the heat increment sufficiently to act as a partial protection against forecast high heat stress (Fuquay, 1981). Reduced blood flow during heat stress may reduce nutrient uptake and mammary blood flow (McGuire et al., 1989). Heat exposed cows have shown 75 % of the DMI compare to thermoneutral condition (McGuire et al., 1989). Additionally, mild and severe heat stress increase energy requirements by 7% and 25%, respectively (NRC, 2001). Collectively, metabolic hot weather can strongly affect animal bioenergetics, with adverse effects on the performance and well-being of livestock. Reduced feed intake, growth performance, immunity or milk production, efficiency, and reproduction are recognized results (Hahn, 1999). Baumgard and Rhoads (2015) reported that 20 to 25 % reduction on DMI in dairy cattle in severe heat stress condition. Under heat stress condition, decreased milk yield, milk protein and fat was observed, whereas somatic cell count in milk was increased (Mohammed et al., 2017). The proliferation of mammary gland cells was decreased and the amount of insulin secretion was increased. Increased insulin cause glucose to be used in tissues other than mammary gland cells, which may result in decreased milk yield (Tao et al., 2011; Wheelock et al., 2010). In the summer season, estrus cycle was

lengthened, estrus activity decreased, and follicular growth was inhibited in Japanese cow (Sakatani et al., 2012). Also, heat stress lowers fertility of breeding in dairy heifers (Al-Katanani et al., 1999).

2. Strategies for alleviation heat stress

Fat supplementation

Several methods for alleviation heat stress have been tried, and there are some effective methods. To increase energy density in concentrate, fat supplementation was effective to alleviation heat stress. Wang et al. (2009) reported that body temperature was decreased by fat supplementation under high temperature condition, and milk yield and milk fat contents were increased by fat supplementation in Holstein. Fat supplementation have effect of reducing the heat increment instead of the grain source, which make fermentation heat increment. However, unsaturated fatty acids can reduce the fermentation of rumen, which can cause problems (NRC, 2007).

Betaine supplmentation

Betaine (trimethylglycine and glycine betaine) is a non-toxic amino acid derivative distributed widely in nature (Huang et al., 2007). Some researchers reported a 14.8% decrease in backfat thickness of pigs fed diets supplemented with betaine, and numerous reports have indicated that betaine may improve growth performance, carcass characteristics and pork quality for pigs by the use of betaine in commercial swine diets (Cadogan et al., 1993). Growth enhancement by betaine was associated with more growth hormone secreted in pulses, decreased concentrations of serum urea nitrogen and increased concentration of serum total protein as osmoregulatory function of betaine (Huang et al., 2007). Under hot condition, dietary betaine supplementation improved DMI and carcass weight (DiGiacomo et al., 2014).

Dietary betaine may be eliciting some energy-sparing responses that are manifested in carcass fat depth and measures of pH, and betaine supplementation may be a useful carcass modifier in growing feedlot steers during summer (DiGiacomo et al., 2014). Betaine reduces ion pumping associated with cell osmolarity retention, resulting in a reduction in energy requirements and an increase in carcass weight (Loxton et al., 2007). Zhang et al. (2014) reported that dietary betaine enhanced milk performances and physiological parameters in dairy cows under heat stress.

Dietary glutamine and γ-Aminobutyric acid supplementation

Dietary glutamine has positive effect to dairy cows by sustaining cow immune reaction in terms of a strengthening of cell-mediated immune response, with improved milk fat, and protein yield (Caroprese et al., 2013). γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the adult mammalian central nervous system, the peripheral nervous system, and some nonneuronal tissues (Watanabe et al., 2002), and has certain physiological functions such as regulating body temperature and feed intake. Several publications have suggested that GABA can alleviate the negative effects of heat stress, resulting in a greater performance in chicks (Dai et al., 2011; Zhang et al., 2012) and pigs (Fan et al., 2007). Feeding 40 mg/kg GABA to dairy cows during the warm summer months alleviated heat stress by reducing rectal temperatures, increased DMI and milk production, and enhanced milk protein and lactose concentrations (Cheng et al., 2014). γ-Aminobutyric acid and its agonists appear to regulate feed intake in some mammalian animals through effects on both the central and peripheral nervous system.

Niacin supplementation

Niacin, nicotinic acid, or vitamin B3 induces vasodilatation at the skin and this increases heat loss at the periphery (Di Constanza et al., 1997). The vasodilatory effects of niacin act through prostaglandin D production by epidermal Langherhans cells (Benyo et al., 2006; Maciejewski et al., 2006). Supplementation of encapsulated niacin (12 g/d) to thermally stressed lactating dairy cows increased evaporative heat loss and this was associated with increased water intake, decreased rectal temperature, vaginal temperatures and respiration rates (Zimbleman et al., 2010).

Dietary chromium supplementation

Chromium occurs widely in nature, it is a transition element, which occurs in a number of oxidation states. Burton (1995) has found that the most stable form is the trivalent state, which is the one involved in glucose tolerance factor. Supplementation dietary organic Cr complexes have resulted in beneficial effects on growth, reproduction, and immune response (Burton et al., 1996). The focal point is the function of Cr in glucose utilization and its consequent impact on utilization of dietary sugars particularly in rapidly growing animals or in those under physiological stress whether due to disease challenge or the demands of high production or reproduction (Al-saiady et al., 2004). Under heat stress, supplementation Cr improved milk yield in dairy cattle.

Antioxidants supplementation

Effects of selenium, vitamin E or their combination on fertility have been variable, with some

studies reporting of an increase in fertility (Arechiga et al., 1998). Given the effectiveness of vitamin E and selenium, as administered in the present study, and the possible importance of antioxidant status for heat-stressed cows, it is important to determine if injection of vitamin E and selenium increases fertility of heat-stressed cows (Arechiga et al., 1998).

Mitigation of heat stress by shelter

Heat stress can be mitigated by actively cooling or by providing shade. Wetting animals with sprinklers, showers or fine mist is most effective in dry climates (Armstrong, 1994). It has a rather limited use in more humid temperate areas, although its effectiveness can be improved when combined with convective cooling (Armstrong, 1994). Many studies have illustrated the beneficial effects of providing shade to heat-stressed cattle in hot climates. Cows with access to shade eat more and rest, drink or linger around the drinking trough less (Shultz, 1984). Positive effects of shade on the performance of cattle kept in hot climates include increased grazing and dry matter intake, resulting in increased weight gain (Gaughan et al., 2010; McDaniel and Roark, 1956; Mitlohner et al., 2001). Conception rate, calf birth weight, milk yield, and milk fat and lactose yield are also increased by shade provision, whereas somatic cell counts are deceased (Collier et al., 1982b; Davison et al.,1988; Roman-Ponce et al.,1977).

3. Effects of cold stress on cattle

Physiological responses to cold stress

Exposure of feedlot beef cattle to cold stress reduces growth performance and feed efficiency through increased maintenance energy for retaining body temperature in cold condition (Ames et al., 1980). For livestock exposed to cold, windy environments, effective temperature must now be predicted by a wind-chill index prepared for humans and designed without regard to external insulation such as-hair or wool (Ames and Insley, 1975). Thus, high wind velocity and low ambient temperature may strongly affect cattle bioenergetics. Ambient temperatures progressively below the lower critical temperature, the heat production of the animal becomes increasingly dependent upon the ambient temperature. This concept has been demonstrated in short-term calorimetric studies of sheep (Graham et al., 1959), and cattle (Blaxter and Wainman, 1961; Blaxter, 1962). Animal's central nervous system about its thermal state regulate a body homeostasis which is a consequence of prior thermal exposure and the balance between the rate of thermogenic processes and the net heat exchange. Decreased productivity in cold condition may be result from the increased basal metabolic rate in cold stress conditions (Young, 1981). Increased basal metabolic required energy, mainly used to maintain body temperature. Cattle will also increase the time spent eating in response to colder temperatures (Redbo et al., 2001). In addition to behavioural responses, cattle undergo physiological changes when exposed to winter weather. Acute cold stress has been shown to increase plasma concentrations of epinephrine and norepinephrine in shorn sheep by two and fivefold, respectively, and to increase the urinary excretion of both epinephrine and norepinephrine (Webster et al., 1969; Thompson et al., 1978). Norepinephrine contributes to an increased thermogenesis, including shivering through somatic innervation as primal source of thermogenesis (Young, 1981). An increased

sympathetic activity could also elicit the cardiovascular and metabolic responses observed during cold exposure, such as increased heart rate, blood hematocrit and plasma concentration of glucose and free fatty acids (Thompson, 1973; McKay et al., 1974). Thyroid function and fat metabolism (as measured by non-esterified fatty acids, NEFA, Broucek et al., 1987) increase in response to cold weather. For example, circulating glucose and non–esterified fatty acid (NEFA) are increased probably by the elevated metabolic heat production and a mobilization of substrates for energy metabolism in the adipose tissue and liver (Mader, 2003). Cold condition may affect rumen capacity and digestibility. Literature relating to the influence of temperature on digestibility in ruminants has been reviewed. Decreases in digestibility of certain diets induced by exposing animals to low environmental temperatures are related to changes in the rate of passage of digesta through the gastrointestinal tract (Christopherson et al., 1979; Christopherson et al., 1983). In cold condition, increases in rumination activity, reticulorumen motility, and rate of digesta passage were observed (Kennedy et al., 1976). The changes in rumen digestive characteristics were associated with a reduced digestion in the reticulorumen, particularly with roughage feeds (NRC, 1981). Rumen fermentation characteristics including VFAs concentrations were also changed by cold exposure (Christopher son and Kennedy, 1983). For some diets cold exposure results in increased urea nitrogen recycling to the rumen, increased efficiency of rumen microbial synthesis and reduced rumen degradation of dietary protein: responses which help to favor the nitrogen economy of the animal even though digestible energy is reduced (Christopherson and Kennecy, 1983).

Effects of cold stress on productivity

Previous reports suggest that the intramuscular fat (IMF) in beef cattle has been deposited from 250 day of age to slaughter (Park et al., 2018; Du et al., 2013). Thus, sufficient energy to hyperplasia and hypertrophy for IMF deposition is important for early fattening stage of cattle, thus, cold exposed cattle spending more energy to maintain homeostasis, may have less beef quality. The energy requirement of adult beef cows is assumed to increase with 0.0007 Mcal/BW0.75 for each degree that the ambient temperature differs from 20 C (NRC, 2000). The change in energy requirement for lactating dairy cows in cold environments is probably minimal because of their high heat production, at least if they are kept dry and unexposed to wind (NRC, 2001). Collectively, cold stress negatively influences on mortality, immune system of calves, backfat thickness and meat quality of beef cattle and milk yield of dairy cow, result from increased basal metabolic energy, which can cause hormonal homeostasis (Gulliksen et al., 2009; Mader, 2003; Broucek et al., 1991). Cold and cold-induced starvation account for 50% of perinatal lamb death (Samson and Slee, 1981). Lambs produced 50 to 60% of their heat through shivering thermogenesis, and 40 to 50% through nonshivering thermogenesis (Alexander and Williams,1968). Brown adipose tissue (BAT) is responsible for nonshivering thermogenesis. Lambs are born with almost 100% BAT unlike other species, which are born with brown and white adipose tissue (Gemmelletal.,1972; Alexander and Bell, 1975). New born calves have fewer tissues produce heat compared to the surface area of the heat than the cattle, and insufficient heat capacity due to no accumulation of fat. Lack of fermentation heat due to lack of microbial community formation in rumen. Newly born calves

are wet and body temperature is easily reduced. Lack of ability to produce heat may make new born calves weaker in cold condition than adult cattle (increase in metabolism, hair growth, vasodilation) (Roland, 2016). When the temperature and wind speed were low, milk yield decreased in dairy cattle Angrecka et al., 2015). Also, milk yield decreased with longer feeding period at low temperature (Broucek et al., 1991). The period of rebreeding became longer after the milking period in the low temperature stress situation (Wiltbank et al., 1962). When cold stress persisted, free fatty acid, glucose, and cholesterol decreased, such changes in blood status were correlated with the decrease in milk yield (Broucek et al., 1991).

4. Strategies for alleviation cold stress

Linoleic acid supplementation

Gibney and L'Estrange (1975) fed feed high in linoleic acid and increased linoleic acid content of fat stores in lambs. Linoleic acid is the major fuel for heat production in brown adipose tissue (Lammogliaetal.,1999). Linoleic and linolenic acid supplementation (sunflower and linseed oil) increased the thermogenic capacity of brown adipose tissue by 75% (Nedergaard et al., 1983). Encinias et al. (2004) reported that feeding high-linoleic safflower seed to gestating ewes would increase brown adipose tissue stores in neonatal lambs and improve lamb survival. Mader et al. (2003) report that if dry flooring is maintained and dry litter is provided, daily gain and feed efficiency are increased by 6% and 7%, respectively, in beef cattle. Wind blockage and in-house shelter can affect marbling scores and reduced backfill thickness (Mader et al., 2003).

Mitigation of cold stress by shelter

Animals kept outside in adverse winter conditions consume more feed but grow slower or produce less milk because less feed energy is available for productive processes. Provision of shelter during inclement weather will improve rates of gains by feedlot cattle and reduce maintenance requirements for the cow herd (ASAE, 1974). An animal can modify the rate of heat exchange, however, by its behavior (shelter seeking, postural changes, etc.) and by changing its thermal insulation through retention or shedding of hair, piloerection of the hair

coat or vasomotor control of blood flow to superficial tissue (Young, 1981). Comparisons of weight gain (McCarrick and Drennan, 1972), carcass quality, estimations of energy demand, immune function and behaviour (Hickey et al., 2002) between steers housed indoors and on outdoor outwintering pads, suggest that in Irish winters growing beef cattle can be housed outdoor, even without wind-shelter. In Canadian winter conditions, weaned beef bull calves provided with shelter have higher gain rates than calves without shelter (Kubisch et al., 1991). shelter significantly improved animal welfare (i.e. it increased the time spent lying down and decreased faecal glucocorticoid, thyroxine and NEFA concentrations) (Van laer et al., 2014).

5. Effects of glycerol or rumen-protected fat supplementation on cattle

Effects of fat supplementation

In ruminants, modified diets (especially concentrate) with low heat increments can also help improve dry matter intake (DMI) and growth performance under hot condition. In hot environments, nutrient requirements are altered during heat stress, which results in a need for reformulation of concentrate (Collier et al., 2006). In dairy cattle, decreased milk production was observed (35% to 50%) as a consequence of heat stress may be potentially recuperated through nutritional management (Rhoads et al., 2010). Some trials have been conducted, such as decreasing fiber intake in order to make the proper rumen capacity, supplementation fat supplementation, because of its high-energy content and low heat increment and implementing increased concentrate diets with caution to avoid metabolic disorders

(Morrison, 1983; Beede and Collier, 1986; West, 1999). To avoid metabolic disorders and maintain proper rumen capacity, ADF and NDF should not be decreased below 18% and 28% of the concentrate, respectively (West, 1994). The supplementation of fat to the concentrate including high energy density and potential to reduce heat increment. The most limiting nutrient for cattle during heat stress is usually low energy utilization efficiency, related to decreased DMI, and a common approach to increase energy density is to reduce forage intake and to increase concentrate intake (Renaudeau et al., 2012). The addition of 3% to 5% fat to the concentrate can be used to ruminant without any toxic effects to ruminal microflora (Palmquist and Jenkins 1980). In hot condition, 25% of ME from a protected tallow had 8 to 13.6 % higher efficiency than those not fed supplemental tallow (Kronfeld et al., 1980).

Effects of rumen protected fat supplementation

The use of rumen-protected fat will increase energy absorption in the small intestine, while minimizing digestion in the rumen, which may lower heat increment, effectively compensating for the increased maintenance energy in hot condition. Although the use of dietary RPF has been widely attempted in previous study (McNamara et al., 2003; Hill and West, 1991). Rumen bypass fat was made by several methods. Crystalline or prilled fatty acids can be made by liquifying and spraying the saturated fatty acids under pressure into cooled atmosphere, so that melting point of the fatty acids is increased and do not melt at ruminal temperature, thus resisting rumen hydrolysis and association with bacterial cells or feed particles (Naik, 2013). Formaldehyde treated protein encapsulated fatty acids is also an affecting means of protecting dietary fat from rumen hydrolysis (Sutton et al., 1983). Fatty

acyl amide can be prepared and used as a source of bypass fat. Butylsoyamide is a fatty acyl amide consisting of an amide bond between soy fatty acids and a butylamine, which increases linoleic acid content of the milk fat (Jenkins, 1998). Fatty acyl amide of sardine oil based complete diet is effective in protecting fat from degradation in rumen and improves the apparent and true dry matter degradability (Ambasankar and Balakrishnan, 2011). Calcium salts of long chain fatty acids (Ca-LCFA) are insoluble soaps produced by reaction of carboxyl group of long chain fatty acids (LCFA) and calcium salts (Ca++) (Naik, 2013). Among all forms of bypass fat, Ca-LCFA is relatively less degradable in rumen (Elmeddah et al., 1991), has highest intestinal digestibility (Dairy Technical Service Staff, 2002) and serve as an additional source of calcium (Naik et al., 2007a; 2007b). Tyagi et al. (2009) reported increase (3.16 vs 3.41; kg/100 kg BW/d) in DM intake in dairy animals fed bypass fat. The digestibility of EE increased significantly, when bypass fat was supplemented in the diet of the dairy animals (Naik et al., 2007b; 2009a; Thakur and Shelke, 2010; Sirohi et al., 2010). The increase in the digestibility of the fat indicates that added fat is more digestible than the basal diet fat or fat supplementation dilutes the endogenous lipid secretions, resulting in more accurate estimate of the true lipid digestibility (Grummer, 1988). According to its higher energy density and its lower metabolic heat when compared with fiber or starch, fat supplementation can be used to reduce heat load and to increase net energy intake in heat-stressed dairy cows (Morrison, 1983; Beede and Collier, 1986; Knapp and Grummer, 1991).

Effects of glycerol supplementation

High fermentable carbohydrate diets can be used under hot conditions to stimulate energy

intake but the effect must be balanced with the potential for rumen acidosis associated with high-grain diets (West, 2003). For example, glycerol is an essential structural component of triglycerides and phospholipids, and glucogenic properties of glycerol are well known (Cori and Shine, 1935). When animals are fasted off, they can use body fat reserves as energy source, and then free fatty acids and glycerol can be released into the bloodstream. In general, some portion of glycerol can enter the gluconeogenesis to be converted to glucose by the liver or kidney so that it can provide energy for cellular metabolism. The caloric value of glycerol was calculated to be equal to that of corn (NE_M=2.2 Mcal/kg; NE_G=1.5 Mcal/kg), but it doesn't contain protein, fat, or fiber (Preston, 2014). Glycerol has been used as a feed additive for ruminants and has been shown to be effective in improving ruminant productivity (Fiorentini et al., 2018; Mach, 2009; Gunn 2010). Mach et al. (2009) reported that when supplement glycerin at up to 12% of DM to isocaloric and isonitrogenous high-concentrate diets fed to Holstein bulls, there were no detrimental effects on performance. These positive effects of glycerol on productivity on cattle may come from three of the principle fates of dietary glycerol in ruminants have been estimated and include absorption through the rumen epithelium (45%), fermentation to volatile fatty acid (VFA, 25%), and escape through the omasal orifice (30%; Werner-Omazic et al., 2015). Boyd et al. (2013) reported that glycerin supplemented to ruminant diets is well known to cause a shift in VFA profiles, favoring C3 production at the expense of C2. Rémond et al. (1993) reported that when glycerin (240g) was ruminally administered to cows fed maize silage, about 35-69% of carbons forming C3 are derived from glycerol. Liu et al. (2014) reported that when supplemented glycerol to diets fed to lactating dairy cows during heat stress period, the glucose concentration was increased

while the NEFA concentration was decreased, which indicated that glycerol increased glucose utilization by peripheral tissues and diminished triglyceride mobilization.

Effects of propylene glycol supplementation

Propylene glycol is a glucogenic precursor that is rapidly absorbed from the rumen for gluconeogenesis in the liver. Administration of propylene glycol makes a small positive contribution to energy status, and its main benefit derives from bolus administration, which increases insulin secretion (Christensen et al., 1997). Propylene glycol can also be converted to propionic acid in the rumen and transported to liver, where it is converted to glucose though the glyconeogenesis pathway (Nielsen and Ingvartsen, 2004). Some investigators have indicated that PG supplementation can reduce serum NEFA and ketone body concentrations (Christensen et al., 1997; Miyoshi et al., 2001; Pickett et al., 2003), and has been reported to increase milk production (Formigoni et al., 1996; Lucci et al., 1998). As described, in the thermal stress condition, cattle show thermoregulatory response, which increase energy consumption, including glucose or NEFA. Little information of effects of glycerol on animal productivity during cold season in Korean cattle steers is available.

REFERENCES

Alexander G, Bell AW. Quantity and calculated oxygen consumption during summit metabolism of brown adipose tissue in newborn lambs. Biol Neonate 1975;26:214–20.

Alexander G, Mills SC, Scott TW. Changes in plasma glucose, lactate, and free fatty acids in lambs during summit metabolism and treatment with catecholamines. J Physiol 1968;198:277–89.

Al-Katanani YM, Webb DW, Hansen PJ. Factors Affecting Seasonal Variation in 90-Day Nonreturn Rate To First Service in Lactating Holstein Cows in a Hot Climate. J Dairy Sci 1999;82:2611-6.

Al-saiady MY, Al-Shaikh MA, Al-Mufarrej SI, et al. Effect of chelated chromium supplementation on lactation performance and blood parameters of Holstein cows under heat stress. Anim Feed Sci Technol 2004;117:223-33.

Ambasankar K, Balakrishnan, V. Influence of protected sardine oil on in vitro rumen fermentation and nutrient digestibility of complete diet. Indian J Anim Sci 2011;81:84-6.

Ames DR, Brink DR, Willms CL. Adjusting protein in feedlot diets during thermal stress. J Anim Sci 1980;50:1–6.

Ames DR, Insley LW. Wind-Chill Effect for Cattle and Sheep. J Anim Sci 1975;40:161–5.

Armstrong DV. Heat stress interaction with shade and cooling. J. Dairy Sci 1994;77:2044–50.

Angrecka S, Herbut P. Conditions for cold stress development in dairy cattle kept in free stall barn during severe frosts. Czech J Anim Sci 2015;60:81-7.

ASAE. Livestock Environment: Proceedings of the International Livestock Environment Symposium. ASAE 1974; St. Joseph, ML

Baumgard LH, Rhoads RP. Ruminant nutrition symposium: Ruminant production and metabolic responses to heat stress. J Anim Sci 2015;90:1855-65.

Beede DK, Collier RJ. Potential nutritional strategies for intensively managed cattle during thermal stress. J Anim Sci 1986;62:543–54.

Benyo Z, Gille A, Bennett CL, Clausen BE, Offermanns S. Nicotinic acid induced flushing is mediated by activation of epidermal langerhans cells. Mol Pharmacol 2006;70:1844-9.

Blackshaw JK, Blackshaw AW. Heat stress in cattle and the effect of shade on production and behaviour: a review. Austral J Experimental Agri 1994;34:285-95.

Boyd J, West JW, Bernard JK. Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. J Dariy Sci 2010;94:4616–22.

Broucek J, Kovalčuj K, Gajdogik D, Sottnik J, Brestensky V. The effect of extreme ambient temperatures on haematological and biochemical parameters of heifers. Vet Med 1987;32:259–68.

Brouček J, Letkovičová M, Kovalčuj K. Estimation of cold stress effect on dairy cows. Int J Biomet 1991;35:29–32.

Burton JL. Supplemental chromium: its benefits to the immune system. Anim Feed Sci Technol, 1995;53:117-33.

Burton JL, Nonnecke BJ, Dubesn PL, Elsasser TH, Mallar BA. Effects of supplemental chromium on production of cytokines by mitogen-stimulated bovine peripheral blood mononuclear cells. J Dairy Sci 1996;79:2237-46.

Cadogan DJ, Campbell RG, Harrison D, Edwards AC. The effects of betaine on the growth performance and carcass characteristics of female pigs. In: E. S. Batterham (ed.), Manipulating Pig Production. Australasian Pig Science Association, 1993, Attwood, Vic., Australia, pp. 219–225.

Caroprese M, Albenzio M, Marino R, Santillo A, Sevi A. Dietary glutamine enhances immune responses of dairy cows under high ambient temperature. J Dairy Sci2013;96:3002-11.

Cheng JB, Bu DP, Wang JQ, et al. Effects of rumen-protected γ-aminobutyric acid on performance and nutrient digestibility in heat-stressed dairy cows. J Dairy Sci 2014;97:5599-607.

Christensen JO, Grummer RR, Rasmussen FE, Bertics SJ. Effect of method of delivery of propylene glycol on plasma metabolites of feed-restricted cattle. J Dairy Sci 1997;80:563-8.

Christopherson RJ, Gonyou HW, Thomson JR. Effects of temperature and feed intake on plasma concentration of thyroid hormones in beef cattle. Can J Anim Sci 1979;59:655–61.

Christopherson RJ, Kennedy PM. Effect of the thermal environment on digestion in ruminants. Can J Anim Sci 1983;63:417–96.

Collier RJ, Dahl GE, VanBaale MJ. Major advances Associated with Environmental Effects on Dairy Cattle. J Dairy Sci 2006;89:1244–53.

Collier RJ, Doelger SG, Head HH, Thatcher WW, Wilcox CJ. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. J Anim Sci 1982;54:309–19.

Conrad JH. Feeding of farm animals in hot and cold environments. In 'Stress Physiology in Livestock, Vol. 2: Ungulates'. Ed. M. K. Yousef. 1985; pp. 205-26. CRC Press: Boca Raton, FL, U.S.A.

Cori CF, Shine WM. The formation of carbohydrate from glycerophosphate in the liver of the rat. Science 1935;82:134-35.

Dai SF, Gao F, Zhang WH, et al. Effects of dietary glutamine and gamma-aminobutyric acid on performance, carcass characteristics and serum parameters in broilers under circular heat stress. Anim Feed Sci Technol 2011;168:51–60.

Davison TM, Silver BA, Lisle AT, Orr WN. The influence of shade on milk production of Holstein–Friesian cows in a tropical upland environment. Aust J Exp Agric 1988; 28:149–54.

Di Costanzo, A, Spain JN, Spiers DE. Supplementation of nicotinic acid for lactating Holstein cows under heat stress conditions. J Dairy Sci 1997;80:1200-6.

DiGiacomo K, Warner RD, Leury BJ, Gaughan JB, Dunshea FR. Dietary betaine supplementation has energy-sparing effects in feedlot cattle during summer, particularly in those without access to shade. Anim Prod Sci 2014;54:450-8.

Du M, Huang Y, Das AK, Yang Q, et al. Meat science and muscle biology symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. J Anim Sci 2013;91:1419–27.

Elmeddah Y, Doreau M, Michalet-Doreau B. Interaction of lipid supply and carbohydrate in the diet of sheep with digestibility and ruminal digestion. J Agri Sci 1991;116:437-45.

Encinias HB, Lardy GP, Encinias AM, Bauer ML. High linoleic acid safflower seed supplementation for gestating ewes: Effects on ewe performance, lamb survival, and brown fat stores. J Anim Sci 2004;82:3654-61.

Fan ZY, Deng JP, Liu GH, et al. Effects of γ-aminobutyric acid on the performance and internal hormone levels in growing pigs. Chin J Anim Nutr 2007;4:350–6.

Finch VA. Body temperature in beef cattle: its control and relevance to production in the

tropics. J Anim Sci 1986;62:531-42.

Fiorentini G, Messana JD, Jose Neto A, et al. Performance and meat quality of Nellore bulls fed crude glycerin combined with soybean oil. Anim Feed Sci Tech 2018;241:45-54.

Formigoni A, Cornil M, Prandi A, et al. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. J Dairy Res 1996;63:11-24..

Fuquay JW. Heat stress as it affects animal production. J Anim Sci 1981;52:164-74.

Gaughan JB, Mader TL, Holt SM, Josey MJ, Rowan KJ. Heat tolerance of Boran and Tuli crossbred steers. J Anim Sci 1999;77:2398-405.

Gaughan JB, Bonner S, Loxton I, et al. Effect of shade on body temperature and performance of feedlot steers. J Anim Sci 2010;88:4056–67.

Gemmell RT, Bell AW, Alexander G. Morphology of adipose cells in lambs at birth and during the subsequent transition of brown to white adipose tissue in cold and warm conditions. J Anat 1972;133:134–64.

Gibney MJ, L'Estrange JL. Effects of dietary unsaturated fat and of protein source on melting point and fatty acid compotition of lamb fat. J Agric Sci 1975;84:291–6.

Grummer RR. Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J Dairy Sci 1988;71:117-23.

Gulliksen SM, Lie KI, Løken T, Østerås O. Calf mortality in Norwegian dairy herds. J Dairy Sci 2009;92:2782–95.

Gunn PJ, Schultz AF, Lake SL, et al. Effects of elevated crude glycerin concentrations on feedlot performance, carcass characteristics, and serum metabolite and hormone concentrations in finishing ewe and wether lambs. Prof Anim Sci 2010;26:298–306.

Hahn GL. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999;77:10–20.

Hales JRS, Findlay JD. Respiration of the ox: normal values and the effects of exposure to hot environments. Respiratory Physiol 1968;4:333-52.

Hassan A, Roussel JD. Effect of protein concentration in the diet on blood composition and productivity of lactating Holstein cows under thermal stress. J Agric Sci Camb 1975;85:409–15.

Hickey MC, French P, Grant J. Out-wintering pads for finishing beef cattle: animal production

and welfare. Anim Sci 2002;75:447–58.

Hill GM, West JW. Rumen protected fat in Kline barley or corn diets for beef cattle: digestibility, physiological, and feedlot responses. J Anim Sci 1991;69:3376–88.

Huang QC, Xu ZR, Han XY, Li WF. Effect of betaine on growth hormone pulsatile secretion and serum metabolites in finishing pigs. J Anim Physiol Anim Nutr 2007;91:85-90.

Jenkins TC. Fatty acid composition of milk from Holstein cows fed oleamide or canola oil. J Dairy Sci 1998;81:794-800.

Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows — a review. Livest Prod Sci 2002;77:59–91.

Kelly RO, Martz FA, Johnson HD. Effects of environmental temperature on ruminal VFA levels with controlled feed intake. J Dairy Sci 1967;50:531–3.

Kennedy PM, Christopherson RJ, Milligan LP. The effect of cold exposure of sheep on digestion, tureen turnover time and efficiency of microbial synthesis. Brit J Nutr 1976;36:231–42.

Knapp DM, Grummer RR. Response of lactating dairy cows to fat supplementation during heat stress. J Dairy Sci 1991;74:2573-9.

Kronfeld DS, Donoghue S, Naylor JM, Johnson K, Bradley CA. Metabolic Effects of Feeding Protected Tallow to Dairy Cows. J Dairy Sci 1980;63:545–52.

Kubisch HM, Makarechian M, Arthur PF. A note on the influence of climatic variables and age on the response of beef calves to different housing types. Anim Sci 1991;52:400–3.

Lammoglia MA, Bellows RA, Grings EE, Bergman JW. Effects of prepartum supplementary fat and muscle hypertrophy genotype on cold tolerance in newborn calves. J Anim Sci 1999;77:2227–33.

Lee DHK. Principles of homeothermic adaptation. In 'Biometeorology'. Ed. W. P. Lowry. 1967; pp. 113-30:Oregon State University Press: Cowallis, OR, U.S.A.

Liu J, Ye G, Huang K, et al. Feeding glycerol-enriched yeast culture improves performance, energy status, and heat shock protein gene expression of lactating Holstein cows under heat stress. J Anim Sci 2014;92:2494–502.

Livestock Conservation Institute (LCI). Patterns of transit losses. Omaha, NE. 1970.

Loxton I, Grant T, Reid D, Lawrence R, Kempe N. The effect of Bos Koolus fed during

summer on the feedlot performance and carcass characteristics of steers. J Dairy Sci 2007;90:455.

Lucci CS, Fonseca LF, Rodrigues PHM, Santos MV, Lima AP. Supplementation of propylene glycol to dairy cows in periparturient period: effects on body condition score, milk yield and first estrus postpartum. J Dairy Sci 1998;81;(Suppl. 1):321(Abstr).

Mach, N., A. Bach, and M. Devant. 2009. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. J. Anim. Sci. 87: 632-638.

Mader TL. Environmental stress in confined beef cattle. J Anim Sci 2003;81:E110–9.

Maciejewski D, Richman JG, Hakak Y, Gaidarov I, Behan DP, Conolly DT. Langerhans cells release prostaglandin D2 in response to nicotinic acid. J Invest Dermatol 2006; 126:2637-46.

McCarrick RB, Drennan MJ. Effect of winter environment on growth of young beef cattle 1. Effects of exposure during winter to rain or wind and rain combined on performance of 9-month-old Friesian steers fed on two planes of nutrition. J Anim Sci 1972;14:97–105.

McGuire MA, Beede DK, Collier RJ, et al. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. J Anim Sci 1991;69:2050-6.

McKay DG, Young BA, Milligan LP. Energy substrates for cold induced thermogenesis. In K. H. Menke, H. L. Lantzech and J. R. Beichl (Ed). Energy Metabolism and Farm Animal 1974; pp.30-42.

McNamara S, Butler T, Ryan DP, et al. Effect of offering rumen-protected fat supplements on fertility and performance in spring–calving Holstein–Friesian cows. Anim Reprod Sci 2003;79:45–56.

Mitlohner FM, Morrow JL, Dailey JW, et al. Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. J Anim Sci 2001;79:2327–35.

Miyoshi S, Pate JL, Palmquist DL. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Anim Reprod Sci 2001;68:29-43.

Morrison SR. Ruminant heat stress effect on production and means of alleviation. J Anim Sci 1983;57: 1594–600.

Naik PK, Saijpaul S, Neelam R. Effect of ruminally protected fat on in vitro fermentation and apparent nutrient digestibility in buffaloes (Bubalus bubalis). Anim Feed Sci Technol 2009a;153:68-76.

Naik PK, Saijpaul S, Neelam R. Evaluation of rumen protected fat prepared by fusion method. Anim Nutr Feed Technol 2007a;7:95-101.

Naik PK, Saijpaul S, Neelam R. Preparation of rumen protected fat and its effect on nutrient utilization in buffaloes. Indian J Anim Nutr 2007b;24:212-15.

Naik PK. Bypass fat in dairy ration - a review. Anim Nutr Feed Technol 2013;13:147-63.

Nedergaard J, Becker W, Cannon B. Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue. J Nutr 1983;113:1717–24.

Nielsen NI, Ingvartsen KL. Propylene glycol for dairy cows: A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Anim Feed Sci Technol 2004;115:191-213.

NRC. Effect of Environment on Nutrient Requirements of Domestic Animals. Washington, DC: National Academy Press; 1981.

NRC. Effect of Environment on Nutrient Requirements of beef cattle. 7th revised. Washington, DC: National Academy Press; 2001

NRC. Effect of Environment on Nutrient Requirements of Dairy cattle. 7th revised. Washington, DC: National Academy Press; 2001

O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH. Metabolic adaptations to heat stress in growing cattle. Domest Anim Endocrinol 2010;38:86–94.

Palmquist DL, Jenkins TC. Fat in lactation rations: a review. J Dairy Sci 1980;63:1–14.

Park SJ, Beak SH, Jung DJS, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle — a review. Asian Australas J Anim Sci 2018;31:1043–61.

Pejman A, Habib A. Heat Stress in Dairy Cows (A Review), Res Zoolo 2012; 2: 31-7.

Pickett MM, Piepenbrink MS, Overton TR. Effects of propylene glycol or fat drench on plasma metabolites, liver composition and production of dairy cows during the periparturient period. J Dairy Sci 2003;86:2113-21.

Redbo I, Ehrlemark A, Redbo-Torstensoon P. Behavioural responses to climatic demands of

dairy heifers housed outdoors. Can J Anim Sci 2001;81:9–15.

Remond B, Souday R, Jouany JP. In vitro and in fermentation of glycerol by rumen microbes. Anim Feed Sci Technol 1993; 41:121–32.

Renaudeau D, Collin A, Yahav S, de Basilio V. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 2012;5:707–28.

Roland L, Drillich M, Klein-Jöbstl D, Iwersen M. Invited review: Influence of climatic conditions on the development, performance, and health of calves. J Dairy Sci 2016;99:2438-52.

Roman-Ponce H, Thatcher WW, Van Horn HH, et al. Physiological and production responses of dairy cattle to a shade structure in a subtropical environment. J Dairy Sci 1977;60:424-31.

Sakatani M, Balboula AZ, Yamanaka K, Takahashi M. Effect of summer heat environment on body temperature, estrous cycles and blood antioxidant levels in Japanese Black cow. Anim Sci J 2012;83:394-402.

Samson DE, Slee J. Factors affecting resistance to induced body cooling in newborn lambs of 10 breeds. Anim Prod 1981;33:59–65.

Schneider PL, Beede DK, Wilcox CJ. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. J Anim Sci 1988;66:112-25.

Shultz TA. Weather and shade effects on cow corral activities. J Dairy Sci 1984;67:868–73.

Sirohi SK, Wali TK, Mohanta R. Supplementation effect of bypass fat on production performance of lactating crossbred cow. Indian J Anim Sci 2010;80:733-36.

Sutton JD, Knight R, Mc Allan AB, Smith RH. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. Brit J Nutr 1983;49:419-32.

Tao S, Bubolz JW, Do Amaral BC, et al. Effect of heat stress during the dry period on mammary gland development. J Dairy Sci 2011;94:5976-86.

Thakur SS, Shelke, SK. Effect of supplementing bypass fat prepared from soybean acid oil on milk yield and nutrient utilization in Murrah buffaloes. Indian J Anim Sci 2010;80:354-57.

Thompson JR, Christopherson RJ, Hammond VA, Hills GA. Effects of acute cold exposure on plasma concentrations of noradrenaline and adrenaline in sheep. Can J Anim Sci

1978;58:23.

Thompson GE. Respiratory system. In 'Stress Physiology in Livestock. Vol. 1: Basic Principles'. Ed. M. K. Yousef. 1984; pp. 155-62. CRC Press: Boca Raton, FL, U.S.A.

Tyagi N, Thakur SS, Shelke SK. Effect of feeding bypass fat supplement on milk yield, its composition and nutrient utilization in crossbred cows. Indian J Anim Nutr 2009;26:1-8.

Van laer E, Moons CPH, Sonck B. Importance of outdoor shelter for cattle in temperate climates. Livest Sci 2014;159:87-101.

Wang Y, Chaves AV, Rigby FL, et al. Effects of hops on ruminal fermentation, growth, carcass traits and shedding of Escherichia coli of feedlot cattle. Livest Sci 2009; 129:135-40.

Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 2002;213:1–47.

Webster AJF, Heitman JH, Hays FL, Olynyk GP. Catecholamines and cold thermogenesis in the sheep. Can J Physiol Pharmacol 1969;47:718.

Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 2015;93:644-55.

Werner Omazic A, Kronqvist C, Zhongyan L, Martens H, Holtenius K. The fate of glycerol entering the rumen of dairy cows and sheep. J Anim Physiol Anim Nutr 2015;99:258-64.

West JW. Effects of heat-stress on production in dairy cattle. J Dairy Sci 2003;86:2131–44.

West JW. Interactions of energy and bovine somatotropin with heat stress. J Dairy Sci 1999;77:2091–102.

West JW. Nutritional strategies for managing the heat-stressed dairy cow. J Anim Sci 1999;77:21–35.

Wiltbank JN, Rowden WW, Ingalls JE, Geegoey KE, Koch RM. Effect of Energy Level on Reproductive Phenomena of Mature Hereford Cows. J Anim Sci 1962;21:219-25.

Young BA. Cold stress as it affects animal production. J Anim Sci 1981;52:154–63.

Zhang M, Zou X, Li H, Dong X, Zhao W. Effect of dietary gamma-aminobutyric acid on laying performance, egg quality, immune activity and endocrine hormone in heat-stressed Roman hens. Anim Sci J 2012;83:141–7.

Zimbleman RB, Baumgard LH, Collier RJ. Effects of encapsulated niacin on evaporative heat

loss and body temperature in moderately heat-stressed lactating Holstein cows. J Dairy Sci 2010;93:2387-94.

CHAPTER THREE

Study 1

Effects of ambient temperature and rumen–protected fat supplementation on growth performance, rumen fermentation and blood parameters during cold season in Korean cattle steers

*This study comprises a part of paper was accepted in Asian-Australasian Journal of Animal Science, as a partial fulfillment of Hyeokjoong Kang's Ph.D program.

1. ABSTRACT

This study was performed to evaluate whether cold ambient temperature and dietary rumen-protected fat (RPF) supplementation affect growth performance, rumen fermentation, and blood parameters in Korean cattle steers. Twenty Korean cattle steers [BW: 550.6 ± 9.14 kg, age: 19.7 ± 0.13 months] were divided into a conventional control diet group (n = 10) and a 0.5% RPF supplementation group (n = 10). Steers were fed a concentrate diet (1.6% BW) and a rice straw diet (1 kg/day) for 16 weeks [January 9 to February 5 (P1), February 6 to March 5 (P2), March 6 to April 3 (P3), and April 4 to May 2 (P4)]. The mean and minimum indoor ambient temperatures in P1 (–3.44 ℃, –9.40 ℃) were lower ($P < 0.001$) than those in P3 (5.87 ℃, –1.86 ℃) and P4 (11.18 ℃, 4.28 ℃). The minimum temperature in P1 fell within the moderate cold-stress (CS) category, as previously reported for dairy cattle, and the

minimum temperatures of P2 and P3 were within the mild CS category. Neither month nor RPF supplementation affected the ADG or G:F ratio ($P > 0.05$). Ruminal NH_3–N concentrations were higher ($P < 0.05$) in cold winter than spring. Plasma cortisol concentrations were lower ($P < 0.05$) in the coldest month than the other months. Serum glucose concentrations were generally higher in colder months than in the other months, but were unaffected by RPF supplementation. RPF supplementation increased both total cholesterol ($P = 0.004$) and HDL concentrations ($P = 0.03$). Korean cattle may not be significantly affected by moderate CS, considering that the growth performance of cattle remained unchanged, although variations in blood parameters were observed among the studied months. RPF supplementation altered cholesterol and HDL concentrations but did not affect growth performance.

Keywords: Ambient temperature, beef cattle, blood metabolites, cold stress, growth, rumen-protected fat

2. INTRODUCTION

Exposure of feedlot beef cattle to cold stress reduces growth performance and feed efficiency due to an increases in the maintenance energy required to retain body temperature (Ames et al., 1980). Several studies have reported that cold stress negatively influences

mortality, the immune system of calves, back fat thickness, the meat quality of beef cattle, and the milk yield of dairy cows (Gulliksen et al., 2009; Mader, 2003; Broucek et al., 1991). Decreased productivity under cold conditions may result from increased basal metabolic intensity (Young, 1981). Moreover, cold stress appears to alter the metabolic and digestive status of animals. For example, increases in circulating glucose and non-esterified fatty acids (NEFAs) are likely triggered by elevated metabolic heat production and mobilization of substrates for energy metabolism in adipose tissue and liver (Mader, 2003). In addition, increases in rumination activity, reticulorumen motility, and the rate of digesta passage have been observed under cold conditions (Kennedy et al., 1976). Such changes in rumen digestive characteristics have been associated with reduced digestion in the reticulorumen, particularly when consuming roughage feed (NRC, 1981). Rumen fermentation characteristics, including volatile fatty acid (VFA) concentrations, are also altered by cold exposure (Christopherson and Kennedy, 1983). However, little information on the effects of cold conditions on growth performance, rumen fermentation, and blood parameters is available for Korean cattle.

Dietary fat supplementation has been reported to alleviate cold stress and increase animal productivity (Hess et al., 2008). Due to the relatively higher caloric density of fat, dietary fat supplementation in ruminants may provide additional energy, to meet elevated energy requirements under cold conditions (NRC, 2007). For example, in lactating dairy cows, dietary fat supplementation was associated with increased circulating glucose, which sufficiently increased substrate availability and thus reduced substrate mobilization from energy reserves (Kronfeld et al., 1980). Therefore, dietary fat supplementation could represent an effective strategy to alleviate cold stress. However, little information is available on the

effects of fat supplementation on alleviating cold stress in Korean cattle. Dietary rumen-protected fat (RPF) has been used as an energy supplement to enhance the productivity of cattle (McNamara et al., 2003; Hill and West, 1991). Therefore, this study was performed to examine the effects of RPF supplementation on the growth performance, rumen fermentation, and blood parameters of Korean cattle steer under cold conditions.

3. MATERIALS AND METHODS

Animals and feeding trial

All experimental procedures involving animals were approved by the Seoul Natio nal University Institutional Animal Care and Use Committee (SNUIACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of the SNUIACUC. The study was conducted at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University, South Korea.

In the feedlot trial, 20 Korean cattle steers with an average age of 19.7 ± 0.13 months and body weight (BW) of 550.6 ± 9.14 kg were used. The steers had been fed commercial early fattening stage concentrate using an automatic feeding station (DeLaval Alpro System; DeLaval, Sweden) and rice straw, following a conventional feeding program. Water was provided freely. During the 2-week adaptation period before the experiment, all animals were fed an experimental control concentrate (approximately 1.6% BW per animal) and rice straw

(1 kg/day/head). Steers were assigned to one of two treatments: the control group and RPF supplementation group. The RPF is prilled form of palm oil, as described (Naik, 2013), and purchased from Ecolex SDN. BHD (Pulau Indah, Selangor**,** Malyasia). The RPF was composed of 99.63% free fatty acids, including 85.48% palmitic acid (C 16:0), 7.05% oleic acid (C 18:1), 3.45% myristic acid (C 14:0), 1.64% linoleic acid (C 18:2), and 1.04% lauric acid (C 12:0), with an energy density of 9,316 kcal/kg (Haneol Corp., Anseong, Republic of Korea). Contents of the distillers dried grains with solubles, corn flour, corn gluten feed, barley stone, and palm oil were also adjusted to make similar non-fiber carbohydrate, neutral detergent fiber, and acid detergent fiber between two diets. Table 1 lists the formula and chemical compositions of the experimental diets. Steers were fed a concentrate diet (1.6% BW) using an automatic feeding station and a rice straw diet (1 kg/day) for 16 weeks [January 9 to February 5 (P1), February 6 to March 5 (P2), March 6 to April 3 (P3), and April 4 to May 2 (P4)]. The daily feed intake of the concentrate was automatically recorded online using a computer with the DeLaval Alpro system. Equal amounts of roughage were provided twice daily (08:00 and 18:00) and residual roughage was weighed before the morning feeding. Concentrate and rice straw samples were collected weekly and stored at –20 °C until analysis. BW was measured before morning feeding on the start day, at 4-week intervals thereafter.

Analysis of chemical composition of feed

The chemical compositions (dry matter, crude protein, ether extract, ash, calcium, and phosphorus) of the concentrate and rice straw were determined using the AOAC method (AOAC, 1996). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of

the rice straw were analyzed using the sequential method with an ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA) and reagents, as described by Van Soest et al. (1991).

Blood collection and measurement of ambient temperature

Blood was collected before feeding (after 9 h of fasting) at approximately 09:00 on the start date, and at 4-week intervals thereafter. Blood was collected via jugular venipuncture with both a non-heparinized vacutainer (20 mL; Becton–Dickinson, Franklin Lakes, NJ, USA) and EDTA-treated vacutainer (20 mL). Serum and plasma were separated by centrifugation at 1,500 × *g* at 4 °C for 15 min and stored at −80 °C until analysis.

Ambient and climate temperatures and relative humidity inside and outside the barn, were recorded in 1-h intervals using four HOBO data loggers (Onset Computer Corp., Bourne, MA, USA). Minimum, mean, and maximum temperatures and corresponding relative humidity data were chosen at every day, and monthly data were average values of 28 days per month. The experimental farm was covered with a roof and the animals were raised indoors; therefore, the animals were protected from precipitation and direct sunlight. Doors were installed on both sides of the barn, which remained open to allow exposure to cold weather. Thus, low winds may have affected the wind-chill temperature.

Rumen fluid collection and analysis

After blood collection, rumen fluid was collected before feeding (after 9 h of fasting) using the oral stomach tube method, as described by Shen et al. (2012). Rumen fluid pH was

measured immediately with a pH meter (Ohaus Corp., Parsippany, NJ, USA). For the VFA analysis, 1 mL of rumen fluid was mixed with 0.2 mL of 25% meta-phosphoric acid and stored at –20 °C until analysis, and an additional 30 mL of rumen fluid was stored at –20 °C for the ruminal ammonia nitrogen (NH_3-N) analysis. NH_3-N concentrations were determined using a modified colorimetric method (Chaney and Marbach, 1962). VFA concentrations were determined by gas chromatography using an Agilent Tech 7890A (Hewlett Packard, Waldbronn, Germany) with a Supelco fused silica capillary column (30 m × 0.25 mm × 0.25 μm).

Blood analysis

The analytical reagents for albumin, glucose, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), calcium, magnesium, and phosphorus were purchased from JW Medical (Seoul, Korea). The analytical reagents for the NEFA analysis were purchased from Wako Pure Chemical (Osaka, Japan). These parameters were analyzed using an automated chemistry analyzer (7180; Hitachi, Tokyo, Japan). Plasma cortisol was analyzed using a Cortisol Salivary HS Enzyme-linked Immunosorbent Assay Kit (cat. no. SLV4635; DRG Instruments, Marburg, Germany). The intra- and inter-assay coefficients of variation for the cortisol kit were 4.0% and 4.6%, respectively, based on bovine plasma samples. All analytical methods were verified in our laboratory, as reported previously (Kang et al., 2016).

Statistical analysis

Differences in climate data by month were analyzed with one-way analysis of variance (ANOVA). Differences in growth performance, blood parameters, and rumen fluid parameters by month and dietary treatment were analyzed using a repeated-measures two-way ANOVA. The statistical model included month, diet, and their interaction. $P < 0.05$ was considered to indicate significance. Pearson's correlation coefficient was calculated for the correlation analysis between daily average temperature and DMI during each period. All statistical tests were performed using R Studio for Windows software (R Studio, Boston, MA, USA).

4. RESULTS AND DISCUSSION

Climate conditions

The mean (P1: –3.44 °C, P2: –0.70 °C) and minimum (P1: –9.40 °C, P2: –5.5 °C) indoor ambient temperatures in January (P1) and February (P2) were lower ($P < 0.001$) than those in April (P4; mean: 11.18 °C, minimum: 4.28 °C; Table 2). In a previous study, cold stress was categorized as mild (0 °C to −6.7 °C), moderate (−7.2 °C to −13.9 °C), or severe (< −13.9 °C) under dry-winter-coat conditions in cattle (Grzych, 2010). Therefore, the minimum temperatures of P1 and both P2 and P3 in this study were considered to represent moderate and mild cold-stress (CS) conditions, respectively. In addition, we measured the average temperature during blood collection (08:00–10:00). The temperatures were –6.42 °C, –0.29 °C, 6.79 °C, 10.8 °C, and 14.0 °C on January 9, February 6, March 6, April 4, and May 2, respectively (Table 5). Then sampling days in January and February were classified as mild

CS conditions, while sampling days in March, April and May were considered as thermo-neutral conditions.

Growth performance

BW was higher ($P < 0.001$) during P4 than P1, reflecting animal age. Moreover, the daily concentrate intake was higher ($P = 0.03$) during P4 than P1 (Table 3). In this study, the daily allowance of concentrate was set at 1.6% BW and was adjusted each month based on BW. Thus, the higher concentrate intake during P4 reflected the increased concentrate allowance, corresponding to a higher BW during P4 than P1. Daily forage intake was higher in P1 than in the other months, and it did not affect growth performances, because most of require energy is supply from concentrate, which did not show difference by months. Additionally, there is no significant correlation between daily average temperature and daily DMI (data in supplementary table 1). RPF supplementation did not affect concentrate and roughage intake during all studied months. Neither month nor RPF supplementation affected the ADG and feed efficiency (G:F ratio; Table 3). In a previous study, dietary RPF supplementation (4.5% fatty acid calcium salts) increased the G:F ratio in beef cattle (Hill and West, 1991). In South Korea, commercial feed for Korean cattle steers has generally higher energy levels to produce highly marbled beef (Park et al., 2018). Additional energy supply through RPF supplementation may not have significant impact on growth performance in mild cold conditions. Other possible explanation for no influence on growth performance by fat addition is an insufficient amount of RPF supplementation (0.5%).

Rumen VFAs and NH_3-N

Neither month nor RPF supplementation affected ruminal pH ($P > 0.05$; Table 4). However, ruminal NH_3-N concentrations were higher ($P < 0.05$) in cold winter (January and February) than spring (March and April) (Table 4). For comparison, in sheep, NH_3 production was dependent on diet and ambient temperature (Kennedy et al., 1982). Moreover, cold exposure did not affect ruminal NH_3 production with barley–canola seed meal and lucerne diets, but reduced NH_3 production with a bromegrass diet. Meanwhile, cold exposure reduced the irreversible loss of both plasma urea and rumen NH_3, and the conversion of plasma urea-nitrogen into rumen NH_3 was greater in cold-exposed sheep than in warm sheep (Kennedy and Milligan, 1978). The same authors reported an increased efficiency of ruminal microbial synthesis under cold exposure in sheep. Thus, the differences in rumen NH_3 concentrations between cold winter and spring in this study may have been related to changes in the irreversible loss of NH_3, conversion of urea into NH_3, or to the efficiency of microbial synthesis upon cold exposure. Meanwhile, RPF supplementation did not affect ruminal NH_3-N concentrations.

Neither month nor RPF supplementation affected C2, C3, iso-C5, C5, or total VFA concentrations in rumen fluid ($P > 0.05$; Table 4). The C2:C3 ratio tended to be lower ($P = 0.07$) in the RPF supplementation group than in the control group. In another study, increasing levels of protected lipids linearly increased the molar proportion of C3, whereas the molar proportion of C2 remained unchanged, resulting in a linear decrease in the C2:C3 ratio (Bines et al., 1978). RPF supplementation decreased ($P = 0.02$) ruminal iso-C4 concentrations, while ruminal C4 concentrations varied by season ($P = 0.02$; Table 4).

Blood cortisol and metabolites

Plasma cortisol concentrations were lower ($P < 0.05$) on the starting day than during the other periods, and RPF supplementation did not affect ($P > 0.05$) cortisol concentrations (Table 5). For comparison, plasma cortisol and corticosterone concentrations were similar among newborn calves at –4.0 °C and 16 °C, although the concentrations varied at –12 °C and –18 °C in one or two animals (Khan et al., 1970). Collectively, blood cortisol concentration does not appear to be a suitable marker of cold stress, although cortisol is commonly used as a general stress marker (Pollard, 1995).

Serum glucose concentrations were generally higher ($P < 0.001$) in colder weather, but were unaffected by RPF supplementation (Table 5). Young (1975) suggested that increased blood glucose results from increased metabolism (e.g., metabolic rate or heart rate) under cold conditions. In humans, lipid and muscle glycogen have major roles in providing the energy required for heat production under cold exposure, whereas plasma glucose has only a minor role (Haman et al., 2002). Meanwhile, in lactating beef cows, fat supplementation did not affect circulating glucose concentrations (Lake et al., 2005).

Month did not affect total cholesterol concentrations ($P > 0.05$; Table 5), and serum HDL concentrations were similar among January, February, March, and early April, albeit that the concentrations in late April were comparatively lower. RPF supplementation increased both total cholesterol ($P = 0.004$) and HDL concentrations ($P = 0.03$; Table 5). In Holstein calves, various types of fat supplementation increased plasma cholesterol and HDL concentrations, with no differences seen in TG and glucose concentrations, during the cold season (Ghasemi

et al., 2017). Furthermore, dietary rumen-protected oleic acid increased blood HDL concentrations in cattle (Lee et al., 2003). Collectively, the increased HDL concentrations observed in this and previous studies may be due to increased amounts of absorbed fat in the small intestine via RPF supplementation. Moreover, changes in posthepatic lipoprotein metabolism, including alterations in HDL concentrations, may contribute to increased cholesterol concentrations with RPF supplementation, as suggested by Duske et al. (2009).

Neither month nor RPF supplementation affected serum TG, LDL, or NEFA concentrations. The effects of RPF supplementation on blood NEFA concentrations in several studies of lactating dairy cows were inconsistent with our results (Bines et al., 1978; Payne et al., 1974), and showed an increase in blood NEFA concentrations with RPF supplementation. Overall, little information is available on the effects of RPF supplementation on NEFA concentrations in beef cattle.

Serum albumin concentrations were higher ($P = 0.017$) on the experimental starting day in the colder month (January) than in the other months in spring, although the concentrations were unaffected by RPF supplementation ($P > 0.05$; Table 4). A previous study revealed higher serum albumin concentrations with changes in the osmotic pressure of body fluid during hotter summer periods than colder winter periods in dairy cows (Payne et al., 1974). Thus, the seasonal variation in circulating albumin concentrations remains unclear.

Serum GOT and GPT concentrations were not affected by month, though GPT concentrations were lower ($P < 0.001$) in the RPF supplementation group, which has not been reported previously (Table 5).

Month and/or RPF supplementation affected blood mineral concentrations (Table 5).

Blood calcium concentrations were higher ($P < 0.001$), while blood magnesium concentrations were lower ($P < 0.001$), on the starting day in January compared with the spring months. In addition, the RPF supplementation group had lower ($P < 0.001$) phosphorus and magnesium concentrations than the control group. In a previous study, serum magnesium levels were lower in hyperthyroid human patients (Ebel and Gunther, 1980). Furthermore, thyroid hormone thyroxine increased under cold conditions in beef cattle (Christopherson et al., 1979). Thus, the lower magnesium concentrations under cold conditions in this study may have been indirectly influenced by higher circulating thyroid hormone levels. However, changes in these mineral concentrations according to RPF supplementation have not been well studied.

5. CONCLUSION

Growth performance (i.e., ADG and G:F ratio) remained unchanged during all studied months (January to May). Furthermore, rumen fermentation parameters, such as C2, C3, and total VFA concentrations, were unaffected by cold conditions and RPF supplementation, although NH_3-N concentrations were altered under cold conditions. Serum glucose and HDL concentrations were generally higher under cold conditions; however, other serum lipid metabolites (e.g., TG, LDL, and NEFA) did not differ among the studied months. These results suggest that Korean cattle may not have been significantly affected by cold conditions in this study, although cold conditions partially affected rumen fermentation and blood parameters. Moreover, RPF supplementation did not affect growth performance or major

rumen VFA (C2, C3, and C4) concentrations, although RPF supplementation resulted in altered cholesterol and HDL concentrations. Further research is warranted to clarify which cold conditions significantly affect the growth performance of Korean cattle.

Table 1. Ingredients of the concentrate and composition of experimental diets (RPF 0.5% supplemented diet) for Korean cattle steers during winter through spring season.

Ingredients, %DM	Control concentrate	RPF concentrate
Ground corn	1.48	0.68
Wheat bran	25.00	25.00
Rice bran	4.88	5.00
Soy hull	8.00	8.00
Urea	0.39	0.35
Salt	0.20	0.20
Molasses	3.50	3.50
Whole cottonseed	3.00	3.00
Cottonseed hull	3.00	3.00
Bentonite	0.30	0.30
Magnesium oxide	0.30	0.30
Sodium bicarbonate	0.45	0.45
Steamed–flaked corn	21.00	21.00
Corn flour	5.48	7.61
Condensed molasses soluble	1.20	1.20
Coconut meal	5.00	5.00
Rapeseed meal	2.41	2.04
Wheat flour	5.00	5.00
Distiller's dried grains with solubles (DDGS)	0.00	0.61
Corn gluten feed	4.78	3.88
Limestone	2.89	2.57
Live yeast	0.07	0.01
Barley stone	1.18	0.00
Rumen protected fat	0.00	0.50
Palm oil	0.30	0.61
Mineral/Vitamin premix[1]	0.19	0.19
Total	100.00	100.00
TDN[2]	71.79	75.36
Chemical composition, % DM		
Concentrate diet		

DM	88.46	88.25
Moisture	11.54	11.75
CP	14.50	14.50
Ether extract (EE)	3.63	5.30
Ash	9.98	7.93
NDF	27.26	27.60
ADF	11.54	11.75
Non-fiber carbohydrate (NFC)	34.28	34.40
Ca	1.45	1.32
P	0.45	0.46
Crude fiber	8.93	9.23
ME[3], Mcal/kg	2.47	2.63
NE[4], Mcal/kg	1.12	1.22

Rice straw, % DM

DM	74.91
CP	3.87
Ether extract (EE)	1.37
Ash	11.46
Ca	0.25
P	0.095
ADF	51.97
NDF	31.52

[1] Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D_3 (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic–DC). Grobic–DC was provided by Bayer Health Care (Leverkusen, Germany).

[2] TDN (%) = NFC + CP + [(EE – 1) x 2.25] + NDF – 7 (NRC, 2007)

[3] ME = [1.01 x (DE) − 0.45] + 0.0046 x (EE−3) (NRC, 2007)

[4] $NE = 1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$ (NRC, 2007)

RPF = rumen-protected fat.

Table 2. Mean, maximum, and minimum values of ambient temperatures, climate temperatures, and relative humidity during winter through spring season.

Items	January(P1)[1]	February(P2)[2]	March(P3)[3]	April(P4)[4]	SE	P–value
Ambient temperature, ℃						
Mean	–3.44[a]	–0.70[b]	5.87[c]	11.18[d]	0.58	< 0.001
Maximum	4.49[a]	5.04[a]	17.78[b]	20.68[c]	1.53	< 0.001
Minimum	–9.40[a]	–5.5[b]	–1.86[c]	4.28[d]	0.49	< 0.001
Climate temperature, ℃						
Mean	–2.91[a]	–0.24[b]	4.68[c]	10.36[d]	0.58	< 0.001
Maximum	8.78[b]	7.00[a]	16.16[c]	18.12[d]	1.59	< 0.001
Minimum	–10.57[a]	–5.78[b]	–3.12[c]	3.88[d]	0.54	< 0.001
Ambient Relative humidity, %						
Mean	75.32	66.35	52.33	57.64	2.12	0.31
Maximum	90.03	83.38	89.95	77.90	2.60	0.27
Minimum	50.51[b]	45.92[b]	19.03[a]	35.95[ab]	2.69	0.012
Climate Relative humidity, %						
Mean	71.39	62.26	54.34	75.37	2.44	0.08
Maximum	91.80	81.42	93.55	106.38	3.36	0.51
Minimum	40.08[b]	38.80[b]	18.39[a]	44.36[b]	3.17	< 0.001

N = 28.

Mean values with different letters differ significantly.

[1] January 9 – February 5 (4 weeks). [2] February 6 – March 5 (4 weeks). [3] March 6 – April 3 (4 weeks). [4] April 4 – May 2 (4 weeks).

Table 3. Growth performance of Korean cattle steers fed either control or RPF–supplemented (0.5%) diet during winter through spring season.

Items	January(P1)[1]		February(P2)[2]		March(P3)[3]		April(P4)[4]		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
Initial body weight, kg	547.2	554.0	571.6	579.0	587.2	595.6	604.4	616.0	5.28	< 0.001	0.34	0.87
Body weight, kg	571.6	579.0	587.2	595.6	604.4	616.0	625.2	636.8	5.57	< 0.001	0.35	0.94
Daily feed intake, kg: DM base												
Total feed intake, kg/d	7.52	7.43	7.24	7.38	7.59	7.51	7.66	7.76	0.07	0.12	0.92	0.75
Concentrate intake, kg/d	6.66	6.57	6.50	6.64	6.84	6.76	6.91	7.01	0.07	0.03	0.93	0.74
Forage intake, kg/d	0.86	0.86	0.74	0.74	0.75	0.75	0.75	0.75	0.01	< 0.001	0.98	0.99
Average daily gain, kg/d	0.87	0.89	0.56	0.59	0.61	0.73	0.77	0.77	0.03	0.39	0.41	0.62
Feed efficiency (G:F ratio)	0.104	0.124	0.077	0.080	0.080	0.097	0.099	0.098	0.01	0.35	0.33	0.60

N = 10/group. RPF = rumen-protected fat

[1] January 9 – February 5 (4 weeks): a mild cold stress range

[2] February 6 – March 5 (4 weeks): a mild cold stress range

[3] March 6 – April 3 (4 weeks): a thermo-neutral range

[4] April 4 – May 2 (4 weeks): a thermo-neutral range

Table 4. Ruminal pH, VFAs and NH3-N of Korean cattle steers fed either control or RPF–supplemented (0.5%) diet during winter through spring season.

Items	February 6		March 5		April 3		May 2		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
Temperature[1], ℃	-6.42		-0.26		6.79		10.8		14.0			
pH	7.21	7.04	6.74	6.50	6.73	6.77	6.85	6.90	0.06	0.46	0.50	0.17
NH_3–N, mg/dL	13.30	8.97	11.06	13.59	5.19	4.57	7.42	3.46	0.69	<0.001	0.40	0.45
C2, mM	36.22	37.84	51.24	52.71	43.89	46.06	46.19	39.34	1.12	0.82	0.87	0.18
C3, mM	10.25	10.98	15.81	17.39	14.48	18.13	13.60	12.63	0.54	0.47	0.22	0.59
C2:C3 ratio	3.62	3.46	3.29	3.11	3.14	2.84	3.46	3.14	0.07	0.24	0.07	0.66
iso–C4, mM	0.71	0.65	0.88	0.81	0.74	0.71	0.86	0.68	0.02	0.63	0.02	0.22
C4, mM	10.28	10.01	13.99	14.99	9.79	10.66	10.27	8.38	0.40	0.02	0.98	0.34
iso–C5, mM	1.10	1.15	1.51	1.27	1.19	1.06	1.36	0.98	0.05	0.62	0.08	0.27
C5, mM	0.79	0.80	1.33	1.37	0.98	1.18	1.10	0.91	0.04	0.87	0.76	0.40
Total VFA, mM	59.35	61.43	84.76	88.55	71.07	77.81	73.38	62.93	1.86	0.86	0.84	0.22

N = 10/group. RPF = rumen-protected fat. January 9 and February 6 are within a mild cold stress range, and other 3 days are within a thermos-neutral range.

[1] Minimum ambient temperatures of sampling times of each day.

Table 5. Plasma cortisol and serum parameters of Korean cattle steers fed either control or RPF–supplemented (0.5%) diet during winter through spring season.

	January 9		February 6		March 5		April 3		May 2		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
Temperature[1], ℃	-6.42		-0.26		6.79		10.8		14.0					
Cortisol, ug/dL	35.6	37.6	64.7	62.2	57.0	41.2	54.3	54.0	59.0	64.5	5.68	0.012	0.55	0.65
Glucose, mg/dL	74.3	72.7	78.9	75.5	76.1	75.6	66.7	66.9	59.0	57.8	0.90	<0.001	0.34	0.75
Cholesterol, mg/dL	170.1	174.6	147.4	168.3	156.1	168.5	154.7	178.4	152.1	180.9	3.09	0.78	0.004	0.21
Triglyceride, mg/dL	14.8	13.6	21.0	20.0	14.4	15.7	16.0	15.8	15.8	15.2	0.43	0.70	0.84	0.76
HDL, mg/dL	101.7	103.9	97.7	105.3	94.3	102.3	93.8	102.1	87.8	92.5	1.39	0.003	0.03	0.75
LDL, mg/dL	23.7	21.9	17.3	18.7	19.1	19.3	19.3	21.7	21.4	25.4	0.61	0.31	0.51	0.13
NEFA, mg/dL	303.8	283.0	193.0	271.4	439.2	374.1	296.4	308.9	264.6	280.7	11.39	0.95	0.97	0.88
Albumin, mg/dL	3.51	3.56	3.36	3.47	3.39	3.53	3.38	3.39	3.38	3.38	0.02	0.017	0.18	0.52
GOT, IU/dL	73.5	71.0	75.9	79.7	79.2	78.2	74.2	72.3	74.2	74.8	1.16	0.86	0.99	0.92
GPT, IU/dL	26.1	23.6	24.1	22.5	25.1	22.2	24.1	21.1	24.6	22.7	0.35	0.18	<0.001	0.95
Calcium, mEq/dL	9.69	9.77	9.47	9.40	9.37	9.78	9.30	9.31	9.34	9.25	0.04	<0.001	0.40	0.58
Phosphorus, mEq/dL	7.92	7.68	8.70	7.95	8.09	7.62	7.83	7.19	8.60	7.84	0.08	0.75	<0.001	0.40
Magnesium, mEq/dL	2.56	2.39	2.74	2.48	2.82	2.63	2.83	2.44	2.89	2.65	0.03	<0.001	<0.001	0.40

N = 10/group. January 9 and February 6 are within a mild cold stress range, and other 3 days are within a thermos-neutral range.
[1] Minimum ambient temperatures of sampling times of each day.
RPF = rumen-protected fat, GOT = glutamic oxalacetic transaminase, GPT = glutamic pyruvate transaminase, HDL = high density lipoprotein, LDL = low density lipoprotein, NEFA = non–esterified fatty acid.

6. REFERENCES

Ames DR, Brink DR, Willms CL. Adjusting protein in feedlot diets during thermal stress. J Anim Sci 1980;50:1–6.

AOAC. Official Methods of Analysis. 15th ed, Association of Official Analytical Chemists, Washington, DC. 1996. p. 210–9.

Bines JA, Brumby PE, Storry JE, Fulford RJ, Braithwaite GD. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cows during early lactation. J Agric Sci Camb 1978;91:135–50.

Brouček J, Letkovičová M, Kovalčuj K. Estimation of cold stress effect on dairy cows. Int J Biomet 1991;35:29–32.

Chaney AL, & Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem 1962;8:130–2.

Christopherson RJ, Gonyou HW, Thompson JR. Effects of temperature and feed intake on plasma concentration of thyroid hormones in beef cattle. Can J Anim Sci 1979;59:655–61.

Christopherson RJ, Kennedy PM. Effect of the thermal environment on digestion in ruminants. Can J Anim Sci 1983;63:417–96.

Duske K, Hammon HM, Langhof AK, et al. Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. J Dairy Sci 2009;92:1670–84.

Ebel H, Gunther T. Magnesium metabolism: a review. J Clin Chem Clin Biochem 1980;18:257–70.

Ghasemi E, Azad–Shahraki M, Khorvash M. Effect of different fat supplements on performance of dairy calves during cold season. J Dairy Sci 2017;100:5319–28.

Grzych M. 2010. Webinar Portal for Forestry and Natural Resources. http://www.forestrywebinars.net/webinars/planning–and–design–of–livestock–watering–systems/Cattle Stress Index Description Accessed Jun 26, 2014.

Gulliksen SM, Lie KI, Løken T, Østerås O. Calf mortality in Norwegian dairy herds. J Dairy Sci 2009;92:2782–95.

Haman F, Péronnet F, Kenny GP, et al. Effect of cold exposure on fuel utilization in humans:

plasma glucose, muscle glycogen, and lipids. J Appl Physiol 2002;93:77–84.

Hess BW, Moss GE, Rule DC. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J Anim Sci 2008;86:E188–204

Hill GM, West JW. Rumen protected fat in Kline barley or corn diets for beef cattle: digestibility, physiological, and feedlot responses. J Anim Sci 1991;69:3376–88.

Kang HJ, Lee IK, Baik M, et al. Effects of ambient temperature on growth performance, blood metabolites, and immune cell populations in Korean cattle steers. Asian Australas J Anim Sci 2016;29:436–43

Kennedy PM, Christopherson RJ, Milligan LP. The effect of cold exposure of sheep on digestion, tureen turnover time and efficiency of microbial synthesis. Brit J Nutr 1976;36:231–42.

Kennedy PM, Milligan LP. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br J Nutr 1978;39:105–17.

Kennedy PN, Christopherson RJ, Milligan LP. Effect of cold exposure on feed protein degradation, microbial protein synthesis and transfer of plasma urea to the rumen of sheep. Br J Nutr 1982;E47:521–35.

Khan MA, Dickson WM, Meyers KM. The effect of low environmental temperature on plasma corticosteroid and glucose concentrations in the newborn calf. J Endocrinol 1970;48:355–63.

Kronfeld DS, Donoghue S, Naylor JM, Johnson K, Bradley CA. Metabolic Effects of Feeding Protected Tallow to Dairy Cows. J Dairy Sci 1980;63:545–52

Lake SL, Scholljegerdes EJ, Atkinson RL, et al. Body condition score at parturition and postpartum supplemental fat effects on cow and calf performance. J Anim Sci 2005;83:2908–17.

Lee H–J, Lee SC, Oh YG, et al. Effects of rumen protected oleic acid in the diet on animal performances, carcass quality and fatty acid composition of Hanwoo steers. Asian Australas J Anim Sci 2003;16:1003–10.

Mader TL. Environmental stress in confined beef cattle. J Anim Sci 2003;81:E110–9.

McNamara S, Butler T, Ryan DP, et al. Effect of offering rumen–protected fat supplements on fertility and performance in spring–calving Holstein–Friesian cows. Anim Reprod Sci 2003;79:45–56.

Naik PK. Bypass fat in dairy ration – A review. Anim Nutr Feed Techol 2013;13:147–63.

NRC. Effect of Environment on Nutrient Requirements of Domestic Animals. Washington, DC: National Academy Press; 1981.

NRC. Nutrient requirements of dairy cattle. 8th rev ed. Washington, DC: National Academy Press; 2007.

Park SJ, Beak SH, Baik M, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle — A review. Asian-Australas J Anim Sci 2018;31:1043–61.

Payne JM, Rowlands GJ, Manston R, Dew SM, Parker WH. A Statistical Appraisal of the Results of the Metabolic Profile Tests on 191 Herds in the B.V.A./A.D.A.S Joint Exercise in Animal Health and Productivity. Br Vet J 1974;130:34–44.

Pollard TM. Use of cortisol as a stress marker: practical and theoretical problems. Am J Hum Biol 1995;7:265–74.

Shen JS, Chai Z, Song LJ, Liu ZX, Wu YM. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. J Dairy Sci 2012;95:5978–84.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74:3583–97.

Young BA. Cold stress as it affects animal production. J Anim Sci 1981;52:154–63.

Young BA. Temperature-Induced Changes in Metabolism and Body Weight of Cattle (Bss taurus). Can J Physiol Pharmacol 1975;53:947–53.

Study 2

Effects of ambient temperature and rumen–protected fat supplementation on growth performance, rumen characteristics, and blood parameters during cold season in early fattening stage of Korean cattle steers

*This study comprises a part of paper was accepted in Asian-Australasian Journal of Animal Science, as a partial fulfillment of Hyeokjoong Kang's Ph.D program.

1. ABSTRACT

This study was performed to evaluate whether cold ambient temperature and rumen–protected fat supplementation affect growth performance, rumen characteristics, and blood metabolic parameters in early fattening stage of Korean cattle steers. Twenty Korean cattle steers [356.5±3.72 kg of body weight (BW), 16.8±0.34 months of age] were divided into a conventional control diet group (n = 10) and a 0.8 % rumen–protected fat (RPF) supplementation group (n = 10). Steers were fed 1.6 % BW of a concentrate diet and 3 kg of a tall-fescue hay for 12 weeks. BW measurement and blood and rumen fluid collection were conducted four times (starting date and 4 weeks intervals). The mean ambient temperature in P1 (–7.37 °C) and P2 (–2.96 °C) were lower ($P < 0.001$) than that in P3 (6.22 °C), respectively. The mean ambient temperature in P1 and P2 were within the mild to moderate cold stress (CS) category range previously reported for dairy cattle, while mean ambient temperature of P3

was under the thermo–neutral range. Neither month nor RPF supplementation affected ($P > 0.05$) average daily gain and gain to feed ratio. In the rumen fluid, C2, C3 and total volatile fatty acid were higher ($P < 0.05$) in the colder month. Blood concentrations of triglyceride, cholesterol, and high density lipoprotein (HDL) were higher ($P < 0.05$) in the colder period. Triglyceride, cholesterol, and HDL were lower ($P < 0.05$) in the colder month. RPF supplementation increased ($P < 0.05$) blood concentration of cholesterol. Korean cattle may not be significantly affected by mild or moderate CS, considering that growth performance of cattle was not changed, although some changes in blood metabolic and ruminal parameters were observed. RPF supplementation may affect some blood lipid metabolites and ruminal VFAs, whereas RPF supplementation did not affect growth performance.

Keywords: Korean cattle steers, cold ambient temperature, rumen–protected fat, growth, blood metabolites, rumen characteristics

2. INTRODUCTION

The purpose of beef farming is to get highest profit with high quality and yield grades, while maintaining the welfare of the animals. In South Korea, there are about 3 months (December through February) of winter season with cold temperature. The average minimum temperature during last 30 years of December and January were −5.6 ℃, and −3.2 ℃, respectively, which can be classified as mild cold stress (CS) condition (Grzych, 2010; KMA, 2007). In this winter season, cattle in Korea may suffer from CS. Cold exposed cattle showed increased basal metabolic intensity and changed digestive capacity (Young, 1981; Kennedy

et al., 1976). In previous studies, cold exposure altered metabolism and negatively influenced on mortality, immune system of calves, back fat thickness and meat quality of beef cattle and milk yield of dairy cow (Gulliksen et al., 2009; Mader, 2003; Brouček et al., 1991). Previous reports suggest that the intramuscular fat (IMF) in beef cattle has been deposited from 250 day of age to slaughter (Park et al., 2018; Du et al., 2013). Therefore, sufficient energy to hyperplasia and hypertrophy for IMF deposition is important for early fattening stage (from 13 to 20 months of age) of cattle. However, little information on the effects of cold condition on growth performance and rumen fermentation and blood metabolite parameters is available for Korean cattle in early fattening stage.

To alleviate CS, several nutritional strategies were applied (Hess et al., 2008). Feeding of rumen-protected fat (RPF), which is inert in rumen, has been used to enhance energy intake without compromising rumen bacterial activity (Jenkins and Palmquist, 1984). Previous studies have reported a positive effect of RPF supplementation in dairy cows and beef cattle (McNamara et al., 2003; Hill and West, 1991). In lactating dairy cows, dietary fat supplementation was associated with increased circulating glucose, and it effectively increased substrate availability for reduced the mobilized substrate from energy reserves (Kronfeld et al., 1980). Therefore, dietary fat supplementation could be a proper strategy for alleviating CS. In previous study during winter season, we conducted similar experiment using 0.5 % RPF supplemented concentrate, but 0.5 % RPF supplementation did not affect growth performance of Korean cattle steer, although some rumen and blood parameters were altered (Kang et al., In revision). Thus, in this study, we increased RPF supplementation levels up to 0.8 %. Winter temperatures in this study were colder compared to those in our previous

study. This study was performed to examine the effects of 0.8% RPF supplementation on growth performance and rumen fermentation and blood parameters during cold environment in early fattening stage of Korean cattle steer.

3. MATERIALS AND METHODS

Animals and feeding trial

All experimental procedures followed our previous study (Kang et al., Submitted). All experimental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of the SNUIACUC. The study was conducted at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University, South Korea.

In the feedlot trial, 20 Korean cattle steers with an average age of 16.8 ± 0.34 months and body weight (BW) of 356.5 ± 3.72 kg were used. The steers had been fed commercial early fattening stage concentrate using an automatic feeding station (DeLaval Alpro System; DeLaval, Sweden) and tall-fescue hay, following a conventional feeding program. Water was provided freely. During the 2-week adaptation period before the experiment, all animals were fed an experimental control concentrate (approximately 1.6 % BW per animal) and tall-fescue hay (3 kg / day / head). Steers were assigned to one of two treatments: the control group and RPF supplementation group (0.8 %). The RPF is prilled form of palm oil, as described (Naik, 2013), and purchased from Ecolex SDN. BHD (Pulau Indah, Selangor, Malyasia). The RPF

was composed of 99.63% free fatty acids, including 85.48% palmitic acid (C 16:0), 7.05% oleic acid (C 18:1), 3.45% myristic acid (C 14:0), 1.64% linoleic acid (C 18:2), and 1.04% lauric acid (C 12:0), with an energy density of 9,316 kcal/kg (Haneol Corp., Anseong, Republic of Korea). Table 6 lists the formula and chemical compositions of the experimental diets. Steers were fed a concentrate diet (1.6 % BW) using an automatic feeding station and a tall-fescue hay (3 kg / day / head) for 12 weeks [January 8 to February 2 (P1), February 3 to March 3 (P2), March 4 to April 1 (P3)]. The daily feed intake of the concentrate was automatically recorded online using a computer with the DeLaval Alpro system. Equal amounts of roughage were provided twice daily (08:00 and 18:00) and residual roughage was weighed before the morning feeding. Concentrate and tall-fescue hay samples were collected weekly and stored at –20 °C until analysis. BW was measured before morning feeding on the start day and at 4-week intervals thereafter.

Rumen fluid collection and analysis

The rumen fluid collection and analysis were followed the same method, as described in Study 1.

Blood analysis

The blood metabolites were measured using the same method, as described in Study 1.

Statistical analysis

The statistical analysis was followed the same method, as described in Study 1.

4. RESULTS AND DISCUSSION

Climate conditions

The mean (P1: –7.37 °C, P2: –2.96 °C) and minimum (P1: –12.52 °C, P2: –8.18 °C) indoor ambient temperatures in January (P1) and February (P2) were lower ($P < 0.001$) than those in March (P3; mean: 6.22 °C, minimum: –2.11 °C; Table 7). In a previous study, CS was categorized as mild (0 °C to −6.7 °C), moderate (−7.2 °C to −13.9 °C), or severe (< −13.9 °C) under dry-winter-coat conditions in cattle (Grzych, 2010). Therefore, the mean temperatures of P1 and P2 were considered as mild cold-stress (CS), whereas that of P3 in this study were considered to represent thermos-neutral conditions, respectively. Mean and minimum ambient temperature in this study were lower than those (mean: January −3.44 °C, February −0.70 °C; minimum: January −9.40 °C, February – 5.50 °C) of our previous study conducted in 2015 winter (Kang et al., in revision), although mean ambient temperature of two studies were classified as same mild CS condition. In addition, we measured the average temperature during blood and rumen fluid collection time (08:00–12:00). The temperatures were –7.92 °C, –3.81 °C, 2.87 °C, and 11.84 °C on January 8, February 4, March 4, and April 1, respectively (Table 9). The first 2 sampling days were classified as moderate and mild CS conditions, while the other 2 days were considered as thermo-neutral conditions.

Growth performance

BW was higher ($P < 0.001$) during P3 compared with P1, reflecting animal age. The daily

concentrate intake was not changed ($P > 0.05$) by month and diet (Table 8). Daily forage intake of P1 was higher ($P < 0.05$) than that of other months. RPF supplementation did not affect concentrate and roughage intake during all experimental periods. Neither month nor RPF supplementation affected ($P > 0.05$) average daily gain (ADG) and gain to feed ratio (G:F ratio) (Table 8). Growth performances were not changed by months, although forage intake was increased during colder months. Additionally, there is no significant correlation between daily average temperature and daily DMI (data in supplementary table 1). The reason for unchanged growth performances may be that most of the energy source is concentrate, and the concentrate intake was not changed during all periods. Thus, the increased forage intake did not affect growth performances. The low critical temperature of Korean cattle was not established, and the cold condition of the experimental region would not affect the growth performances. In the previous studies, dietary RPF supplementation (4.5 % of Ca salts of fatty acid) increased G:F ratio in beef cattle (Hill and West, 1991). In this study, no improvement of growth performance may be due to the fact that amount (0.8 %) of RPF supplementation was not sufficient to affect growth performance. Our previous study during winter season in Korean cattle steer also showed similar results that neither month nor 0.5 % of RPF supplementation did affect growth performance (Kang et al., in revision).

Rumen NH_3–N and VFAs

Neither month nor RPF supplementation affected ($P > 0.05$) ruminal pH and NH_3–N concentrations (Table 9). The C2, C3, and total VFA concentrations were higher ($P < 0.05$) in colder month (Table 9). Increased C3 concentrations by cold exposure were reported in

several sheep studies (Kennedy et al., 1976; Kelly and Christopherson, 1989; Kennedy and Milligan, 1978; Kennedy, 1985), indicating consistent results with our study. Kennedy and Milligan (1978) suggested that the increased proportions of C3 in the total VFA and reduced digestion of cell wall contents in the stomach in cold-exposed sheep are indicative of a reduced dependence of microbes on fermentation of cellulose and hemicellulose. However, decreased ruminal C2 and total VFA concentrations by cold exposure were reported in sheep studies (Kennedy et al., 1976; Kelly and Christopherson, 1989; Kennedy and Milligan, 1978; Kennedy, 1985), showing inconsistent results with current study. In our previous study with Korean cattle steer (average 19.7 age of months, BW of 550.6 kg), cold condition did not affect C2, C3, and total VFA concentrations (Kang et al., in revision), showing inconsistent results with this study. Inconsistent results of VFA concentrations among studies may be due to different animal species and variation in animal age, cold conditions, etc. Cold condition did not affected ($P > 0.05$) C4, iso C4, C5 concentrations and C2:C3 ratio in rumen fluid.

RPF supplementation decreased ($P < 0.05$) C4 and iso C5 concentrations, but did not affect ($P > 0.05$) total VFA, C2, C3, iso C4, and C5 concentrations and C2:C3 ratio in rumen fluid (Table 4). In previous dairy cow study, increasing level of protected lipid decreased total VFA and C4 concentrations, which are consistent with our result, but it increased C3 concentration (Bines et al., 1978).

Blood metabolites

Serum glucose concentration were generally higher ($P < 0.01$) on colder weather, and were unaffected ($P > 0.05$) by RPF supplementation (Table 10). The same result was observed

in our previous study (Kang et al., Submitted). As we mentioned in previous study, higher glucose levels during cold season are likely due to increased metabolism in the cold condition, such as metabolic rate or heart rate (Young, 1975). In lactating beef cows, fat supplementation did not affect circulating glucose concentrations (Lake et al., 2005).

Serum TG, total cholesterol, and HDL concentrations were lower ($P < 0.05$) on colder months (Table 10). Acute cold exposure has reduced the concentration of TG in arterial blood of young steers, but increased oxidation of free fatty acid by the shivering leg (Bell and Thompson, 1979). Such changes of lipid metabolism under cold condition may affect blood lipid metabolism-related parameters. In our recent study, cold condition generally did not affect blood cholesterol, HDL, and TG concentrations (Kang et al., in revision). Thus, current results showed larger changes in lipid metabolites during cold conditions compared to previous experiment (Kang et al., in revision), which may be related to differences in animal age, BW, and climate conditions between two studies.

RPF supplementation increased ($P < 0.01$) total cholesterol concentrations, but other lipid metabolites were not altered ($P > 0.05$) by RPF supplementation (Table 10). In our previous study with Korean cattle study, 0.5% RPF supplementation also increased total cholesterol and HDL concentrations (Kang et al., in revision). In Holstein calves, various types of fat supplementation also increased the concentrations of plasma cholesterol concentrations with no differences in TG and glucose concentrations during the cold season (Ghasemi et al., 2017). Neither month nor RPF supplementation affected circulating NEFA concentrations. In lactating dairy cow, fat supplementation increased blood NEFA concentrations, showing inconsistent result with our study (Duske et al., 2009). Little

information is available on the effects of RPF on NEFA concentrations in beef cattle. Neither month nor RPF supplementation affected ($P > 0.05$) serum concentrations of albumin, GOT and GPT (Table 10).

5. Conclusion

Growth performance (i.e., ADG and G:F ratio) remains unchanged during all studied months (January to March). However, rumen C2, C3, and total VFA concentrations were affected by cold conditions. Serum TG, cholesterol and HDL concentrations were generally lower under cold conditions; however, NEFA concentrations did not differ among the studied months. These results suggest that growth performance of Korean cattle may not have been significantly affected by cold conditions in this study, although cold conditions partially affected rumen fermentation and blood parameters. RPF supplementation did not affect growth performance and major rumen VFA (C2 and C3) concentrations, whereas C4 concentrations were decreased by RPF supplementation. RPF supplementation increased blood cholesterol concentrations.

Table 6. Ingredients of the concentrate and composition of experimental diets (RPF 0.8% supplemented diet) for Korean cattle steers during winter through spring season.

Items	Control concentrate	RPF concentrate
Ingredients, % DM		
Steamed-flaked corn	15.30	13.59
Wheat bran	20.00	20.00
Rice bran	0.66	6.24
Salt	0.59	0.35
Soy hull	2.00	2.00
Molasses	4.00	4.00
Ammonium chloride	0.32	0.10
Palm meal	10.00	10.00
Condensed molasses soluble	1.50	1.10
Coconut meal	10.00	10.00
Live yeast	0.01	0.01
Ground corn	8.00	8.00
Corn gluten feed	20.00	20.00
Beat pulp	2.57	0.86
Limestone	2.86	2.76
Bentonite	2.00	0.00
Rumen protected fat	0.00	0.80
Mineral/Vitamin premix [1]	0.19	0.19
Total	100.00	100.00
Chemical Composition, % Dry matter		
Concentrate diet		
Dry matter	88.55	88.25
Crude protein (CP)	14.50	14.50

Ether extract (EE)	3.63	5.38
Ash	9.98	7.90
Calcium	1.32	1.16
Phosphorus	0.53	0.58
Neutral detergent fiber (NDF)	27.26	27.55
Acid detergent fiber (ADF)	11.54	11.75
Non fiber carbohydrate (NFC)	34.28	33.96
Rumen undegradable protein	5.02	5.24
TDN[2]	71.79	75.42
ME[3], Mcal/kg	2.75	2.91
NE[4], Mcal/kg	1.19	1.31
Tall-fescue hay, % Dry matter		
Dry matter	89.11	
Crude protein (CP)	6.56	
Ether extract (EE)	1.58	
Ash	5.87	
Calcium	0.32	
Phosphorus	0.11	
Acid detergent fiber (ADF)	65.84	
Non fiber carbohydrate (NFC)	39.48	

[1]Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D_3 (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic–DC). Grobic–DC was provided by Bayer Health Care (Leverkusen, Germany).

[2]TDN = Total digestible nutrients (%) = NFC + CP + [(EE – 1) x 2.25] + NDF – 7 (NRC, 2007).

[3]ME = Metabolizable energy = [1.01 x (DE) − 0.45] + 0.0046 x (EE−3) (NRC, 2007).

[4]NE = Net energy = 1.42 ME − 0.174 ME^2 + 0.0122 ME^3 − 1.65 (NRC, 2007).

Table 7. Mean, maximum, and minimum values of ambient temperatures and climate temperatures during winter through spring season.

Items	January (P1) [1]	February (P2) [2]	March (P3) [3]	SEM	P-value
Ambient temperature, ℃					
Mean	-7.37[a]	-2.96[b]	6.22[c]	0.45	< 0.001
Maximum	-1.80[a]	2.62[b]	12.4[c]	0.86	< 0.001
Minimum	-12.52[a]	-8.18[b]	-2.11[c]	0.59	< 0.001
Climate temperature, ℃					
Mean	-8.79[a]	-4.30[b]	5.75[c]	0.41	< 0.001
Maximum	-2.72[a]	1.71[b]	11.8-[c]	0.75	< 0.001
Minimum	-13.59[a]	-9.94[b]	-3.29[c]	0.60	< 0.001

[a-c] Mean values with different letters differ ($P < 0.05$).

[1] January 8 – February 2 (4 weeks).

[2] February 3 – March 3 (4 weeks).

[3] March 4 – April 1 (4 weeks).

Table 8. Growth performance of Korean cattle steers fed either control or RPF[1]–supplemented (0.8%) diet during winter through spring season.

Items	January (P1) [2]		February (P2) [3]		March (P3) [4]		SEM	P value		
	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Age, month	16.9	16.8	17.8	17.7	18.8	18.7	0.40	0.87	< 0.001	0.70
Initial body weight, kg	357.8	355.3	375.3	376.3	394.0	392.8	4.57	0.76	< 0.001	0.46
Body weight, kg	375.3	376.3	394.0	392.8	413.0	415.2	4.89	0.80	< 0.001	0.69
Feed intake, kg: DM base										
Total feed intake, kg/d	6.69	6.80	6.99	7.08	6.80	6.63	0.14	0.99	0.84	0.65
Concentrate intake, kg/d	4.36	4.39	4.89	4.95	4.87	4.79	0.08	0.95	0.13	0.82
Forage intake, kg/d	2.33	2.41	2.10	2.13	1.93	1.84	0.06	0.90	< 0.001	0.38
Average daily gain, kg/d	0.65	0.78	0.64	0.57	0.67	0.83	0.06	0.58	0.27	0.73
Feed efficiency (G:F ratio)	0.097	0.115	0.092	0.081	0.098	0.125	0.009	0.26	0.57	0.49

N = 10/group.

[1]RPF = rumen-protected fat.

[2] January 8 – February 2 (4 weeks).

[3]February 3 – March 3 (4 weeks).

[4]March 4 – April 1 (4 weeks).

Table 9. Ruminal pH, VFAs and NH3-N of Korean cattle steers after 3 h feeding fed either control or RPF–supplemented (0.8%) diet during winter through spring season.

Items	January 8		February 4		March 4		April 1		SEM	P value		
	Control	RPF[1]	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Temperature[2], ℃	–7.92		–3.81		2.87		11.84					
pH	6.14	6.29	6.31	6.10	6.56	6.54	5.97	6.15	0.56	0.34	0.57	0.80
NH_3-N, mg/dl	5.64	5.89	6.81	5.99	6.54	6.18	5.78	6.15	0.87	0.65	0.48	0.87
C2, mM	87.3	87.2	60.3	54.1	57.6	53.9	66.8	58.5	2.10	0.31	0.009	0.32
C3, mM	28.7	29.2	16.3	14.4	13.2	12.2	16.1	17.2	0.91	0.86	< 0.001	0.87
C2 to C3 ratio	3.05	3.04	3.89	3.96	4.49	4.41	4.20	3.64	0.54	0.76	0.21	0.74
Iso C4, mM	0.62	0.54	0.73	0.61	0.66	1.37	0.77	0.62	0.12	0.70	0.60	0.90
C4, mM	14.5	13.9	10.9	9.8	12.1	11.0	13.8	9.0	0.45	0.011	0.25	0.046
Iso C5, mM	0.84	0.72	1.13	0.72	0.69	0.64	0.77	0.65	0.04	0.049	0.20	0.44
C5, mM	1.44	1.32	1.17	0.80	1.04	0.85	1.27	1.04	0.06	0.107	0.60	0.81
Total VFA	133.4	132.8	90.5	80.4	85.3	79.9	99.4	86.9	3.27	0.31	0.006	0.34

N = 10/group.

[1]RPF = rumen-protected fat.

[2]Average ambient temperatures of sampling times of each day.

Table 10. Serum parameters of Korean cattle steers before feeding fed either control or RPF–supplemented diet (0.8%) during winter through spring season.

Items	January 8		February 4		March 4		April 1		SEM	P value		
	Control	RPF[1]	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Glucose, mg/dL	72.3	88.0	78.9	80.7	73.3	76.7	74.8	80.6	1.23	0.11	0.008	0.67
Triglyceride, mg/dL	10.7	12.5	16.4	17.0	20.2	19.6	17.4	19.1	0.74	0.86	0.007	0.65
Cholesterol, mg/dL	109.3	137.0	122.0	158.2	136.8	157.8	161.2	196.7	5.48	0.008	0.0012	0.52
HDL, mg/dL	68.4	73.8	83.8	75.8	81.8	82.3	89.0	91.7	2.12	0.59	0.03	0.31
GOT, IU/dL	57.3	80.5	62.4	65.8	53.5	58.1	58.9	92.9	4.04	0.075	0.98	0.31
GPT, IU/dL	17.6	24.8	20.4	20.2	16.9	17.7	20.5	22.8	0.50	0.13	0.24	0.75
Albumin, mg/dL	2.99	3.25	3.50	3.15	3.03	3.00	3.37	3.44	0.06	0.34	0.80	0.22
NEFA, mg/dL	228.5	278.7	193.8	132.4	195.6	215.1	234.4	293.5	14.8	0.59	0.70	0.41

N = 10/group.

[1]RPF = rumen-protected fat.

HDL = high density lipoprotein. GOT = glutamic oxalacetic transaminase. GPT = glutamic pyruvate transaminase. NEFA = non–esterified fatty acid.

6. REFERENCES

Bell AW, Thompson GE. Free fatty acid oxidation in bovine muscle in vivo: effects of cold exposure and feeding. Ame J Physiol Endocrinol Metab 1979;237:E309–15.

Bines JA, Brumby PE, Storry JE, Fulford RJ, Braithwaite GD. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cows during early lactation. J Agri Sci 1978;91:135–50.

Brouček J, Letkovičová M, Kovalčuj K. Estimation of cold stress effect on dairy cows. Int J Biomet 1991;35:29–32.

Du M, Huang Y, Das AK, Yang Q, et al.. Meat science and muscle biology symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. J Anim Sci 2013;91:1419–27.

Duske K, Hammon HM, Langhof AK, et al. Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. J Dairy Sci 2009;92:1670–84.

Ghasemi E, Azad–Shahraki M, Khorvash M. Effect of different fat supplements on performance of dairy calves during cold season. J Dairy Sci 2017;100:5319–28.

Gulliksen SM, Lie KI, Løken T, Østerås O. Calf mortality in Norwegian dairy herds. J Dairy Sci 2009;92:2782–95.

Grzych M. 2010. Webinar Portal for Forestry and Natural Resources. http://www.forestrywebinars.net/webinars/planning–and–design–of–livestock–watering–systems/Cattle Stress Index Description Accessed Jun 26, 2014.

Hess BW, Moss GE, Rule DC. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J Anim Sci 2008;86:E188–204.

Hill GM, West JW. Rumen protected fat in Kline barley or corn diets for beef cattle: digestibility, physiological, and feedlot responses. J Anim Sci 1991;69:3376–88.

Jenkins TC, Palmquist DL. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J Dairy Sci 1984;67:978–86.

Kang HJ, Piao MY, Baik M, et al. Effects of ambient temperature and rumen–protected fat supplementation on growth performance, rumen fermentation and blood parameters during cold season in Korean cattle steers. Asian–Australas J Anim Sci 2018;in publish.

Kang HJ, Lee IK, Baik M, et al. Effects of ambient temperature on growth performance, blood

metabolites, and immune cell populations in Korean cattle steers. Asian Australas J Anim Sci 2016;29:436–43

Kelly JM, Christopherson RJ. The apparent digestibility of dry matter, organic matter and nonammonia nitrogen in the forestomach, small intestine, and large intestine of weathers exposed to a cold environment. Can J Anim Sci 1989;69:911–9.

Kennedy PM, Christopherson RJ, Milligan LP. The effect of cold exposure of sheep on digestion, tureen turnover time and efficiency of microbial synthesis. Brit J Nutr 1976;36:231–42.

Kennedy PM, Milligan LP. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br J Nutr 1978;39:105–17.

Kennedy PM. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behavior in sheep given four forage diets in the chopped, or ground and pelleted form. Brit J Nutr 1985;53:159–73.

Korea Meteorological Administration, KMA. Annual climatological report of Korea. 2017.

Kronfeld DS, Donoghue S, Naylor JM, Johnson K, Bradley CA. Metabolic Effects of Feeding Protected Tallow to Dairy Cows. J Dairy Sci 1980;63:545–52.

Lake SL, Scholljegerdes EJ, Atkinson RL, et al. Body condition score at parturition and postpartum supplemental fat effects on cow and calf performance. J Anim Sci 2005;83:2908–17.

Mader TL. Environmental stress in confined beef cattle. J Anim Sci 2003;81:E110–9.

McNamara S, Butler T, Ryan DP, et al. Effect of offering rumen-protected fat supplements on fertility and performance in spring–calving Holstein–Friesian cows. Anim Reprod Sci 2003;79:45–56.

Naik PK. Bypass fat in dairy ration – A review. Anim Nutr Feed Techol 2013;13:147–63.

NRC. Nutrient requirements of dairy cattle. 8th rev ed. Washington, DC: National Academy Press; 2007.

Park SJ, Beak SH, Baik M, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle — A review. Asian-Australas J Anim Sci 2018;31:1043–61.

Young BA. Temperature-Induced Changes in Metabolism and Body Weight of Cattle (Bss

taurus). Can J Physiol Pharmacol 1975;53:947–53.

Young BA. Cold stress as it affects animal production. J Anim Sci 1981;52:154–63.

Study 3

Effects of temperature and glycerol supplementation as feed additive on growth performance, rumen characteristics, and blood parameters during winter season in growing stage of Korean cattle steers

*This study will be published in elsewhere as a fulfillment of Hyeokjoong Kang's Ph.D program

1.ABSTRACT

This study was performed to evaluate whether cold condition and glycerol supplementation affect growth performance, rumen characteristics, and serum metabolites in growing stage of Korean cattle steers. Twenty Korean cattle steers [217.0 ± 7.63 kg of BW, 7.7 ± 0.07 months of age] were divided into a conventional control diet group (n = 10) and a 6.0 % glycerol supplementation group (n = 10). Control and glycerol supplementation group were top-dressed with 0.15 kg of wheat bran/d/head and 0.4 kg of 60% glycerol-adsorbed wheat bran/d/head, respectively. Steers were fed 1.5 % BW of a concentrate diet and 3.0 kg of a timothy hay for 16 weeks [January 10 to February 7 (P1), February 8 to March 7 (P2), March 8 to April 4 (P3), April 5 to May 1 (P4), of 2017]. Mean ambient temperatures of January and February (-5.09 ℃ and -0.08 ℃) were categorized within the mild cold stress range (Webinar Portal for Forestry and Natural Resources, 2014), whereas mean ambient temperatures of March and April were 5.57 ℃ and 14.7 ℃, respectively. Cold environment did not affect (*P*

> 0.05) average daily gain (ADG), whereas gain to feed ratio (G:F ratio) were higher ($P <$ 0.05) in the colder period. Glycerol supplementation numerically increased ($P > 0.05$) both ADG and G:F ratio. C3 concentration in the rumen was higher ($P < 0.05$) in the glycerol supplementation group than that of control group, and C2/C3 ratio was lower ($P < 0.05$) in the glycerol supplementation group compared with control group. Cold exposure did not affect ($P > 0.05$) ruminal pH, NH_3-N, and VFAs. In the cold period, glucose, triglyceride and NEFA were higher ($P < 0.05$) than those of thermo-neutral period. Glucose, NEFA, and triglyceride were not changed ($P > 0.05$) by glycerol supplementation. Korean cattle may not be significantly affected by moderate CS, considering that the growth performance of cattle remained unchanged, although variations in blood glucose, NEFA, and triglyceride were observed among the studied months. Glycerol supplementation altered ruminal C3, C5, and C2:C3 ratio, but did not affect growth performance. Beef cattle, ambient temperature, cold stress, glycerol, growth, blood metabolites, rumen parameters

2. INTRODUCTION

Many studies have reported that extremely low temperature climate condition negatively influence on growth performance, reproduction rate, beef quality, milk production, overall health, mortality, the immune system of calves, back fat thickness and wellbeing of cattle (Mader, 2003; Young, 1981; Hahn 1995; Gulliksen et al., 2009). In the cold condition, cattle consume more energy source to maintain body temperature, which results in reduced feed efficiency and daily gain (Birkelo et al., 1991). Thus, cold exposed cattle have increased basal metabolic rate and changed digestive capacity (Young, 1981; Kennedy et al., 1976). In these situation, a change in the energy source accompanied, such as glucose and lipid metabolites (Kang et al., in publish). In addition, increases in rumination activity, reticulorumen motility, and the rate of digesta passage have been reported under cold conditions (Kennedy et al., 1976). Such changes in rumen digestive characteristics is related with reduced digestion in the reticulorumen, particularly when consuming roughage feed (NRC, 1981). Rumen fermentation characteristics, including volatile fatty acid (VFA) concentrations, are also influenced by cold exposure (Christopherson and Kennedy, 1983). However, little information on the effects of cold conditions on growth performance, rumen fermentation, and blood parameters is available for Korean cattle steers in growing stage.

Glycerol, a by-product with biodiesel is a colorless, odorless, sticky, sweet-tasting liquid. Because glycerol is a liquid form at room temperature, it is difficult to store or transport for feeding to cattle. Glycerol has been used as a feed additive for ruminants and has been shown to be effective in improving ruminant productivity (Fiorentini et al., 2018; Mach et al., 2009;

Gunn et al., 2010). In other experiments, drenching method was used to feed glycerol for cows (Ferraro et al., 2016), but drenching method is difficult to commercialized, because of requirement of a lot of labor and stress can be applied to cattle. In previous study, we used glycerol as ingredient in the concentrate feed, which was difficult to add over 3.0 % due to the quality of the pellets (Kang et al., 2017). Thus, in this experiment, we made a glycerol-absorbed wheat bran for easier transportation, storing, and feeding to cattle. Glycerol, the 3 carbon backbone in triglyceride, is also known as substrate for gluconeogenesis in the liver and kidney, which can provide energy for cellular metabolism, and mainly fermented into C3 in rumen by specific microbes (Werner-Omazic et al., 2015; Wolin et al. 1997). As described above, in the cold stress situation, glucose and NEFA were more consumed. In this situation, it would be possible to reduce cold stress by supplementation glycerol, with gluconeogenic effect and fermentation characteristic. Little information about effect of glycerol supplementation on the Korean cattle steer in growing stage during cold season is known.

3. MATERIALS AND METHODS

Animals and feeding trial

All experimental procedures followed our previous study (Kang et al., 2017). All experi mental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of the SNUIACU C. The study was conducted at the University Animal Farm of the College of Agriculture and

Life Sciences, Pyeongchang Campus of Seoul National University, South Korea.

In the feedlot trial, 20 Korean cattle steers with an average age of 7.7 ± 0.07 months and body weight (BW) of 217.0 ± 7.63 kg were used. The steers had been fed commercial early fattening stage concentrate using an automatic feeding station (DeLaval Alpro System; DeLaval, Sweden) and timothy hay, following a conventional feeding program. Water was provided freely. During the 2-week adaptation period before the experiment, all animals were fed an experimental control concentrate (approximately 1.5 % BW per animal) and timothy hay (3 kg / day / head). According to individual BW, age and average daily gain (ADG), Steers were assigned to one of two treatments: control group (150 g of wheat bran) and glycerol supplementation group (400 g of 99.7 % purified glycerol–adsorbed wheat bran). Glycerol is a colorless, odorless, viscous, and sweet-tasting liquid. In order to apply the glycerol to diet fed to Korean cattle conveniently and effectively, glycerol mixture is made by adsorbing glycerol to the wheat bran at the ratio of 60 % of glycerol to 40 % of wheat bran on DM basis. Cattles in the glycerol group received glycerol at 6.0 % of concentrate DM supply daily. The supplements were poured on top of the feed trough for individual cattle. Table 11 lists the formula and chemical compositions of the experimental diets. Steers were fed a concentrate diet (1.5 % BW) using an automatic feeding station and a timothy hay (3 kg / day / head) for 16 weeks [[January 10 to February 7 (P1), February 8 to March 7 (P2), March 8 to April 4 (P3), April 5 to May 1 (P4), of 2017]. The daily feed intake of the concentrate was automatically recorded online using a computer with the DeLaval Alpro system. Equal amounts of roughage were provided twice daily (08:00 and 18:00) and residual roughage was weighed before the morning feeding. Concentrate and timothy hay samples were collected

weekly and stored at –20 °C until analysis. BW was measured before morning feeding on the start day and at 4-week intervals thereafter.

Rumen fluid collection and analysis

The rumen fluid collection and analysis were followed the same method, as described in Study 1.

Blood analysis

The blood metabolites were measured using the same method, as described in Study 1.

Statistical analysis

The statistical analysis was followed the same method, as described in Study 1.

4. RESULTS AND DISCUSSION

Climate conditions

The mean (P1: –5.09 °C, P2: –0.08 °C) and minimum (P1: –10.3 °C, P2: –6. 8 °C) indoor ambient temperatures in January (P1) and February (P2) were lower ($P < 0.001$) than those in March and April (P3; mean: 5.57 °C, minimum: –1.89 °C, P4; mean: 14.7 °C, minimum: 6.56 °C Table 12). In a previous study, CS was categorized as mild (0 °C to −6.7 °C), moderate (−7.2 °C to −13.9 °C), or severe (< −13.9 °C) under dry-winter-coat conditions in cattle (Grzych, 2010). Therefore, the mean temperatures of P1 and P2 were considered as mild cold-

stress (CS), whereas that of P3 and P4 in this study were considered to represent thermo-neutral conditions, respectively. In addition, we measured the average temperature during blood and rumen fluid collection time (08:00–12:00). The temperatures were –5.33 °C, –6.38 °C, 1.03 °C, 17.71 °C, and 24.38 °C on January 10, February 7, March 7, April 4 and March 1, respectively (Table 14). Then sampling days in January and February were classified as mild CS conditions, while sampling days in March, April and May were considered as thermo-neutral conditions.

Growth performance

BW was higher ($P < 0.001$) during P4 compared with P1, reflecting animal age. In this study, the daily allowance of concentrate was set at 1.5 % BW and was adjusted each month based on BW. Thus, the higher concentrate intake during P4 reflected the increased concentrate allowance, corresponding to a higher BW during P4 than P1. The cattle almost ate glycerol-absorbed wheat bran (0.4 kg/day/head for glycerol supplementation group) and wheat bran (0.15kg/day/head for control group). The studies with glycerol supplementation or inclusion in feedlot diets at 10% DM did not show interferences in the DMI (Mach et al., 2009; Lage et al., 2014). The daily concentrate and forage intake were higher ($P < 0.05$) by colder months (Table 13), whereas, percentage of concentrate intake relative to the offered amount was higher ($P < 0.05$) in the colder month. Percentage of forage intake relative to the offered amount was lower ($P < 0.05$) in the colder month (Table 13). Additionally, there is no significant correlation between daily average temperature and daily DMI (data in supplementary table 1). Glycerol supplementation did not affect concentrate and forage intake during all experimental periods. ADG was not changed by cold condition, but G:F was higher ($P < 0.05$) in the colder months. Glycerol supplementation did not affected ($P > 0.05$) average daily gain (ADG) and G:F ratio (Table 13). Result of growth performance is similar as our previous study in growing stage of Korean cattle steers [10.6 age of months, 230.4 kg of BW] during summer season, regardless of the temperature condition, the lower the age of the moon, the higher the feed efficiency. (Kang et al., in revision). Considering increased percentage of DMI and increased G:F ratio during colder months, other factors rather than temperature may affect growth performances. Other factors, such as heredity, diet and age,

may affect the growth performance of cattle (Park et al., 2018). In this study, genetic factor or diet is similar among experimental months, but the age of month is obviously different by each period. Goonewardene et al. (1981) showed that highest growth rate about 0 to 300 age of days, since then, the growth rate decreases as the age increases. Thus, higher weight gain and better feed efficiency during hotter months may be due to age effect rather than THI effect in this study. Parsons et al. (2009) reported that glycerol supplementation can have positive effects on ADG, and G:F ratio when included at less than 10% of livestock diets. However, negative effects on ADG and feed efficiency were also observed when glycerol was included in finishing heifer diets at 16% of DM (Parsons et al., 2009). Ladeira et al. (2016) reported that there is no improvement of ADG and G:F ratio by 60g or 120g/kg of glycerol supplementation in young bulls. In this study, there are no improvement of growth performance by glycerol supplementation, although numerical increases of ADG and G:F were observed in glycerol supplementation group with increased TDN and the changes in ruminal C3 and C2:C3 ratio, as described below part. As other glycerol supplementation experiments showed positive improvement of growth performance, increasing the number of animals may show significant results.

Rumen NH_3–N and VFAs

Cold period did not affect ($P > 0.05$) ruminal pH, NH3-N, and VFAs (Table 4), In the sheep studies, increased C3 and decreased ruminal C2 and total VFA concentrations by cold exposure were reported (Kennedy, 1976; Kelly and Christopherson,1989; Kennedy and Milligan1978; Kennedy, 1985). Although our previous study with Korean cattle steer (average

19.7 age of months, BW of 550.6 kg), cold condition did not affect C2, C3, iso-C5, C5, or total VFA concentrations in rumen fluid (Kang et al., in publish), showing consistent results with this study. Inconsistent results of VFA concentrations among studies may be due to different animal species and variation in animal age, cold conditions, etc.

The C3 and C5 were increased ($P < 0.05$) by glycerol supplementation, whereas iso-C4 and C2:C3 ratio were decreased ($P < 0.05$) by glycerol supplementation. The ruminal pH, NH_3-N, C2, C4, iso C5, and total VFA were not changed ($P > 0.05$) by glycerol supplementation (Table 14). As similar with our results, Remond et al. (1993) reported that decreased C2 and increased C4 concentration in their *in vitro* experiment, whereas C3 was not increased. In our study, the C2 concentration have decreased tendency (P = 0.066) by glycerol supplementation (Table 14). Lee et al. (2011) reported that decreased C2 and increased C3 concentration in their *in vitro* experiment, and Castagnino et al. (2018) also observed similar results in Nellore bulls. Consistent with our results, decreased C2:C3 ratio was observed (Lee et al., 2011; Castagnino et al., 2018). In this study, decreased tendency of C2 and increased C3 trends may affect decreased C2:C3 ratio. These result confirm that glycerol is mainly fermented to C3. *Selenomonas* species was reported as major fermenters of glycerol, with main product being C3 (Krehbiel, 2008). Trabue et al. (2007) reported that a large portion of glycerol was fermented to C3, although fermentation of glycerol also produced some C5.

Blood metabolites

Serum glucose concentration were generally higher ($P < 0.01$) on colder weather, and

were unaffected ($P > 0.05$) by glycerol supplementation (Table 15). The same result was observed in our previous study (Kang et al., in publish). As we observed in previous study, higher blood glucose concentration during cold season are mainly due to increased metabolism in the cold condition, such as metabolic rate or heart rate (Young, 1975). As similar to our result, Sauer et al. reported that blood glucose and NEFA were not changed with 3.6 % of glycerol supplementation. DeFrain et al. (2004) failed to show any glucogenic effect of glycerol, although they observed an increased C3 production and decreased the ratio of C2 to C3 in the rumen.

Serum TG and NEFA concentration were generally higher ($P < 0.05$) on colder months (Table 15). Blood NEFA concentrations in dairy cattle often increase when feed intake cannot support their energy requirements, requiring the mobilization of NEFA by lipolysis of fat depots to support energy demand (Bauman and Currie, 1980). NEFA concentration was increased under low temperature conditions (Broucek et al., 1987a, 1987b). Calves in a cold environment also had higher NEFA concentrations than those in a warm indoor environment (Nonnecke et al., 2009). Taken together, increased NEFA concentrations during colder weather may help generate energy to maintain body temperature and growth. The triglyceride concentrations were elevated in cold exposed Brahman calves (Godfrey et al. 1991). Godfrey et al., (1991) also suggested that Brahman calves may not be able to utilize the energy constituents of blood to maintain body temperature along with the elevated concentrations of glucose. Such changes of lipid metabolism under cold condition may affect blood lipid-related parameters. In this study, we observed similar result, which is elevated glucose, NEFA, and triglyceride in colder month, thus, it means that the cattle may have used more energy to

maintain homeostasis. Different from this study, cold condition generally did not affect blood triglyceride and NEFA concentrations in our previous Korean cattle study, (Kang et al., in publish). These different change by cold exposure may indicate that differences in animal age, BW, and climate conditions between two studies may related with. Neither Month nor Glycerol supplementation affected ($P > 0.05$) cholesterol, HDL, GOT, GPT, and albumin concentration (Table 15). In study 5, glycerol supplementation increased cholesterol and HDL, but in this study, the change of cholesterol and HDL concentration was observed, whereas NEFA was decreased by glycerol supplementation. Little information about relationship between glycerol supplementation and lipid metabolites are available. Some researchers have observed that glycerol supplementation have no glucogenic effects (DeFrain et al., 2004), thus, glycerol may be carried to blood flow from rumen as back bone of lipid metabolites. The follow-up study on glycerol transport form in cattle is required.

5. CONCLUSION

Growth performance (ADG and G:F ratio) was not worsen during colder months. (January to February). However, ruminal C2 concentration and C2:C3 ratio were decreased by glycerol supplementation, but C3 concentration was increased by glycerol supplementation. Serum glucose, triglyceride and NEFA were generally higher under cold conditions; however, cholesterol, and HDL concentrations did not differ among the studied months. These results suggest that growth performance of Korean cattle may not have been significantly affected by cold conditions in this study, although cold conditions partially

affected rumen fermentation and blood parameters. Glycerol supplementation did not affect growth performance, although increase major rumen VFA, especially C3, concentrations. However, glycerol supplementation made the numerical improvement of G:F ratio in Korean cattle steers in growing stage, suggesting the possibility of follow-up research.

Table 11. Ingredient of concentrate and composition of experimental diets (glycerol 6.0% as feed additive) for Korean cattle steers during winter through spring season.

	Control concentrate
Ground corn	12.331
wheat	8.000
Wheat bran	20.000
Wheat flour	20.000
Salt	0.400
Molasses	2.800
Ammonium chloride	0.308
Palm meal	13.000
Condensed molasses soluble	1.500
Soybean meal	5.000
Amaferm	0.020
Corn gluten feed	12.670
Limestone	3.133
Barley stone	0.643
Mineral/Vitamin premix[1]	0.195
Total	100.000
Chemical composition, %	
Protein	16.000
Ether extract (EE)	3.000
Ash	8.000
NPN	0.883
NDF	25.904
ADF	19.57
Moisture	11.831

NFC	35.214	
RUP	5.135	
TDN [2]	70.217	
ME [3]	2.67	
NE [4]	1.14	
Timothy hay, % DM		
DM	95.55	
CP	6.34	
Ether extract (EE)	8.47	
Ash	49.97	
Ca	0.56	
P	0.12	
NDF	71.99	
ADF	44.41	
Supplements, g/day/head	Control	Glycerol
Wheat bran	150	150
Glycerol	—	250
Total TDN	71.11	75.46

[1] Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D_3 (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic–DC). Grobic–DC was provided by Bayer Health Care (Leverkusen, Germany).

[2] TDN (%) = NFC + CP + [(EE – 1) x 2.25] + NDF – 7 [NRC, 2007]

[3] ME = [1.01 x (DE) − 0.45] + 0.0046 x (EE−3) [NRC, 2007]

[4] $NE = 1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$ [NRC, 2007]

Table 12. Mean, maximum, and minimum values of ambient temperatures during winter through spring season.

Items	January (P1)1	February (P2)[2]	March (P3)[3]	April (P4)[4]	SE	P-value
Ambient temperature, ℃						
Mean	-5.09	-0.08	5.57	14.7	0.76	<0.001
Maximum	1.83	9.27	15.9	25.2	1.09	<0.001
Minimum	-10.3	-6.8	-1.89	6.56	0.94	<0.001

[a-d] Mean values with different letters within same row differ ($P < 0.05$).

[1]10th January - 7th Fabruary (4 weeks).

[2]7th February - 7th March (4 weeks).

[3]7th March - 4th April (4 weeks).

[4]4th April - 1st March (4 weeks).

Table 13. Growth performance of growing Korean cattle steers fed either control or glycerol–supplemented (6.0% as feed additive) diet during winter through spring season.

Items	January (P1)[1]		February (P2)[2]		March (P3)[3]		April (P4)[4]		SE	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol		Diet	Month	Interaction
Age, month	7.6	7.8	8.5	8.7	9.4	9.6	10.3	10.5	0.34	0.49	< 0.001	0.45
Initial body weight, kg	217.3	216.3	243.4	243.1	275.1	279.5	298.7	306.7	12.4	0.16	< 0.001	0.32
Body weight, kg	243.4	243.1	275.1	279.5	298.7	306.7	325.9	332.8	12.1	0.24	< 0.001	0.48
Daily feed intake[3], kg: DM base												
Total feed intake, kg/d, DM base	5.60	5.00	5.86	6.02	6.30	6.31	6.69	6.67				
Concentrate intake, kg/d	2.90	2.50	3.07	3.23	3.49	3.46	3.83	3.81	0.02	0.96	<0.001	0.92
Forage intake, kg/d	2.70	2.50	2.79	2.79	2.81	2.85	2.86	2.86	0.05	0.35	<0.001	0.039
[5]Concentrate intake, %	98.0	94.7	92.3	97.0	91.9	90.5	93.8	90.8	0.66	0.39	0.007	0.53
[6]Forage intake, %	94.1	87.4	97.4	97.3	98.2	99.6	99.9	99.8	0.62	0.35	<0.001	0.039
[7]Top dressing, %	100.0	98.2	100.0	100.0	100.0	100.0	100.0	100.0				
Average daily gain, kg/d	0.93	0.96	0.95	1.07	0.84	0.97	0.97	0.93	0.02	0.19	0.66	0.54
G:F ratio	0.162	0.169	0.158	0.167	0.129	0.150	0.142	0.137	0.003	0.22	<0.001	0.59

N = 10/group. RPF = rumen–protected fat

[1]10th January - 7th Fabruary (4 weeks). [2]7th February - 7th March (4 weeks). [3]7th March - 4th April (4 weeks). [4]4th April - 1st March (4 weeks).

[5, 6, 7] Percentage of feed intake relative to the offered amount

Table 14. Ruminal pH, VFAs and NH_3-N of Korean cattle steers after 3 h of feeding fed either control or glycerol–supplemented (6.0% as feed additive) diet during winter through spring season.

Items	January 10		February 7		March 7		April 4		May 1		SE	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol		Diet	Month	Interaction
Temperature[1], ℃	-5.33		-6.38		1.03		17.71		24.38					
pH	6.54	6.35	6.50	6.67	6.25	6.39	6.61	6.49	6.63	6.58	0.48	0.54	0.32	0.75
NH_3-N, mg/dL	10.45	9.01	11.23	11.97	10.60	9.12	13.82	12.39	10.65	12.53	1.98	0.56	0.73	0.56
C2, mM	90.5	68.1	78.6	74.1	69.7	60.5	76.5	69.7	114.8	73.6	5.34	0.066	0.34	0.48
C3, mM	24.3	25.1	19.6	23.9	22.5	20.4	19.3	27.2	21.6	23.6	0.76	0.046	0.65	0.55
Iso C4, mM	0.98	0.80	0.82	0.75	0.66	0.61	0.83	0.66	0.90	0.84	0.03	0.03	0.93	0.71
C4, mM	18.9	19.2	16.9	19.3	14.5	14.7	14.6	17.1	17.1	19.4	0.52	0.088	0.39	0.54
Iso C5, mM	1.24	1.00	0.96	1.00	0.72	0.74	0.87	1.67	1.04	1.05	0.12	0.50	0.83	0.48
C5, mM	2.14	2.35	1.87	2.86	1.51	1.50	1.40	2.08	1.80	2.16	0.10	0.012	0.10	0.99
C2:C3 ratio	3.74	2.78	4.07	3.17	3.20	3.19	4.07	2.61	5.65	3.24	0.27	0.011	0.18	0.26
Total VFA, mM	138.0	116.6	118.8	121.9	109.5	98.5	113.5	118.4	157.3	120.7	5.74	0.22	0.47	0.62

N = 10/group.

1 THI of sampling time of each day.

Table 15. Serum parameters of Korean cattle steers before feeding fed either control or glycerol–supplemented (6.0% as feed additive) diet during winter through spring season.

Items	January 10		February 7		March 7		April 4		May 1		SE	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol		Diet	Month	Interaction
Glucose, mg/dL	97.7	99.7	101.4	100.4	116.4	105.1	84.4	75.5	84.3	89.4	2.01	0.43	<0.001	0.93
NEFA, mg/dL	468.6	293.2	194.9	189.1	303.7	286.0	254.6	136.8	187.0	114.0	27.01	0.091	0.007	0.83
Triglyceride, mg/dL	24.4	26.6	22.4	25.0	29.0	25.0	21.2	17.9	21.7	22.2	0.79	0.69	0.02	0.42
Cholesterol, mg/dL	122.7	130.1	118.0	120.4	138.4	149.4	123.1	123.3	136.1	138.8	3.65	0.53	0.30	0.79
HDL, mg/dL	89.9	91.3	92.6	91.4	102.0	104.7	90.1	85.2	93.8	93.1	1.79	0.80	0.97	0.73
GOT, IU/dL	84.3	76.3	73.9	74.0	88.4	79.1	66.9	79.6	70.9	86.5	2.88	0.45	0.82	0.096
GPT, IU/dL	27.9	27.1	26.6	25.3	32.1	31.3	26.2	26.1	27.8	29.9	0.58	1.00	0.51	0.34
Albumin, mg/dL	3.3	3.4	3.2	3.3	4.0	3.8	3.4	3.4	3.2	3.7	0.06	0.29	0.058	0.26

N = 10/group.

RPF = rumen protected fat, GOT = glutamic oxalacetic transaminase, GPT = glutamic pyruvate transaminase, HDL = high density li poprotein, LDL = low density lipoprotein, NEFA = non–esterified fatty acid.

6. REFERENCES

Birkelo, C. P., Johnson, D. E., & Phetteplace, H. P. (1991). Maintenance requirements of beef cattle as affected by season on different planes of nutrition. J Anim Sci, 69, 1214-22.

Broucek J, Kovalčuj K, Gajdogik D, Sottnik J, Brestensky V. The effect of extreme ambient temperatures on haematological and biochemical parameters of heifers. Vet Med 1987a;32:259–68.

Broucek J, Kovalčuj K, Gajdogik D, Brestensky V. Changes in biochemical indicators in dairy cows induced by extremely low temperatures. Vet Med 1987b;32:449–58

Castagnino PS, Dallantonia EE, Riorentini G, San Vito E, Messana JD, Lima LO, Simioni TA, Berchielli TT. Changes in ruminal fermentation and microbial population of feedlot Nellore cattle fed crude glycerin and virginiamycin. Anim Feed Sci Tech 2018;242:69-76.

Christopherson RJ, Kennedy PM. Effect of the thermal environment on digestion in ruminants. Can J Anim Sci 1983;63:417–96.

DeFrain JM, Hippen AR, Kalscheur KF, Jardon PW. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance J Dairy Sci 2018;87:4195-206.

Ferraro SM, Mendoza GD, Miranda LA, Gutierrez CG. In vitro ruminal fermentation of glycerol, propylene glycol and molasses combined with forages and their effect on glucose in insulin blood plasma concentrations after and oral drench in sheep. Anim feed Sci Tech 2016;213:74-80.

Fiorentini G, Messana JD, Jose Neto A, et al. Performance and meat quality of Nellore bulls fed crude glycerin combined with soybean oil. Anim Feed Sci Tech 2018;241:45-54.

Parsons GL, Shelor MK, Drouillard JS. Performance and carcass traits of finishing heifers fed crude glycerin. J Anim Sci 2009;87:653-57.

Godfrey RW, Smith SD, Guthrie MJ, Stanko RL, Neuendorff DA, Randel RD. Physiological responses of newborn bos lndicus and bos lndicus x bos taurus calves after exposure to cold. J Anim Sci 1991 69 258-263

Goonewardene LA, Berg RT, Hardin RT. A growth study of beef cattle. Can J Anim Sci 1981;61:1041–8.

Grzych M. 2010. Webinar Portal for Forestry and Natural Resources. http://www.forestrywebinars.net/webinars/planning–and–design–of–livestock–watering–systems/Cattle Stress Index Description Accessed Jun 26, 2014.

Gulliksen SM, Lie KI, Løken T, Østerås O. Calf mortality in Norwegian dairy herds. J Dairy Sci 2009;92:2782–95.

Gunn PJ, Schultz AF, Lake SL, et al. Effects of elevated crude glycerin concentrations on feedlot performance, carcass characteristics, and serum metabolite and hormone concentrations in finishing ewe and wether lambs. Prof Anim Sci 2010;26:298–306.

Hahn GL. Environmental influences on feed intake and performance of feedlot cattle. In: Proc. Symp. Intake by Feedlot Cattle; Oklahoma State Univ., Stillwater; 1995. p. 207–25.

Kang HJ, Piao MY, Lee IK, et al. Effects of ambient temperature and dietary glycerol addition on growth performance, blood parameters and immune cell populations of Korean cattle steers. Asian Australas J Anim Sci 2017;30:505–13.

Kang HJ, Piao MY, Baik M, et al. Effects of ambient temperature and rumen–protected fat supplementation on growth performance, rumen fermentation and blood parameters during cold season in Korean cattle steers. 2018. AJAS. In publish.

Kang HJ, Piao MY, Baik M, et al. Effects of hot temperature and rumen–protected fat supplementation on growth performance, rumen characteristics, and blood parameters in growing stage of Korean cattle steers. 2018, AJAS, in revision.

Kelly JM, Christopherson RJ. The apparent digestibility of dry matter, organic matter and nonammonia nitrogen in the forestomach, small intestine, and large intestine of weathers exposed to a cold environment. Can J Anim Sci 1989;69:911–9.

Kennedy PM, Christopherson RJ, Milligan LP. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Brit J Nutr 1976;36:231.

Kennedy PM, Milligan LP. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br J Nutr 1978;39:105–17.

Kennedy PM. Influences of cold exposure on digestion of organic matter, rates of passage of digesta in the gastrointestinal tract, and feeding and rumination behavior in sheep given four forage diets in the chopped, or ground and pelleted form. Br J Nutr 1985;53:159–73.

Krehbiel CR. Ruminal and physiological metabolism of glycerin. J Anim Sci 2008;86:E392 (Abstr.).

Ladeira MM, Carvalho JRR, Chizzotti ML, et al. Effect of increasing levels of glycerin on growth rate, carcass traits and liver gluconeogenesis in young bulls. Anim Feed Sci Tech 2016;219:241-48.

Lage JF, Berchielli TT, San Vito R, et al. Fatty acid profile, carcass and meat quality traits of young Nellore bulls fed crude glycerin replacing energy sources in the concentrate. Meat Sci 2014;96:1158-64.

Lee SY, Lee SM, Seo S, et al. Glycerol as a feed supplements for ruminants: In vitro fermentation characteristics and methane production. Anim feed sci tech 2011;166-167:269-74.

Mader TL. Environmental stress in confined beef cattle. J Anim Sci 2003;81:E110–9.

Mach N, Bach A, Devant M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. J Anim Sci 2009;87:632-38.

Nonnecke BJ, Foote MR, Horst RL, et al. Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves. J Dairy Sci 2009;92: 6134-43.

Park SJ, Beak SH, Baik M, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle — A review. Asian-Australas J Anim Sci 2018;31:1043–61.

Remond B, Souday R, Jouany JP. In vitro and in fermentation of glycerol by rumen microbes. Anim Feed Sci Technol 1993; 41:121–32.

Sauer FD, Erfle JD, Fisher LJ. Propylene glycol and glycerol as a feed additive for lactating dairy cows: an evaluation of blood metabolite parameters. Canadian J Anim Sci 1973;53:265-71.

Trabue S, Scoggin K, Tjandrakusuma S, Rasmussen MA, Reilly PJ. Ruminal Fermentation of Propylene Glycol and Glycerol. J Agric Food Chem 2007;55:7043−51.

Werner Omazic A, Kronqvist C, Zhongyan L, Martens H, Holtenius K. The fate of glycerol entering the rumen of dairy cows and sheep. J Anim Physiol Anim Nutr 2015;99:258-64.

Wolin MJ, Miller TL, Stewart CS. Microbe-microbe interactions. 2015. Pp 467-91 in The Rumen Microbial Ecosystem. 2nd ed. Hobson PN and Stewart CS, ed. Blackie Academic & Professional. London, UK.

Young BA. Cold stress as it affects animal production. J Anim Sci 1981;52:154–63.

Young BA. Temperature-Induced Changes in Metabolism and Body Weight of Cattle (Bss taurus). Can J Physiol Pharmacol 1975;53:947–53.

CHAPTER FOUR

Study 4

Effects of hot temperature and rumen–protected fat supplementation on growth performance, rumen characteristics, and blood parameters in growing stage of Korean cattle steers

*This study will be published in elsewhere as a fulfillment of Hyeokjoong Kang's Ph.D program

1. ABSTRACT

This study was performed to evaluate whether hot temperature and rumen– protected fat (RPF) supplementation affect growth performance, rumen characteristics, and serum metabolites in growing stage of Korean cattle steers. Twenty Korean cattle steers [230.4 ± 4.09 kg of BW, 10.7 ± 0.09 months of age] were divided into a conventional control diet group (n = 10) and a 0.8 % RPF supplementation group (n = 10). Steers were fed 1.5 % BW of a concentrate diet and 4 kg of a tall fescue hay for 16 weeks [July 10 to August 6 (P1), August 7 to September 3 (P2), September 4 to October 1 (P3), October 2 to 30 (P4), of 2015). Mean temperature–humidity index (THI) in P1 (76.8), P2 (76.3), and P3 (75.9) were higher ($P < 0.001$) than those in P4 (50.9), respectively. The mean THI in P1–3 were within the alert heat stress (HS) category range according to previously reported categories of feedlot cattle, and mean THI of P4 were under the thermo–neutral range. Neither month nor RPF supplementation affected (*P*

> 0.05) ADG and G:F ratio. Either month or RPF supplementation affected concentration of glucose, albumin, and high–density lipoprotein (HDL). Concentrations of albumin and glucose tended ($P < 0.10$) to decrease, but HDL was increased ($P < 0.01$) by RPF supplementation. Neither month nor RPF did not affect ruminal pH and NH_3–N and volatile fatty acid concentrations, whereas C2:C3 ratio was changed ($P < 0.05$) by month. Korean cattle during growing stage may not be significantly affected by alert HS, considering that growth performance of cattle was better at hotter months, although some changes in blood metabolites were observed. The RPF supplementation changed some blood lipid and carbohydrate metabolites, whereas it does not affect growth performance.

Keywords: Beef cattle, ambient temperature, heat stress, rumen–protected fat, growth, blood metabolites

2. INTRODUCTION

Animal productivity, (e.g. growth performance, milk yield, or meat quality) is affected by various factors, such as genetic, management, nutritional, and environment factors (Park et al., 2018). Heat stress (HS) is one of important stress type, which can negatively affect amount of dry matter intake (DMI), growth performance, feed efficiency (G:F ratio), overall health, milk production, or reproduction (Hahn, 1995; Mader et al., 1997). Measuring body temperature or respiration rate is the most appropriate way to determine if cattle have been exposed to HS in a hot environment, but it is not efficient method for commercial farms having large number of cattle (Mader et al., 2002; Mader, 2003). Therefore, an indicator for measuring the HS was developed, which is known as temperature–humidity index (THI) (LCI, 1970; Hahn, 1999). In general, HS can affect animal energy metabolism, which reduce metabolic heat production and maintains a normal body temperature (Hahn, 1983). These changes inevitably affect the energy metabolite of blood. In previous studies, some changes in blood parameters and reduced productivity were observed in a hot climatic condition in both of beef and dairy cattle (Kang et al., 2017; West, 2003). Changed metabolic status of cattle under HS condition is associated with hormonal imbalance (Beede and Collier, 1986). These chains of metabolic responses allow cattle to waste energy for maintaining homeostasis instead of weight gain or other production performance. In addition, HS can depress rumination, reticulo-rumen motility, and ruminal activity, which make slow the fractional rate of digesta passage in the gastrointestinal tract (Kadzere et al., 2002). Depressed rumen activity by HS especially reflect decreases in DMI and VFA production in the rumen (Kadzere et al., 2002; Kelly et al., 1967). To alleviate negative response for HS, providing additional energy

should be considered.

To decrease HS and cold stress and to increase animal productivity, several nutritional attempts including dietary fat supplementation have been tried (Hess et al., 2008; Kang et al., 2018; McNamara et al., 2003). Whereas, the ruminal activity is depressed by heat condition, therefore, addition of unprotected forms of fat may decrease rumen fermentation and may adversely affect rumen motility. Therefore, in this study, we used dietary rumen–protected fat (RPF) as a supplementary source of fat in concentrate diet, which may increase energy absorption in the small intestine, while minimizing fermentation of fat in the rumen, and effectively compensating for the increased maintenance energy in HS condition. Although the use of dietary RPF has been widely attempted in previous studies (McNamara et al., 2003; Hill and West, 1991), the effects of the RPF supplementation on growth performance, rumen characteristics, and blood parameters in growing stage of Korean cattle under HS condition are largely unknown.

3. MATERIALS AND METHODS

Animals and feeding trial

All experimental procedures involving animals were approved by the Seoul Natio nal University Institutional Animal Care and Use Committee (SNUIACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of the SNUIACUC. The study was conducted at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University, South

Korea.

In the feedlot trial, 20 Korean cattle steers with an average age of 10.7 ± 0.09 months and weight of 230.4 ± 4.09 kg were used. Steers had been fed commercial growing stage concentrate diet using an automatic feeding station (DeLaval Alpro system; DeLaval, Sweden) and timothy hay, following a conventional feeding program. Water was provided freely. During the 2-week adaptation period before the experiment, all animals were fed an experimental control concentrate (approximately 1.5 % BW per animal) and a timothy hay (5 kg / day / head). Steers were assigned to one of two treatments: the control group and RPF supplementation group. The RPF is prilled form of palm oil, as described (Naik, 2013), and purchased from Ecolex SDN. BHD (Pulau Indah, Selangor**,** Malyasia). The RPF was composed of 99.63% free fatty acids, including 85.48% palmitic acid (C 16:0), 7.05% oleic acid (C 18:1), 3.45% myristic acid (C 14:0), 1.64% linoleic acid (C 18:2), and 1.04% lauric acid (C 12:0), with an energy density of 9,316 kcal/kg (Haneol Corp., Anseong, Republic of Korea). Table 16 lists the formula and chemical compositions of the experimental diets. Steers were fed a concentrate diet (1.5 % BW) using an automatic feeding station and a timothy hay diet (5 kg/day) for 16 weeks [July 10 to August 6 (P1), August 7 to September 3 (P2), September 4 to October 1 (P3), and October 2 to 30 (P4)]. The daily feed intake of the concentrate was automatically recorded online using a computer with the DeLaval Alpro system. Equal amounts of roughage were provided twice daily (08:00 and 18:00) and residual roughage was weighed before the morning feeding. Concentrate and timothy hay samples were collected weekly and stored at –20 °C until analysis. BW was measured before morning feeding on the start day and at 4-week intervals thereafter.

Analysis of chemical composition

The chemical composition was measured using the same method, as described in Study 1.

Blood collection and measurement of ambient temperature

The blood sample was collected using the same method, as described in Study 1.

Ambient and climate temperatures, and relative humidity inside and outside the barn, were recorded in 1-h intervals using four HOBO data loggers (Onset Computer Corp., Bourne, MA, USA); and monthly average values of minimum, mean, and maximum temperatures, in combination with humidity, were calculated using daily data. From the automatic recording of the temperature and humidity data, the THI [modified from (Thom, 1959)] was calculated as previously described (Hahn, 1999). The experimental farm was covered with a roof and the animals were raised indoors and both side doors were closed. Therefore, the effects of rain, direct sunlight and wind could be discounted, and both humidity and temperature, which calculated as THI, were major factors in the climatic conditions.

Rumen fluid collection and analysis

The rumen fluid collection and analysis were followed the same method, as described in Study 1.

Blood analysis

The blood metabolites were measured using the same method, as described in Study 1.

Statistical analysis

The statistical analysis was followed the same method, as described in Study 1.

4. RESULTS AND DISCUSSION

Climate conditions

The mean (P1: 76.8, P2: 76.3, P3: 75.9) and maximum (P1: 81.8, P2: 81.3, P3: 80.3) indoor THI in July (P1), August (P2) and September (P3) were higher ($P < 0.001$) than those in October (P4; mean: 50.9, maxmimum: 63.7; Table 2). We also presented outdoor climate temperature to show outside climate conditions of experimental barn (Table 17). In a previous study, HS has been categorized as thermos-neutral (under 74), alert (75 to 78), danger (79 to 83), and emergency (over 84) under low wind-speed and solar radiation conditions in beef cattle (Hahn, 1999). Therefore, ambient mean THI of P1, P2 and P3 in this study were considered to represent alert HS conditions, but P4 was considered as thermo–neutral. We also measured the average temperature and relative humidity at blood and rumen fluid collection time (0800 to 1200), then calculated THI of each sampling day. The THIs were 78.3, 76.3, 68.6, 55.3, and 42.2 on July 10, August 7, September 3, October 1, and October 30, respectively (Table 19). The first two blood sampling days were classified as alert HS condition, while the other 3 days were considered as thermo-neutral conditions.

Growth performance

Body weight was higher ($P < 0.001$) during P4 compared with P1, reflecting animal age. The daily concentrate intake was higher ($P = 0.03$) during P4 compared to P1 (Table 18). In our study, the daily allowance of the concentrate was set at 1.5 % of BW, and was adjusted each month based on BW. Thus, the higher concentrate intake during P4 simply reflects the increased concentrate allowance corresponding to a higher BW during P4 compared to P1. Daily forage intake of P1 was lower than that of other months. Whereas percentage intake of concentrate, forage and total feed relative to the offered amount were lower ($P < 0.05$) in the hotter months, although RPF did not affect ($P > 0.05$) the percentage intake (Table 20). Additionally, there is no significant correlation between daily average temperature and daily DMI (data in supplementary table 1). Under hot condition, DMI is generally decreased (Hahn, 1995). RPF supplementation tended ($P = 0.07$) to decrease concentrate intake and decreased ($P < 0.01$) forage intake. In dairy study, the decreased DMI was observed by protected lipids supplementation (Bines et al., 1978). ADG and G:F ratio were higher ($P < 0.001$) during hotter months. RPF supplementation did not affect ($P > 0.05$) ADG and G:F ratio (Table 18). Considering decreased percentage of DMI and increased growth performance during hotter months, other factors rather than THI may affect growth performances. Other factors, such as heredity, diet and age, may affect the growth performance of cattle (Goonewardene et al., 1981). In this study, genetic factor or diet is similar among experimental months, but the age of month is obviously different by each period. Goonewardene et al. (1981) showed that highest growth rate about 0 to 300 age of days, since then, the growth rate decreases as the age increases. Thus, higher weight gain and better G:F ratio during hotter months may be due to age effect rather than THI effect in this study.

Rumen VFAs and NH_3–N

Ruminal pH was not changed by temperature or RPF supplementation. Also, the ruminal NH_3–N, and all VFA parameters (C2, C3, C4, iso–C4, C5, iso–C5, and total VFAs) were not changed ($P > 0.05$) by temperature or RPF supplementation (Table 19). C2:C3 ratio was lowest ($P < 0.05$) at experimental starting day with the highest THI (Table 19). Similarly, HS resulted in depression of the ruminal VFAs and C2:C3 ratio (Moody et al., 1967; Tajima et al., 2007). Depression of ruminal VFAs in hot conditions was result from a greater VFA metabolism and utilization from arterial blood (Martz et al., 1971).

Blood metabolites

Circulating glucose was lower ($P < 0.001$) at the hotter condition, and tended to be decreased ($P = 0.08$) by RPF supplementation (Table 20). Previous studies also reported lowered blood glucose concentration in high temperature conditions (Kang et al., 2017; O'brien et al., 2010). Decreased blood glucose at the hotter condition can be explained by altered hormonal homeostasis, such as insulin, or rapid utilization of blood glucose by increased respiratory rate due to duration of hyperthermia (O'brien et al., 2010; Hassan and Roussel, 1975). Indeed, glucose may become a primary energy source for heat–stressed cattle (Febbraio, 2001). Blood NEFA concentration was higher ($P < 0.001$) only on experimental starting day, and NEFA was not changed ($P > 0.05$) by RPF supplementation (Table 20). Blood NEFA concentrations in dairy cattle often increase when feed intake cannot support their energy requirements, requiring the mobilization of NEFA by lipolysis of fat depots to support energy demand (Bauman and Currie, 1980). Highest NEFA concentration with highest THI

at experimental starting day in this experiment may thus result from changed hormonal status (Beede and Collier, 1986), such as catecholamine and glucocorticoids that typically promote adipocyte lipolysis and NEFA mobilization (O'brien et al., 2010).

Blood HDL, TG, and cholesterol concentrations were lower ($P < 0.05$) in the hotter period compared with thermos–neutral period (Table 20). Similarly, our previous study showed that cholesterol and HDL concentrations were lower ($P < 0.05$) under high temperature conditions (Kang et al., 2017). Ronchi et al. (1999) suggested that lower blood cholesterol results from increased lipid utilization by peripheral tissues. Moreover, HS increases adipose tissue lipoprotein lipase (Criston, 1988), indicating adipose tissue of under heat stressed animals has an increased capacity to liberate fatty acids from circulating TGs for storage (Baumgard, 2012). Decreased HDL concentration under heat stressed beef cattle is not clear, while increased lipoprotein lipase may be a one of reasons. HDL concentration was increased ($P < 0.01$) by RPF supplementation (Table 20), but TG and cholesterol were not changed ($P > 0.05$) by RPF supplementation. Lee et al., (2003) reported that rumen–protected oleic acid in the diet increases blood HDL concentration, which may be derived from absorbed RPF in the small intestine.

Blood GPT concentrations were lower ($P < 0.05$) in the hotter period compared to thermo–neutral period, but those were not changed by RPF supplementation (Table 20). As with our study, heat exposed ewes showed lower level of GOT and GPT, probably as a result of the reduction of thyroid hormone secretion to decrease the endogenous body heat production (West, 1999; Caroprese et al., 2012). Little information is available for the effects of dietary fat supplementation on blood GOT and GPT in beef cattle. Blood albumin

concentration showed variation ($P < 0.001$) among months, and also tended to decrease ($P = 0.08$) by RPF supplementation (Table 20). Lowest blood albumin concentration in the hotter month (August 7) is similar with our previous Korean cattle study, which is result from physiological activity aimed at maintaining blood osmolality during hot conditions (Kang et al., 2017). The effect of RPF supplementation on serum albumin concentration in beef cattle is not clear.

5. CONCLUSION

Growth performance (ADG and G:F ratio) was not worsen during hotter months. Rumen fermentation parameters such as rumen pH, NH_3–N, and VFAs concentrations were not significantly affected by HS conditions and RPF supplementation, although C2:C3 ratio was changed by months. Blood parameters were more sensitive to HS condition than rumen parameters. Serum TG, cholesterol, HDL, and glucose were lower at hotter condition, and other serum parameters such as NEFA, GOT, and GPT were altered by months. Serum glucose, HDL, and albumin were changed by RPF supplementation. Thus, Korean cattle in growing stage may not be significantly affected by HS conditions in this study, although rumen fermentation and blood parameters were partially affected. The RPF supplementation did not affect the growth performance and major rumen VFA concentrations, although the RPF supplementation changed glucose and HDL concentrations. Further research is warranted to clarify HS conditions that significantly affect growth performance during growing stage of Korean cattle.

Table 16. Ingredient of concentrate and composition of experimental diets (RPF 0.8% supplemented diet) for Korean cattle steers during summer through fall season.

	Control concentrate	RPF concentrate
Ingredient of concentrate		
Ground corn	17.02	16.78
Wheat bran	20.00	20.00
Rice bran	5.00	5.00
Salt	0.37	0.37
Alfalfa–pellet	1.50	1.50
Molasses	3.80	3.80
Ammonium chloride	0.50	0.50
Palm meal	10.00	10.00
Condensed molasses soluble	1.50	1.50
Coconut meal	10.00	10.00
Distiller's dried grains with solubles (DDGS)	4.71	9.34
Live yeast	0.01	0.01
Corn flour	4.20	4.00
Soy hull	16.84	12.34
Rumen–protected fat	0.00	0.80
Limestone	3.04	3.06
Antimicrobial agent	0.05	0.05
Barley stone	1.20	0.69
Mineral/Vitamin premix[1]	0.26	0.26
Total	100.00	100.00
Chemical composition, % DM		
Concentrate		
DM	89.44	89.25
CP	15.00	15.00
Ether extract (EE)	4.10	5.23

Ash	9.47	8.83
NDF	24.43	24.23
ADF	35.91	35.11
Non fiber carbohydrate (NFC)	30.98	30.75
Ca	1.05	0.95
P	0.40	0.48
TDN[2] %	70.39	72.50
ME[3], Mcal/kg	2.69	2.79
NE[4], Mcal/kg	1.15	1.22

Timothy hay, % DM	
DM	90.63
CP	6.06
Ether extract (EE)	1.55
Ash	5.89
Ca	0.35
P	0.19
ADF	66.76
NDF	38.50

[1] Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D_3 (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic–DC). Grobic–DC was provided by Bayer Health Care (Leverkusen, Germany).

[2] TDN (%) = NFC + CP + [(EE – 1) x 2.25] + NDF – 7 [NRC, 2007]

[3] ME = [1.01 x (DE) − 0.45] + 0.0046 x (EE−3) [NRC, 2007].

[4] NE = 1.42 ME − 0.174 ME^2 + 0.0122 ME^3 − 1.65 [NRC, 2007].

RPF = rumen-protected fat.

Table 17. Mean, maximum, and minimum values of ambient temperatures, climate temperatures, relative humidity, and temperature–humidity index[1] during summer through fall season.

Items	July (P1)[2]	August (P2)[3]	September (P3)[4]	October (P4)[5]	SE	P-value
Ambient temperature, °C						
Mean	24.9[a]	23.4[a]	18.4[b]	9.8[c]	0.55	<0.001
Maximum	30.8[a]	32.9[a]	28.8[ab]	19.8[c]	0.65	<0.001
Minimum	21.3[a]	18.1[b]	11.7[c]	3.75[d]	0.66	<0.001
Climate temperature, °C						
Mean	24.2[a]	21.4[b]	16.7[c]	10.4[d]	0.57	<0.001
Maximum	30.2[a]	28.1[a]	24.9[b]	17.5[c]	0.63	<0.001
Minimum	20.6[a]	17.0[b]	11.0[c]	5.3[d]	0.64	<0.001
Ambient Relative humidity, %						
Mean	100.4[a]	67.0[b]	71.3[b]	72.7[b]	2.41	<0.001
Maximum	120.3[a]	97.9[b]	102.3[b]	99.9[b]	1.68	<0.001
Minimum	71.4[a]	68.0[a]	64.8[ab]	30.4[b]	3.90	<0.001
Climate Relative humidity, %						
Mean	102.1[a]	102.8[a]	97.4[b]	83.0[c]	1.33	<0.001
Maximum	122.9[a]	127.6[a]	127.6[a]	106.6[b]	1.14	0.003
Minimum	70.9[a]	71.1[a]	72.3[a]	49.8[b]	1.95	<0.001
Ambient THI						
Mean	76.8[a]	76.3[a]	75.9[a]	50.9[b]	0.94	<0.001
Maximum	81.8[a]	81.3[a]	80.9[a]	63.7[b]	0.83	<0.001
Minimum	72.0[a]	71.5[a]	71.1[a]	38.9[b]	1.25	<0.001
Climate THI						
Mean	70.9[a]	71.1[a]	72.3[a]	51.4[b]	1.01	<0.001
Maximum	81.1[a]	80.7[a]	80.3[a]	61.6[b]	0.86	<0.001
Minimum	70.6[a]	70.1[a]	69.8[a]	40.9[b]	1.28	<0.001

[a-d] Mean values with different letters within same row differ ($P < 0.05$).

[1] Temperature–humidity index = THI = 0.8×temperature + [(relative humidity×0.01) × (temperature−14.4)] + 46.4 (Hahn, 1999).

[2] July 10 to August 6 (4 weeks). [3] August 7 to September 3 (4 weeks). [4] September 4 to October 1 (4 weeks). [5] October 2 to October 30 (4 weeks).

Table 18. Growth performance of growing Korean cattle steers fed either control or RPF–supplemented (0.8%) diet during summer through fall season.

Items	July[1]		August[2]		September[3]		October[4]		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
Age, month	10.6	10.7	11.5	11.6	12.5	12.6	13.4	13.5	0.35	< 0.001	0.87	
Initial body weight, kg	231.3	229.4	259.1	257.4	288.6	282.7	300.2	294.2	2.59	< 0.001	0.45	0.68
Body weight, kg	259.1	257.4	288.6	282.7	300.2	294.2	319.7	312.5	2.67	< 0.001	0.51	0.34
Daily feed intake[3], kg: DM base												
Total feed intake, kg/d	6.93	6.84	7.48	7.36	8.00	7.80	8.32	8.03	0.29	<0.001	0.46	0.76
Percentage of total feed intake[5], %	77.0	77.0	75.6	74.9	87.1	85.6	87.5	85.2	1.24	<0.001	0.62	0.72
Concentrate intake, kg/d	3.18	3.13	3.58	3.59	3.93	3.79	4.22	4.05	0.24	<0.001	0.07	0.34
Percentage of concentrate intake[6], %	85.4	84.7	75.4	75.7	93.8	92.7	93.8	91.9	1.76	0.009	0.81	0.86
Forage intake, kg/d	3.76	3.72	3.90	3.77	4.08	4.01	4.09	3.98	0.08	0.011	0.0014	0.09
Percentage of forage intake[7], %	71.5	71.8	75.7	74.0	81.6	80.2	81.9	79.6	1.46	0.011	0.66	0.77
Average daily gain, kg/d	0.99	1.00	1.05	0.90	0.75	0.76	0.70	0.65	0.01	<0.001	0.93	0.02
G:F ratio	0.144	0.147	0.142	0.123	0.094	0.099	0.085	0.083	0.0003	<0.001	0.42	0.03

N = 10/group. RPF = rumen–protected fat

[1] July 10 to August 6 (4 weeks). [2] August 7 to September 3 (4 weeks). [3] September 4 to October 1 (4 weeks). [4] October 2 to October 30 (4 weeks).

[5, 6, 7] Percentage of feed intake relative to the offered amount

Table 19. Ruminal pH, VFAs and NH_3-N of Korean cattle steers after 3 h of feeding fed either control or RPF–supplemented (0.8%) diet during summer through fall season.

Items	July 10		August 7		September 4		October 2		October 30		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
THI[1]	78.3		76.3		68.6		55.3		42.2					
pH	6.88	7.02	6.74	7.10	6.84	6.54	6.73	6.86	6.64	6.50	0.05	0.65	0.78	0.70
NH_3-N, mg/dL	11.7	12.7	8.60	8.66	11.8	10.2	10.9	9.91	8.48	9.08	0.51	0.50	0.59	0.77
C2, mM	67.3	77.2	66.3	65.4	58.6	59.9	69.8	60.5	69.7	65.3	1.44	0.32	0.39	0.82
C3, mM	25.7	27.2	19.3	15.4	17.2	22.2	19.2	18.7	26.9	20.4	0.69	0.54	0.62	0.73
Iso C4, mM	0.52	0.64	0.53	0.51	0.66	0.87	0.67	0.72	0.67	0.70	0.03	0.90	0.60	0.72
C2:C3 ratio	2.62	2.84	3.44	4.25	3.41	2.70	3.64	3.24	2.59	3.20	0.08	0.04	0.54	0.48
C4, mM	15.5	11.9	11.9	10.8	12.1	11.9	13.1	12.0	10.5	11.4	0.40	0.46	0.55	0.64
Iso C5, mM	0.80	0.89	1.21	0.82	0.99	0.84	0.76	0.65	0.84	1.09	0.04	0.44	0.29	0.37
C5, mM	1.54	1.22	1.07	1.60	1.14	0.95	1.17	1.44	1.06	1.17	0.02	0.81	0.60	0.45
Total VFA, mM	111.4	119.1	100.3	94.5	90.7	96.7	104.7	94.0	109.7	100.1	1.79	0.57	0.84	0.64

N = 10/group. RPF = rumen–protected fat

[1] THI = Temperature–humidity index = 0.8×temperature + [(relative humidity×0.01) × (temperature−14.4)] + 46.4 (Hahn, 1999).

[1] THI of sampling time of each day.

Table 20. Serum parameters of Korean cattle steers before feeding fed either control or RPF–supplemented (0.8%) diet during summer through fall season.

Items	July 10		August 7		September 3		October 1		October 30		SE	P–value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Month	Diet	Interaction
Glucose, mg/dL	65.4	63.3	69.7	68.6	72.5	66.5	81.1	77.1	82.3	79.0	0.95	<0.001	0.08	0.72
NEFA, mg/dL	526.5	393.8	246.9	196.9	265.3	241.6	255.1	253.9	258.3	302.7	13.9	<0.001	0.17	0.03
Triglyceride, mg/dL	14.4	13.5	16.3	16.3	15.8	15.8	20.2	16.1	17.2	16.8	0.40	0.017	0.27	0.70
Cholesterol, mg/dL	99.6	111.8	110.6	100.0	125.3	136.1	137.3	142.2	140.4	152.9	0.68	<0.001	0.14	0.72
HDL, mg/dL	65.8	77.7	68.6	69.0	78.1	88.5	83.4	92.0	89.7	94.7	0.98	<0.001	0.006	0.67
GOT, IU/dL	75.1	72.1	74.6	62.3	81.2	69.5	78.2	75.1	77.4	67.9	1.25	0.005	0.56	0.77
GPT, IU/dL	21.9	23.5	24.1	24.7	28.1	28.5	27.7	30.4	27.2	26.4	0.37	<0.001	0.31	0.72
Albumin, mg/dL	3.30	3.23	3.03	2.76	3.35	3.30	3.56	3.47	3.55	3.37	0.02	<0.001	0.08	0.81

N = 10/group.

RPF = rumen–protected fat, GOT = glutamic oxalacetic transaminase, GPT = glutamic pyruvate transaminase, HDL = high density lipoprotein, LDL = low density lipoprotein, NEFA = non–esterified fatty acid.

6. REFERENCES

Baines JA, Brumby PE, Storry JE, Fulford RJ, Braithwaite GD. The effect of protected lipids on nutrient intakes, blood and rumen metabolites and milk secretion in dairy cow during early lactation. J Agric Sci Camb 1978;91:135–50.

Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci 1980;63:1514–29.

Baumgard LH. Ruminant nutrition symposium: ruminant production and metabolic responses to heat stress. J Anim Sci 2012;90:1855–65.

Beede DK, Collier RJ. Potential nutritional strategies for intensively managed cattle during thermal stress. J Anim Sci 1986;62:543–54.

Caroprese M, Albenzio M, Bruno A, et al. Effects of shade and flaxseed supplementation on the welfare of lactating ewes under high ambient temperatures. Small Ruminant Res 2012;102:177–85.

Christon R. 1988. The effect of tropical ambient temperature on growth and metabolism in pigs. J Anim Sci 1988;66:3112–23.

Febbraio MA. Alterations in energy metabolism during exercise and heat stress. Sports Med 2001;31:47–59.

Goonewardene LA, Berg RT, Hardin RT. A growth study of beef cattle. Can J Anim Sci 1981;61:1041–8.

Hahn GL. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999;77:10–20.

Hahn GL. Environmental influences on feed intake and performance of feedlot cattle. In: Proc. Symp. Intake by Feedlot Cattle; Oklahoma State Univ., Stillwater; 1995. p. 207–25.

Hahn GL. Management and housing of farm animals in hot environment. in Stress Physiology of Livestock. Vol. 2, Boca Raton, FL; 1983. p. 151–73.

Hassan A, Roussel JD. Effect of protein concentration in the diet on blood composition and productivity of lactating Holstein cows under thermal stress. J Agric Sci Camb 1975;85:409–15.

Hess BW, Moss GE, Rule DC. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J Anim Sci 2008;86:E188–204.

Hill GM, West JW. Rumen protected fat in Kline barley or corn diets for beef cattle: digestibility, physiological, and feedlot responses. J Anim Sci 1991;69:3376–88.

Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows — a review. Livest Prod Sci 2002;77:59–91.

Kang HJ, Piao MY, Lee IK, et al. Effects of ambient temperature and dietary glycerol addition on growth performance, blood parameters and immune cell populations of Korean cattle steers. Asian Australas J Anim Sci 2017;30:505–13.

Kang HJ, Piao MY, Park SJ, et al. Effects of ambient temperautre and rumen-protected fat supplementation on growth performance, rumen fermentation, and blood parameters during cold season in Korean cattle steers. Asian Australas J Anim Sci;2018:Submitted.

Kelly RO, Martz FA, Johnson HD. Effects of environmental temperature on ruminal VFA levels with controlled feed intake. J Dairy Sci 1967;50:531–3.

Lee H-J, Lee SC, Oh YG, et al. Effects of rumen protected oleic acid in the diet on animal performances, carcass quality and fatty acid composition of Hanwoo steers. Asian Australas J Anim Sci 2003;16:1003–10.

Livestock Consevation Institute (LCI). Patterns of transit losses. Omaha, NE. 1970.

Mader TL, Fell LR, McPhee MJ. Behavior response of non–Brahman cattle to shade in commercial feedlots. In Proc. 5th Int. Livestock Environment Symp. Amer. Soc. Agric. Eng., St. Joseph, MI; 1997. p. 795–802.

Mader TL, Holt SM, Hahn GL, Davis MS, Spiers DE. Feeding strategies for managing heat load in feedlot cattle. J Anim Sci 2002;80:2373–82.

Mader TL. Environmental stress in confined beef cattle. J Anim Sci 2003;81:E110–9.

Martz FA, Mishra M, Campbell JR, Daniels LB, Hilderbrand E. Relation of ambient temperature and time postfeeding on ruminal, arterial and venous volatile fatty acids, and lactic acid in Holstein steers. J Dairy Sci 1971;54:520–5.

McNamara S, Butler T, Ryan DP, et al. Effect of offering rumen-protected fat supplements on fertility and performance in spring–calving Holstein–Friesian cows. Anim Reprod Sci 2003;79:45–56.

Moody EG, Van Soest PJ, McDowell RE, Ford GL. Effect of high temperature and dietary fat on performance of lactating cows. J Dairy Sci 1967;50:1909–19.

Naik PK. Bypass fat in dairy ration – A review. Anim Nutr Feed Techol 2013;13:147–63.

NRC. Nutrient requirements of dairy cattle. 8th rev ed. Washington, DC: National Academy Press; 2007.

O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH. Metabolic adaptations to heat stress in growing cattle. Domest Anim Endocrinol 2010;38:86–94.

Park SJ, Beak SH, Jung DJS, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle — a review. Asian Australas J Anim Sci 2018;31:1043–61.

Ronchi B, Bernabucci U, Lacetera N, Verini Supplizi A, Nardone A. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. Zootech Nutr Anim 1999;25:11–20.

Tajima K, Nonaka I, Hihuchi K, et al. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 2007;13:57–64.

Thom EC. The discomfort index. Weatherwise. 1959; 12:57–9.

West JW. Effects of heat-stress on production in dairy cattle. J Dairy Sci 2003;86:2131–44.

West JW. Nutritional strategies for managing the heat stressed dairy cow. J Anim Sci 1999;77:21–35.

Study 5

Effects of temperature and glycerol supplementation on growth performance, rumen characteristics, and blood parameters during summer season in fattening stage of Korean cattle steers

*This study will be published in elsewhere as a fulfillment of Hyeokjoong Kang's Ph.D program

1. ABSTRACT

This study was performed to evaluate whether hot temperature and glycerol supplementation affect growth performance, rumen characteristics, and serum metabolites in fattening stage of Korean cattle steers. Twenty Korean cattle steers [466.9 ± 10.02 kg of BW, 23.5 ± 0.07 months of age] were divided into a conventional control diet group (n = 10) and a 3.0 % glycerol supplementation group (n = 10). Steers were fed 1.6 % BW of a concentrate diet and 1.5 kg of a rice straw for 12 weeks [July 5 to August 2 (P1), August 3 to August 30 (P2), August 31 to September 26 (P3), of 2016]. Mean temperature–humidity index (THI) in P1 (75.02) and P2 (77.50) were higher ($P < 0.001$) than that in P3 (67.63), respectively. The mean THI in P1 and P2 were within the alert heat stress (HS) category range according to previously reported categories of feedlot cattle, and mean THI of P3 was under the thermo-neutral range. Month did not affect ($P < 0.05$) ADG and G:F ratio, while glycerol supplementation improved ADG

(P = 0.056) and G:F ratio (P < 0.05). Ruminal pH and NH_3–N were not changed by both month and glycerol. Ruminal C2:C3 ratio was generally higher (P = 0.051) in hotter month, whereas C5 concentration was lower (P < 0.001) in hotter months. Ruminal C2:C3 ratio was increased (P < 0.001) by glycerol supplementation, whereas C5 concentration was decreased (P < 0.05) by glycerol supplementation. The concentrations of ruminal C2, C3, C4, C5, and total VFA were lowered (P < 0.05) in hotter condition. C5 was increased (P < 0.05) by glycerol supplementation. In the hotter month, blood cholesterol was lower (P < 0.05) than those in other periods. Glycerol supplementation increased (P < 0.05) blood cholesterol and high-density lipoprotein. Neither month nor glycerol affected (P > 0.05) blood concentrations of glucose, non-esterified fatty acid, and triglyceride. Korean cattle may not be significantly affected by alert HS, considering that the growth performance of cattle remained unchanged, although variations in blood and ruminal parameters were observed among the studied months. Glycerol supplementation improved growth performances with changed blood cholesterol and HDL concentrations and ruminal C2:C3 ratio.

Keywords: Beef cattle, ambient temperature, heat stress, glycerol, growth, blood metabolites, rumen parameters

2. INTRODUCTION

Heat stress (HS) can negatively affect the amount of dry matter intake (DMI), growth performance, feed efficiency (G:F ratio), overall health, milk production, or reproduction (Hahn, 1995; Mader et al., 1997). In general, these negative effects are the result from animal energy metabolism changed, such as hormonal change including catecholamine or glucocorticoids, and reduced metabolic heat production to maintain a normal body temperature (Beede and Collier, 1986; Hahn, 1995). Accordingly, these changes of metabolic responses allow cattle to waste energy for maintaining homeostasis instead of weight gain or other production performance (Kang et al., 2017; West, 2003). Depressed in rumination, reticulorumen motility, and ruminal activity were observed in hot climate condition which make slow the fractional rate of digesta passage in the gastrointestinal tract and decrease in DMI and VFA production in the rumen (Kadzere et al., 2002, Kelly et al., 1967). In our previous study of Korean cattle steers, some changes in blood metabolites and ruminal parameters were observed in hot climate (Kang et al., in revision).

Animals respond differently to hot condition, depending on the level of hotness. Previous studies have shown that how temperature and humidity contribute to heat stress. For this, temperature humidity index (THI) was developed to numerically represent heat stress level (LCI, 1970; Hahn, 1999). To alleviate negative response for HS, providing additional energy should be considered.

In the hot condition, lowered blood glucose is caused by altered hormonal homeostasis, such as insulin, or rapid utilization of blood glucose by increased respiratory rate due to the duration of hyperthermia (O'brien et al., 2010; Hassan and Roussel, 1975). Indeed, glucose

may become a primary energy source for heat–stressed cattle (Febbraio, 2001). Therefore, excessive use of glucose in the heat stress condition can be considered as an important factor affecting the productivity of the animal. Therefore, we tried to reduce the heat stress by adding glycerol in the concentrate, which can be used as a precursor of glucose. Glycerol potentially serves as a gluconeogenic substrate for ruminants (Chung et al., 2007). In the rumen, glycerol can be fermented to C3 that can act as a glucogenic precursor in ruminants. Absorbed glycerol into rumen epithelium can be converted to glycerol-3-phosphate via phosphorylation, then enters gluconeogenesis in the liver (Krehbiel, 2008). There have been several experiments to evaluate the effect of glycerol in heat stress situations in dairy cattle, and positive effects on milk fat and digestibility have been reported (Liu et al., 2014; Boyd et al., 2010). However, little information of the effect of glycerol was not evaluated in the hot condition in beef cattle, especially in Korean cattle steers. Thus, the purpose of this experiment is to evaluate the effects of the ambient temperature and glycerol supplementation on growth performance, rumen characteristics, and blood parameters in the fattening stage of Korean cattle during summer season.

3. MATERIALS AND METHODS

Animals and feeding trial

All experimental procedures involving animals were approved by the Seoul Natio nal University Institutional Animal Care and Use Committee (SNUIACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of

the SNUIACUC. The study was conducted at the University Animal Farm of the College of Agriculture and Life Sciences, Pyeongchang Campus of Seoul National University, South Korea.

Twenty Korean cattle steers with an average age of 23.5 ± 0.07 months and weight of 467 ± 10.0 kg were used. Steers had been fed commercial growing stage concentrate diet using an automatic feeding station (DeLaval Alpro system; DeLaval, Sweden) and rice straw, following a conventional feeding program. Water was provided freely. During the 2-week adaptation period before the experiment, all animals were fed an experimental control concentrate (approximately 1.6 % BW per animal) and a rice straw (1.5 kg/day/head). Steers were assigned to one of two treatments: the control group and the glycerol supplementation group. Crude glycerol inclusion level (3.0%) was chosen, based energy value of crude glycerol and proper pellet quality: inclusion of more than 5% glycerol reduced pellet quality due to its viscosity and decomposition. The glycerol-supplemented diet was made by adding 99.7% purified glycerol (Palm Oleo SDN. BHD., Selangor, Malaysia) adsorbed ground wheat to give final 3% glycerol during the pelleting process of a concentrate. Table 21 lists the formula and chemical compositions of the experimental diets. Steers were fed a concentrate diet (1.6 % BW) using an automatic feeding station and a rice straw (1.5 kg/day) for 12 weeks [July 5 to August 2 (P1), August 3 to August 30 (P2), August 31 to September 26 (P3), of 2016]. The daily feed intake of the concentrate was automatically recorded online using a computer with the DeLaval Alpro system. Equal amounts of roughage were provided twice daily (08:00 and 18:00) and residual roughage was weighed before the morning feeding. Concentrate and rice straw samples were collected weekly and stored at –20 °C until analysis.

BW was measured before morning feeding on the start day, at 4-week intervals thereafter.

Analysis of chemical composition

The chemical composition was measured using the same method, as described in Study 1.

Blood collection and measurement of ambient temperature

The blood sample was collected using the same method, as described in Study 1.

Ambient and climate temperatures and relative humidity inside and outside the barn, were recorded in 1-h intervals using four HOBO data loggers (Onset Computer Corp., Bourne, MA, USA). Minimum, mean, and maximum temperatures and corresponding relative humidity data were chosen at every day, and monthly data were average values of 28 days per month. From the automatic recording of the temperature and humidity data, the THI (modified from Thom, (1959)) was calculated as previously described (Hahn 1999). The experimental farm was covered with a roof and the animals were raised indoors and both side doors were closed. Therefore, the effects of rain, direct sunlight and wind could be discounted, and both humidity and temperature, which calculated as THI, were the major factors in the climatic conditions.

Rumen fluid collection and analysis

The rumen fluid collection and analysis were followed the same method, as described in Study 1.

Blood analysis

The blood metabolites were measured using the same method, as described in Study 1.

Statistical analysis

The statistical analysis was followed the same method, as described in Study 1.

4. RESULTS AND DISCUSSION

Climate conditions

The mean (P1: 75.0, P2: 77.5) indoor THI in July (P1) and August (P2) were higher ($P < 0.001$) than those in September (P3: 66.8, Table 22). In a previous study, HS has been categorized as thermo-neutral (under 74), alert (75 to 78), danger (79 to 83), and emergency (over 84) under low wind-speed and solar radiation conditions in beef cattle (Hahn, 1999). Therefore, ambient mean THI of P1 and P2 in this study were considered to represent alert HS conditions, but P3 was considered as thermoneutral. Compared to our previous experiment during 2015 summer, this summer condition is less hot and short. The mean THI of July, August, and September of 2015 (76.8, 76.3, and 75.9, respectively) were classified as alert HS (Kang et al., in revision), while in this experiment, only two months (P1 to P2: July to August) were under alert HS condition. We also measured the average temperature and relative humidity at blood and rumen fluid collection time (0800 to 1200), then calculated THI of each sampling day. The THIs were 75.2, 76.7, 65.6, and 53.5 on July 5, August 2,

August 30, and September 26, respectively (Table 24). The first two blood sampling days were classified as alert HS conditions, while the other two days were considered as thermo-neutral conditions. In this study, ambient THI was slightly lower than previous study (78.3, 76.3, 68.6, and 55.3 on July 10, August 7, September 3, and October 1, of 2015, respectively), but it is in a similar HS category (Kang, 2018 Submitted; summer RPF supple).

Growth performance

No interaction between month and diet was observed for all items of growth performance and feed intake, rumen and blood parameters (Tables 18, 19, and 20). Body weight was higher ($P < 0.001$) during P3 compared with P1, reflecting animal age. Forage and concentrate intake was not changed ($P > 0.05$) by month and glycerol supplementation (Table 23). However, only in the P2, daily average THI showed negative correlation with daily DMI ($P < 0.05$; data in supplementary table 1), which may indicate that high THI condition affect daily DMI. In our study, the daily allowance of the concentrate was set at 1.6 % of BW and was adjusted each month based on BW. In our previous study during summer season (with 10.6 month of age and 230.4 kg of BW of Korean cattle steers), the amount of concentrate and forage intake and growth performance (ADG and G:F ratio) were decreased in hotter months (Kang, in revision). THI condition in this experiment was lower and shorter than those of previous study (Kang, in revision). Also, older aged cattle are more resistant to HS. Thus, the HS level of in this experiment may not significantly affect the feed intake and growth performance.

Glycerol supplementation improved ADG ($P = 0.056$) and G:F ratio ($P = 0.03$) (Table 18). Parsons et al. (2009) reported that glycerol supplementation can have positive effects on

ADG, and G:F ratio when included at less than 10% of livestock diets. However, negative effects on ADG and feed efficiency were also observed when glycerol was included in finishing heifer diets at 16% of DM (Parsons et al., 2009).

Rumen VFAs and NH3–N

Ruminal pH and NH_3-N were not changed ($P > 0.05$) by temperature or glycerol supplementation. The ruminal C2, C3, C4, C5, and total VFA were lower ($P < 0.05$) in hotter months (Table 24). Similarly, HS resulted in depression of the ruminal VFAs and C2:C3 ratio (Moody et al., 1967; Tajima et al., 2007). Depression of ruminal VFAs in hot conditions may result from a greater VFA metabolism and utilization from arterial blood (Martz et al., 1971). Collier et al. (1982) suggested that decreases in forage intake in heat stress condition contribute to decreased VFA production and may contribute to alteration in C2:C3 ratio, but in this experiment, amount of forage intake was not observed, thus VFA depression in this experiment may be explained by combined effects of heat stress on ruminal buffering and efficiency of microbial VFA production (West, 1999). Glycerol supplementation decreased ($P < 0.05$) ruminal C2:C3 ratio and increased ruminal C5 and iso-C5 concentrations ($P < 0.05$; Table 19). The C2 concentration was tended to decrease ($P = 0.06$) by glycerol supplementation. Other ruminal VFAs (C3, C4, and iso-C4) were not changed ($P > 0.05$) by glycerol supplementation (Table 24). Lee et al. (2011) reported that decreased C2 concentration, and increased C3 concentration in their *in vitro* experiment. Consistent with our results, decreased C2:C3 ratio was observed (Lee et al., 2011). In this study, decreased C2 trends may affect decreased C2:C3 ratio. DeFrain et al. (2004) reported an increased C3

production and decreased the C2:C3 ratio in the rumen. Trabue et al. (2007) reported that a large portion of glycerol was fermented to C3, although fermentation of glycerol also produced some C5.

Blood metabolites

Circulating glucose and NEFA were not changed ($P > 0.05$) by month or glycerol supplementation (Table 25). Compared to our previous study that showed lower glucose level in hotter month (Kang et al., 2017), in this experiment, the glucose concentration was not changed by temperature. Similary, O'brien et al. (2010) also reported that no alteration of glucose level in hot condition. In this study, animal age is older than the previous experiment, also showed relatively lower THI than previous study. Therefore, cattle in this study may be more resistance to the HS, or the lower THI may not affect blood glucose concentration. As similar to our result, Sauer et al. reported that blood glucose and NEFA were not changed with 3.6 % of glycerol supplementation. DeFrain et al. (2004) failed to show any glucogenic effect of glycerol. Similarly, Terré et al. (2011) and Mach et al. (2009) observed no differences in plasma glucose concentrations when glycerol included up to 10 % or 12 % DM in high-concentrate diets fed to finishing lambs or Holstein bulls, respectively. Ronchi et al. (1999) and Rhoads et al. (2009) reported that NEFA was not changed by hot climate conditions. But, other researchers reported that basal plasma NEFA levels are typically reduced in heat-stressed cattle (Shwartz et al., 2009). Possible explanation of the the lack of an increase in basal NEFA levels in heat-stressed caws may be the increased basal and stimulated insulin levels as a potent antilipolytic hormone (Vernon, 1992).

Blood cholesterol was lower ($P < 0.05$) in the hotter period compared with thermoneutral period (Table 25). Other blood lipid parameters, including TG, and HDL were not changed ($P > 0.05$) by month (Table 25). Similarly, our previous study showed that cholesterol and HDL concentrations were lower ($P < 0.05$) under high-temperature conditions (Kang et al., in revision). Ronchi et al. (1999) suggested that lower blood cholesterol results from increased lipid utilization by peripheral tissues. Blood cholesterol and HDL concentration were increased ($P < 0.05$) by glycerol supplementation, whereas TG concentration remains unchanged ($P > 0.05$, Table 25). There is little information on the effect of glycerol supplementation on blood HDL and cholesterol in Korean cattle steers, and the reason for the elevated HDL and cholesterol by glycerol supplementation is unknown. In the previous study, glycerol supplementation did not alter TG and cholesterol (Adamski et al., 2011). Clariget et al. (2016) reported that plasma concentrations of total protein and albumin were not affected when cows were fed diets supplemented glycerol.

Blood GPT was higher ($P < 0.05$) in the hotter months compared with thermo-neutral months, whereas GOT was not changed ($P > 0.05$) by months (Table 25). In Korean cattle calves, increased GOT and GPT levels under hot condition was observed (Kim et al., 2018). In the previous study, GOT and GPT levels as liver damage markers were increased in hot condition in human (Panteghini, 1990). Blood GOT was increased ($P < 0.05$) by glycerol supplementation, whereas GOT was not changed ($P > 0.05$) by glycerol supplementation. In the previous study, GOT and GPT were also not changed by glycerol supplementation in Simmental cow (Adamski et al., 2011). Little information is available on the effects of glycerol supplementation on blood GOT and GPT in beef cattle.

Neither month nor glycerol supplementation affect ($P > 0.05$) blood albumin, calcium, and phosphate concentrations. Our previous studies, blood calcium, phosphate, and albumin concentration were higher in hotter condition (Kang et al., 2017; Kang et al., in revision; Table 26, Figure 1). One study suggested that calcium and magnesium bound to serum protein, therefore, albumin concentration may tend to affect the concentration of those minerals (Payne et al., 1974). Therefore, in this study, both blood minerals and albumin were not changed. Adamski et al. (2011) reported that glycerol supplementation did not change albumin concentration. The effect of glycerol supplementation on serum albumin, calcium, and phosphate concentrations in beef cattle remains unclear.

5. CONCLUSION

Growth performance (ADG and G:F ratio) did not worsen during hotter months, but glycerol supplementation improved the growth performance throughout experimental periods. Lowered ruminal C2, C3, C4, C5 and total VFAs in the hotter months were observed. The C2:C3 ratio was decreased by glycerol supplementation, whereas Iso-C5 and C5 concentrations were increased by glycerol supplementation. Serum cholesterol was lower at the hotter months. Serum cholesterol and HDL were increased by glycerol supplementation. Korean cattle in fattening stage may not be significantly affected by HS conditions in this study, considering no decrease of DMI and growth performance, although rumen fermentation and blood parameters were partially affected. The glycerol supplementation improved the growth performance, along with decreased ruminal C2:C3 ratio and increased iso-C5 and C5

without changes in serum glucose and NEFA concentrations. Further research is warranted to clarify HS conditions that significantly affect growth performance during fattening stage of Korean cattle.

Table 21. Ingredients of concentrate and composition of experimental diets (glycerol 3.0% supplemented diet) for Korean cattle steers during summer through fall season.

	Control concentrate	Glycerol concentrate
Ingredients of concentrate		
Ground corn	3.99	5.52
Wheat flour	15.22	17.84
Wheat bran	2.14	1.56
Rice bran	10	15
Cotton seed Hull	5	5
Salt	0.38	0.36
Urea	0.34	0.35
Molasses	3.8	0.8
Ammonium chloride	0.5	0.5
Palm meal	12.31	2
Condensed molasses soluble	0.8	0.8
Soybean meal	1.93	4.97
Distiller's dried grains with solubles	10.7	10.7
Glycerol	**0**	**3**
Amaferm[1]	0.01	0.01
Soybean hull	7.17	7.23
Corn Gluten Feed	23	21.4
Limestone	2.46	2.71
Antimicrobial agent	0.05	0.05
Mineral/Vitamin premix[2]	0.20	0.20
Total	100	100
Chemical composition of concentrate, % DM		
Moisture	10.36	9.88
Ether extract	5.03	5.29

Crude protein	17.97	18
Ash	8.51	8.36
Neutral detergent fiber	36.23	35.19
Acid detergent fiber	26.54	25.35
Calcium	0.86	0.89
Phosphors	0.46	0.52
Non fiber carbohydrate	28.61	32.2
Rumen undegradable protein	5.81	5.81
Total digestible nutrient	71.15	74.65
Metabolizable energy[4] ,Mcal/kg	2.73	2.88
Net energy[5],Mcal/kg	1.18	1.29

Rice straw, % DM	
Moisture	21.81
Ether extract	6.48
Crude protein	0.12
Ash	10.96
Neutral detergent fiber	69.84
Acid detergent fiber	41.56
Calcium	0.27
Phosphors	0.07

[1] Amaferm: Aspergillus oryzae fermentation extract (Biozyme, Joseph, Missouri, USA).

[2] Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D_3 (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic–DC). Grobic–DC was provided by Bayer Health Care (Leverkusen, Germany).

[3] TDN (%) = NFC + CP + [(EE – 1) x 2.25] + NDF – 7 (NRC, 2007)

[4] ME = [1.01 x (DE) − 0.45] + 0.0046 x (EE−3) (NRC, 2007).

[5] NE = 1.42 ME − 0.174 ME^2 + 0.0122 ME^3 − 1.65 (NRC, 2007).

Table 22. Mean and maximum values of environmental temperatures, relative humidity, and temperature–humidity index during summer through fall season

Items	July (P1)[1]	August (P2)[2]	September (P3)[3]	SE	P
Temperature, ℃					
Mean	25.23a	27.51a	20.50b	0.45	<0.001
Maximum	30.35a	32.29a	27.31b	0.63	<0.001
Relative humidity, %					
Mean	76.05a	67.83b	75.97a	0.86	<0.001
Maximum	86.89a	82.49b	85.32a	0.42	<0.001
THI[4]					
Mean	75.02a	77.50a	67.63b	0.62	<0.001
Maximum	84.74a	87.19a	79.46b	0.71	<0.001

[a-b]Mean values with different letters within same row differ (P<0.05). N = 28.

[1, 2, 3] July 5 to August 2 (P1), August 3 to August 30 (P2), August 31 to September 26 (P3), respectively

[4]Temperature–humidity index (THI) = 0.8×temperature + [(relative humidity×0.01) × (temperature−14.4)] + 46.4 (Hahn, 1999)

Table 23. Growth performance of growing Korean cattle steers fed either control or glycerol–supplemented (3.0%) diet during summer through fall season.

Items	July (P1) [1]		August (P2) [2]		September (P3) [3]		SE	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol		Month	Diet	Interaction
Age, month	23.5	23.5	24.4	24.4	25.3	25.3	0.10	< 0.001	0.89	0.78
Initial body weight, kg	466.8	467.0	480.5	487.5	495.3	505.7	12.62	< 0.001	0.54	0.87
Body weight, kg	480.5	487.5	495.3	505.7	511.0	528.5	13.43	< 0.001	0.68	0.80
Daily feed intake, DM base										
Total feed intake, kg/d	6.88	6.97	7.15	7.32	7.15	7.35	0.17	0.41	0.71	0.89
Concentrate intake, kg/d	5.71	5.80	5.98	6.15	5.98	6.17	0.17	0.42	0.71	0.90
Forage intake, kg/d	1.17	1.17	1.17	1.17	1.17	1.17	0.002	0.90	0.51	0.79
Average daily gain, kg/d	0.49	0.73	0.53	0.65	0.56	0.81	0.032	0.55	0.056	0.96
Gain to feed ratio	0.062	0.092	0.065	0.078	0.069	0.097	0.006	0.53	0.03	0.99

N = 10/group.

[1] July 5 to August 2 (P1).

[2] August 3 to August 30 (P2).

[3] August 31 to September 26 (P3).

Table 24. Ruminal pH, volatile fatty acids and NH_3-N of Korean cattle steers after 3 h feeding fed either control or glycerol–supplemented (3.0%) diet during summer through fall season.

Items	July 5		August 2		August 30		September 26		SEM	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol		Month	Diet	Interaction
THI[1]	75.2		76.7		65.6		53.5					
pH	6.21	6.40	6.50	6.78	6.34	6.28	6.61	6.54	0.07	0.54	0.25	0.74
NH_3-N, mg/dL	6.29	8.82	11.8	10.8	8.85	4.64	6.81	7.20	0.84	0.067	0.73	0.36
C2, mM	57.2	45.9	69.6	66.9	86.3	63.5	69.1	73.8	4.19	<0.001	0.06	0.42
C3, mM	14.3	13.0	16.9	20.3	23.4	21.0	16.8	25.9	1.58	<0.001	0.11	0.06
Iso C4, mM	0.82	1.07	1.09	1.17	1.03	0.90	0.96	1.16	0.04	0.50	0.10	0.66
C2:C3 ratio	4.21	3.81	4.14	3.47	3.78	3.09	4.26	2.88	0.18	0.051	<0.001	0.12
C4, mM	11.7	11.7	16.9	18.0	20.0	16.3	19.2	23.1	1.39	<0.001	0.65	0.48
Iso C5, mM	0.99	1.61	1.41	1.54	1.19	1.12	1.15	2.09	0.13	0.21	0.004	0.45
C5, mM	0.94	1.21	1.57	1.98	1.72	1.63	1.55	2.41	0.16	<0.001	0.007	0.28
Total VFA, mM	86.0	74.5	107.4	109.8	133.6	104.4	108.7	128.4	6.92	<0.001	0.55	0.25

N = 10/group.

[1]THI = Temperature–humidity index = 0.8 × temperature + [(relative humidity × 0.01) × (temperature−14.4)] + 46.4 (Hahn, 1999).

[1]THI of sampling time of each day.

Table 25. Serum parameters of Korean cattle steers before feeding fed either control or glycerol–supplemented (3.0%) diet during summer through fall season.

Items	July 5		August 2		August 30		September 26		SE	P–value		
	Control	Glycerol	Control	Glycerol	Control	Glycerol	Control	Glycerol		Month	Diet	Interaction
Glucose, mg/dL	70.8	72.2	80.2	78.8	81.7	79.5	66.2	66.2	2.26	0.64	0.93	0.94
NEFA, mg/dL	204.8	232.2	103.8	97.9	157.6	148.0	127.5	153.7	16.4	0.76	0.83	0.996
Triglyceride, mg/dL	22.8	16.7	29.2	26.8	24.2	21.0	29.3	21.8	1.54	0.91	0.21	0.85
Cholesterol, mg/dL	157.5	169.5	160.2	179.2	194.7	198.8	183.7	205.0	6.28	0.003	0.034	0.86
HDL, mg/dL	92.3	108.3	85.4	102.0	100.7	120.5	91.3	111.6	4.13	0.12	0.014	0.68
GOT, IU/dL	65.3	75.3	59.4	69.2	62.2	73.0	59.3	73.4	2.29	0.86	0.008	0.57
GPT, IU/dL	23.2	23.5	22.4	21.2	20.8	21.0	18.2	19.4	0.64	0.034	0.895	0.537
Albumin, mg/dL	4.38	4.48	4.56	4.60	3.85	3.63	4.25	4.40	0.12	0.15	0.95	0.87
Calcium, mEq/dL	7.87	7.83	8.10	8.20	8.15	8.38	7.68	7.80	0.084	0.903	0.660	0.76
Phosphate, mEq/dL	9.25	8.35	8.96	8.12	9.07	8.45	8.18	8.34	0.15	0.45	0.074	0.209

N = 10/group.

GOT = glutamic oxalacetic transaminase, GPT = glutamic pyruvate transaminase, HDL = high density lipoprotein, NEFA = non–esterified fatty acid.

Table 26. Correlation coefficient between daily average temperature or THI? and DMI during experimental period.

month	Correlation coefficient
[1]July (P1)	-0.18
[2]August (P2)	-0.27*
[3]September (P3)	0.04

N=28. Significant correlation is indicated as *$P < 0.05$.

[1] July 5 to August 2 (P1).

[2]August 3 to August 30 (P2).

[3]August 31 to September 26 (P3).

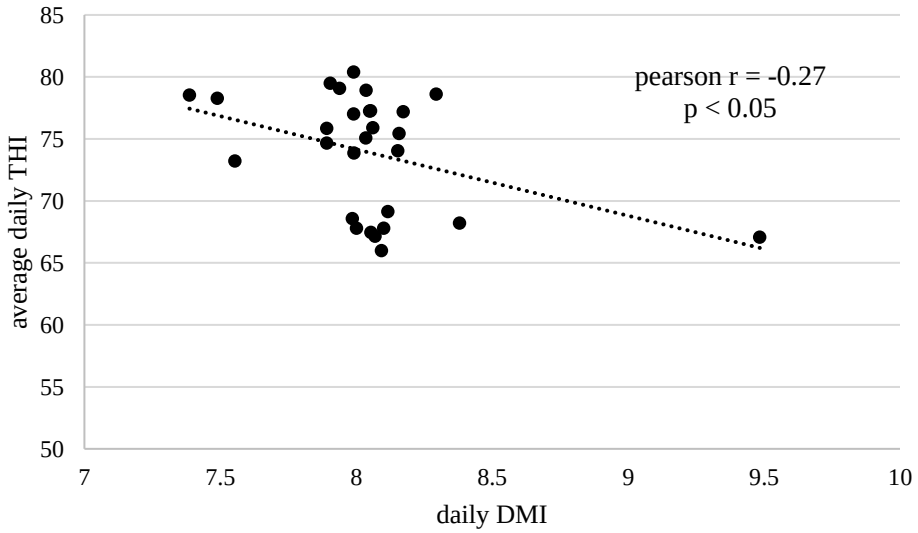

Figure 1. Correlation coefficient between daily THI and DMI during August 3 to August 30.

6. REFERENCES

Adamski M, Kupczynski R, Chaldek G, Falta D. 2011. Influence of propylene glycol and glycerin in Simmental cows in periparturient period on milk yield and metabolic changes. Archiv Tierzucht 54 2011;3:238-48.

Beede DK, Collier RJ. Potential nutritional strategies for intensively managed cattle during thermal stress. J Anim Sci 1986;62:543–54.

Boyd J, West JW, Bernard JK. Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. J Dariy Sci 2010;94:4616–22.

Chung YH, Rico DE, Martinez CM et al. Effects of Feeding Dry Glycerin to Early Postpartum Holstein Dairy Cows on Lactational Performance and Metabolic Profiles J Dairy Sci 2007;90:5682–91.

Clariget JM, Román L, Clariget RP, et al. Supplementation with a mixture of whole rice bran and crude glycerin on metabolic responses and performance of primiparous beef cows. R. Bras. Zootec 2016;45:16-25.

Collier RJ, Beede DK, Thatcher WW, Israel LA, Wilcox CJ. Influences of environment and its modification on dairy animal health and production. J Dairy Sci 1982;65:2213–27.

DeFrain JM, Hippen AR, Kalscheur KF, Jardon PW. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance J Dairy Sci 2004;87:4195-06

Febbraio MA. Alterations in energy metabolism during exercise and heat stress. Sports Med 2001;31:47–59.

Hahn GL. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999;77:10–20.

Hahn GL. Environmental influences on feed intake and performance of feedlot cattle. In: Proc. Symp. Intake by Feedlot Cattle; Oklahoma State Univ., Stillwater; 1995. p. 207–25.

Hassan A, Roussel JD. Effect of protein concentration in the diet on blood composition and productivity of lactating Holstein cows under thermal stress. J Agric Sci Camb 1975;85:409–15.

Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows — a review. Livest Prod Sci 2002;77:59–91.

Kang HJ, Piao MY, Baik M, et al. Effects of hot temperature and rumen–protected fat

supplementation on growth performance, rumen characteristics, and blood parameters in growing stage of Korean cattle steers. Asian–Australas J Anim Sci, 2018. in revision.

Kang HJ, Piao MY, Lee IK, et al. Effects of ambient temperature and dietary glycerol addition on growth performance, blood parameters and immune cell populations of Korean cattle steers. Asian-Australas J Anim Sci 2017;30:505–13.

Kang HJ, Piao MY, Baik M, et al., Effects of ambient temperature and rumen–protected fat supplementation on growth performance, rumen fermentation and blood parameters during cold season in Korean cattle steers. Asian–Australas J Anim Sci, 2018. in publish.

Kelly RO, Martz FA, Johnson HD. Effects of environmental temperature on ruminal VFA levels with controlled feed intake. J Dairy Sci 1967;50:531–3.

Kim WS, Lee JS, Lee HG, et al. Correlation between blood, physiological and behavioral parameters in beef calves under heat stress Asian-Australas J Anim Sci 2018;31919-25.

Krehbiel CR. Ruminal and physiological metabolism of glycerin. J Anim Sci 2008;86:E392 (Abstr).

Lee SY, Lee SM, Seo S, et al. 2011. Glycerol as a feed supplements for ruminants: In vitro fermentation characteristics and methane production. Anim Feed Sci Technol, 166–167:269–74

Liu J, Ye G, Huang K, et al. Feeding glycerol-enriched yeast culture improves performance, energy status, and heat shock protein gene expression of lactating Holstein cows under heat stress. J Anim Sci 2014;92:2494–502.

Livestock Conservation Institute (LCI). Patterns of transit losses. Omaha, NE. 1970.

Mach N, Bach A, Devant M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. J Anim Sci 2009;87:632-38.

Mader TL, Fell LR, McPhee MJ. Behavior response of non–Brahman cattle to shade in commercial feedlots. In Proc. 5th Int. Livestock Environment Symp. Amer. Soc. Agric. Eng., St. Joseph, MI; 1997. p. 795–802.

Martz FA, Mishra M, Campbell JR, Daniels LB, Hilderbrand E. Relation of ambient temperature and time postfeeding on ruminal, arterial and venous volatile fatty acids, and lactic acid in Holstein steers. J Dairy Sci 1971;54:520–5.

Moody EG, Van Soest PJ, McDowell RE, Ford GL. Effect of high temperature and dietary fat on performance of lactating cows. J Dairy Sci 1967;50:1909–19.

NRC. Nutrient requirements of dairy cattle. 8th rev ed. Washington, DC: National Academy Press; 2007.

O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH. Metabolic adaptations to heat stress in growing cattle. Domest Anim Endocrinol 2010;38:86–94.

Panteghini M, Aspartate aminotransferase isoenzymes. Clin Biochem 1990;23:311–9.

Parsons GL, Shelor MK, Drouillard JS. Performance and carcass traits of finishing heifers fed crude glycerin. J Anim Sci 2009;87:653-657

Rhoads ML, Rhoads RP, Baungard LH, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. production, metabolism and aspects of circulating somatotropin. J Dairy Sci 2009;92:1986-97.

Ronchi B, Bernabucci U, Lacetera N, Verini Supplizi A, Nardone A. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. Zootech Nutr Anim 1999;25:11–20.

Shwartz G, Rhoads, ML, VanBaale MJ, Rhoads RP, Baumgard LH. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. J Dairy Sci 2009;92:935–42.

Tajima K, Nonaka I, Hihuchi K, et al. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 2007;13:57–64.

Terréa M, Nuddab A, Casadoc P, Bach A. The use of glycerine in rations for light lamb during the fattening period. Anim Feed Sci Technol 2011;164 :262–67

Thom EC. The discomfort index. Weatherwise. 1959; 12:57–9.

Trabue S, Scoggin K, Tjandrakusuma S, Rasmussen MA, Reilly PJ. Ruminal Fermentation of Propylene Glycol and Glycerol, J. Agric. Food Chem.2007;55:7043−51

Vernon RG. Effects of diet on lipolysis and its regulation. Proc Nutr Soc 1992;51:397–408.

West JW. Effects of heat-stress on production in dairy cattle. J Dairy Sci 2003;86:2131–44.

West JW. Nutritional strategies for managing the heat-stressed dairy cow. J Anim Sci 1999;77:21–35.

Supplementary table 1. Correlation coefficient between daily average temperature and DMI during each period in study 1 to 4.

	month	Correlation coefficient
Study 1	January	-0.04
	February	-0.02
	March	-0.15
	April	-0.11
Study 2	January	-0.14
	February	-0.22
	March	-0.06
Study 3	January	-0.06
	February	-0.19
	March	0.04
	April	0.09
Study 4	July	-0.13
	August	-0.05
	September	-0.10
	October	0.07

N=28.

CHAPTER FIVE

General conclusion

Effect of cold stress

In the study 1, 2, and 3, 3 feedlot trials were conducted during winter through spring. Cold periods of the three trials were classified as mild CS. Growth performance (i.e., ADG and G:F ratio) was not decreased by months in the 3 trials. Thus, the climate condition of experimental region was not significant cold condition on Korean cattle steers, although the climate condition classified as mild cold stress in the criterion of foreign beef cattle. In rumen characteristics, there are no change of VFAs in study 1 and 3, whereas C2, C3, and total VFAs were higher in only study 2. Circulating glucose was higher in all of the 3 trials, but other parameters did not show common changes. These results suggest that Korean cattle may not have been significantly affected by cold conditions in this study, although cold conditions partially affected rumen fermentation and blood parameters. It can be deduced that glucose is mainly used for thermoregulating during the colder period, because glucose is commonly increased during the cold period in 3 trials. Therefore, feed additives that can promote glucose production may be effective in winter season.

Effect of heat stress

In the study 4 and 5, 2 feedlot trials were conducted during summer through fall.

THI conditions of the 2 trials were classified as alert HS. Growth performance (i.e., ADG and G:F ratio) was not decreased by months in the 2 trials. Thus, the climate condition of experimental region was not significant hot condition on Korean cattle steers, although the climate condition classified as alert heat stress range in the criterion of dairy cattle. In the Study 4, only C2:C3 ratio was lowered by hotter months, whereas, in the study 5, C2, C3, C4, C5 and total VFAs were lowered by hotter months. In contrast to winter, circulating glucose was lower by hotter months, but not changed in study 5. Blood cholesterol was lowered by hotter months in the two trials. Lowered triglyceride and HDL concentrations by hotter months were observed in Study 4, whereas not changed in study 5. These results suggest that Korean cattle may not have been significantly affected by hot conditions in this study, although hot conditions partially affected rumen fermentation and blood parameters. Lower blood cholesterol, triglyceride, and HDL during hotter months may result from increased lipid utilization by peripheral tissues. Thus, it can be assumed that dietary ingredient for supplying lipid metabolites may be useful during hotter period. However, it was difficult to control the temperature condition in the experimental region, and the body weight of the animals in the feedlot trials was not constant. Additional experiments will need to collect basal data of effects of hot or cold condition on Korean cattle.

Effect of RPF supplementation

In the study 1 (RPF 0.5%, cold condition), study 2 (RPF 0.8%, cold condition), and study 4 (RPF 0.8%, hot condition), 3 feedlot trials were conducted. Although growth performance (i.e., ADG and G:F ratio) was not promoted in the 3 trials, lipid metabolites were

increased by RPF supplementation. In study 1 and 4, blood cholesterol was increased, and in study 1 and 2, HDL was increased by RPF supplementation. In the 3 trials, rumen characteristics (pH, NH_3-N and VFAs) were not changed by RPF supplementation, which means that the RPF was actually difficult to digest in the rumen. Therefore, RPF supplementation can be used to supplement the energy without changing the rumen environment.

Effect of glycerol supplementation

In study 3 (glycerol 6.0%, cold condition) and study 5 (glycerol 3.0%, hot condition), 2 feedlot trials were conducted. In the study 5, 3 % glycerol supplementation in concentrate increased growth performance (i.e., ADG and G:F ratio), whereas not in study 3. Decreased C2 and C2:C3 ratio by glycerol supplementation was observed in the 2 trials. Glycerol can be fermented mainly into C3 in the rumen, only in the study 3, increased ruminal C3 was observed. Although glycerol is known to has a glucogenic effect, circulating glucose was not changed by glycerol supplementation in the 2 trials. Blood cholesterol and HDL were increased by glycerol supplementation in study 5. Considering decreased C2 and C2:C3 ratio in the rumen, it can be assumed that acidosis can be prevented or reduced by glycerol supplementation.

Summary in Korean

극단적으로 높거나 낮은 외기 온도는 소의 생산성을 감소시키는데, 이는 반추위 능력의 감소나 포도당 또는 non-estrified fatty acid (NEFA) 의 추가적인 소모와 같은 생리적 항상성의 변화에서 기인한다. 글리세롤은 포도당 신생합성의 전구체로 사용될 수 있고, 반추위 보호 지방(RPF)은 반추위 환경에 영향을 미치지 않으면서 추가적인 에너지 공급원으로 사용될 수 있다. 따라서 RPF와 글리세롤 첨가는 덥거나 추운 조건에서 소에게 긍정적인 영향을 미칠 가능성이 있다. 그러나 RPF나 글리세롤 첨가가 추위 또는 더위 환경에서 한우에 미치는 영향에 대한 연구는 거의 없으며, 추위 또는 더위 환경이 한우에게 미치는 영향에 대한 기초 데이터 역시 부족한 상황이다. 따라서 본 연구는 더위 및 추위 환경, 그리고 글리세롤 또는 RPF 첨가가 한우 거세우의 성장, 반추위 및 혈액 성상에 미치는 영향을 평가하기 위해 수행되었다. 이를 위해, 저온 및 고온 조건에서 총 5회의 사양실험이 수행되었는데, 각 실험에 대한 조건은 다음과 같다 [study 1 : 저온 조건, RPF (0.5 %); study 2, 저온 조건, RPF (0.8 %); study 3,

저온 조건, glycerol-absorbed wheat bran (6 %); study 4, 고온 조건, RPF (0.8 %); study 5, 고온 조건, 글리세롤 (3 %)]. 결과적으로, 추위 또는 고온 조건은 한우 거세우의 성장을 저하시키지 않았다. 혈중 포도당은 더 추운시기에 증가하는 반면, 주요 반추위 VFA는 변화하지 않았다. 고온 조건에서는 혈중 콜레스테롤이 감소하고 반추위 VFA가 감소하는 경향을 보였다. 혈중 HDL과 콜레스테롤은 RPF 첨가에 의해 증가되었지만, RPF 첨가 (0.5 또는 0.8 %) 는 한우 거세우의 성장을 향상시키지 않았고, 반추위 환경에 영향을 미치지 않았다. 글리세롤 첨가 (농후사료 내 3%) 는 한우 거세우의 성장을 향상시켰지만, 혈중 포도당 농도에 영향을 주지 않았다. 글리세롤 첨가는 반추위 C2 농도와 C2:C3 비율을 감소시키고 C3 농도를 증가시켰다. 결론적으로, 저온 및 고온 조건은 성장에 영향을 미치지 않았지만, 일부 혈액 및 반추위 성상을 변화시켰다. 글리세롤 첨가 (농후사료 내 3%) 은 반추위내 주요 VFA 변화와 함께 일당증체량 및 사료효율을 향상시켰다. RPF 첨가는 성장에 영향을 미치지 않았으며, 혈중 지질 대사물질의 농도를 상승시켰으나 반추위 환경에는 영향을 미치지 않았다.

Study 1. 겨울철 외기 온도와 반추위 보호 지방 첨가가 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 미치는 영향

이 연구는 겨울에 낮은 외기온도와 반추위 보호 지방 첨가가 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 영향을 미치는지 알아보기 위해 수행되었다. 한우 거세우 20 마리 (550.6 ± 9.14 kg, 19.7 ± 0.13개월령)를 대조구 (10 마리)와 0.5 % 반추위 보호 지방 첨가구 (10 마리) 로 나누었다. 2015년 1월 9일부터 2월 5일까지 (P1), 2월 6일부터 3월 5일까지 (P2), 3월 6일부터 4월 3일까지 (P3), 4월 4일부터 5월 2일 (P4) 까지 16주 동안 실험을 수행하였으며, 하루에 농후사료를 체중의 1.6 %, 볏짚을 1kg 급이하였다. P3 (5.87 ℃, -1.86 ℃) 및 P4 (11.18 ℃, 4.28 ℃) 에 비해 P1 (-3.44 ℃, -9.40 ℃)의 평균 최저 기온은 낮았다 ($P < 0.001$). P1의 최저 온도는 mild cold stress (CS) 범주에 속하며 P2 및 P3의 최소 온도는 thermo-nuetral 범주에 속한다. 전반적으로 반추위 보호지방 첨가 및 외기 온도가 일당증체량 또는 사료효율에 영향을 미치지 않았다 ($P> 0.05$). 반추위 암모니아태 질소 농도는 추운 겨울에 봄보다 높았다 ($P <0.05$). 혈장 cortisol 농도는 가장 추운 달에 다른 달들보다 낮았다 ($P <0.05$). 혈장 포도당

농도는 일반적으로 다른 달들보다 추운 달에 더 높았지만 반추위 보호지방 첨가에 의해 영향을 받지 않았다. 반추위 보호지방 첨가는 총 콜레스테롤 (P = 0.004)과 HDL 농도 (P = 0.03)를 증가시켰다. 실험 기간 동안 혈액 매개 성상의 변화가 관찰되었지만 소의 성장은 변함이 없었으므로, 한우 거세우는 mild한 CS에 의해 유의미한 영향을 받지 않았다고 볼 수 있다. 반추위 보호지방 첨가는 혈중 콜레스테롤과 HDL 농도를 변화 시켰지만 성장에는 영향을 미치지 않았다.

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Study 2. 겨울철 외기 온도와 반추위 보호 지방 첨가가 비육전기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 미치는 영향

본 연구는 겨울철 추운 온도와 반추위 보호 지방 첨가가 비육전기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 영향을 미치는지 평가하기 위해 수행되었다. 한우 거세우 20 마리 (356.5 ± 3.72 kg, 16.8 ± 0.34개월령)를 대조구 (10 마리)와 0.8 % 반추위 보호 지방 첨가구 (10 마리) 로 나누었다. 2016년 1월 8일부터 2월 2일까지 (P1), 2월 3일부터 3월 3일까지 (P2), 3월 4일부터 4월 1일까지 (P3) 까지 12주 동안 실험을 수행하였으며, 하루에 농후사료를 체중의 1.6 %, 톨페스큐를 3kg 급이하였다. P1 (-7.37 ° C)과 P2 (-2.96 ° C)의 평균 대기 온도는 각각 P3 (6.22 ° C)보다 낮았다 ($P < 0.001$). P1과 P2의 평균 온도는 mild~moderate cold stress 범위 내에 있었고 P3의 평균 온도는 thermo-neutral 범위였다. 온도 및 반추위 보호지방 첨가는 일당증체량과 사료 효율에 영향을 미치지 않았다 ($P > 0.05$). 반추위 내 C2, C3 및 총 휘발성 지방산은 추운 달에 더 높았다 ($P < 0.05$). 혈중 중성 지방, 콜레스테롤 및 고밀도 지단백질 (HDL)의 농도는 추운시기에 더 높았다 ($P < 0.05$). 혈중 중성 지방, 콜레스테롤 및 HDL은

더 추운 달에 더 낮았다 (P < 0.05). 반추위 보호지방 첨가는 콜레스테롤의 혈중 농도를 증가시켰다 (P < 0.05). 일부 혈액 성상의 변화가 관찰되었지만, 가축의 성장은 변하지 않았다는 점을 감안할 때 mild~moderate cold stress가 가축에 미치는 영향은 크지 않은 것으로 볼 수 있다. 반추위 보호지방 첨가는 동물의 성장에 영향을 미치지 않는 반면, 일부 혈액 지질 대사 산물 및 반추위 휘발성 지방산에 영향을 줄 수 있다.

Study 3. 겨울철 외기 온도와 글리세롤 첨가가 육성기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 미치는 영향

본 연구는 겨울철 외기 온도 및 글리세롤 첨가가 육성기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 영향을 미치는지 평가하기 위해 수행되었다. 한우 거세우 20 마리 (BW 217.0 ± 7.63kg, 7.7 ± 0.07 개월)를 대조구 (n = 10)와 6.0 % 글리세롤 첨가구 (n = 10)로 나누었다. 대조구는 일일 0.15 kg의 밀기울을, 글리세롤 첨가구는 0.4 kg의 60 % glycerol-adsorbed 밀기울을 탑드레싱 방식으로 급이하였다. 2017년 1월 10일부터 2월 7일까지 (P1), 2월 8일부터 3월 7일까지 (P2), 3월 8일부터 4월 4일까지 (P3), 4월 5일부터 5월 1일까지 (P5) 까지 16주 동안 실험을 수행하였으며, 하루에 농후사료를 체중의 1.5 %, 티모시 건초를 3kg 급이하였다. 1월과 2월의 평균 대기 온도 (-5.09 ℃와 -0.08 ℃)는 mild cold stress 범위였고, 3월과 4월의 평균 기온은 각각 5.57 ℃와 14.7 ℃ 로 thermo-neutral 범위였다. 추위 환경은 일당증체량에 영향을 미치지 않았지만, 사료 효율은 추운시기에 더 높았다 ($P < 0.05$). 글리세롤 첨가는 일당증체량과 사료효율 모두를 수치적으로 증가시켰다 ($P > 0.05$). 대조구에 비해 글리세롤

첨가 군에서 반추위 C3 농도가 높았으며 글리세롤 첨가 군에서 C2 : C3 비율이 대조구보다 낮았다 ($P < 0.05$). 저온 환경은 반추위 pH, NH3-N 및 VFA에는 영향을 미치지 않았다 ($P > 0.05$). 추위 기간에 혈중 포도당, 중성 지방 및 NEFA농도가 높았다 ($P < 0.05$). 연구 기간 동안 혈당, NEFA, 중성지방의 변화가 관찰되었지만 소의 성장 능력은 변함이 없었으므로, 한우 거세우는 mild cold stress에 유의미한 영향을 받지 않은 것으로 볼 수 있다. 글리세롤 첨가는 반추위 C3, C5 및 C2:C3 비율을 변화 시켰지만 성장에는 영향을 미치지 않았다.

Study 4. 여름철 고온 및 반추위 보호지방 첨가가 육성기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 미치는 영향

본 연구는 고온 및 반추위 보호지방 첨가가 육성기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 영향을 미치는지 평가하기 위해 수행되었다. 한우 거세우 20 마리 (BW 230.4 ± 4.09 kg, 10.7 ± 0.09 개월)를 대조구 (10 마리)와 0.8% 반추위 보호지방 첨가구 (10 마리) 로 나누었다. 2015년 7월 10일부터 8월 6일까지 (P1), 8월 7일부터 9월 3일까지 (P2), 9월 4일부터 10월 1일까지 (P3), 10월 2일부터 10월 30일 (P4) 까지 16주 동안 실험을 수행하였으며, 하루에 농후사료를 체중의 1.5 %, 톨페스큐를 4kg 급이하였다. P1 (76.8), P2 (76.3), P3 (75.9)의 평균 온습도지수 (THI) ($P < 0.001$)는 P4 (50.9)보다 높았다. P1-3의 평균 THI는 alert heat stress 범주 내에 있었고, P4의 평균 THI는 thermo-neutral 범위였다. 실험 전 기간 동안 반추위 보호지방 첨가 및 외기온도가 일당증체량 및 사료효율에 영향을 미치지 않았다 ($P > 0.05$). 온도 및 반추위 보호지방 첨가는 혈중 포도당, 알부민 및 고밀도 지단백질 (HDL)의 농도에 영향을 미쳤다. 반추위 보호지방 첨가에 의해 혈중 알부민과 포도당 농도는 감소하는 경향을

보였으나 ($p < 0.10$), HDL은 반추위 보호지방 첨가에 의해 증가 하였다 ($P < 0.01$). 온도 및 반추위 보호지방은 반추위 pH와 NH_3-N 및 휘발성 지방산 농도에 영향을 미치지 않았다. 육성기 한우 거세우의 혈액 대사 산물의 변화가 관찰되었지만, 더운 계절에 한우의 성장이 더 좋았다는 점을 감안할 때, 더위 스트레스에 유의미한 영향을 받지 않았다고 볼 수 있다. 반추위 보호지방 첨가는 혈중 지질과 탄수화물 대사 산물을 변화시켰지만 성장에는 영향을 미치지 않았다

Study 5. 여름철 온도 및 글리세롤 첨가가 비육중기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 미치는 영향

본 연구는 여름철 고온 및 글리세롤 첨가가 비육전기 한우 거세우의 성장, 반추위 특성 및 혈액 성상에 영향을 미치는지 평가하기 위해 수행되었다. 한우 거세우 20 마리 (466.9 ± 10.02 kg, 23.5 ± 0.07개월령)를 대조구 (10 마리)와 3 % 글리세롤 첨가구 (10 마리) 로 나누었다. 2016년 7월 5일부터 8월 2일까지 (P1), 8월 3일부터 8월 30일까지 (P2), 8월 31일부터 9월 26일까지 (P3) 까지 12주 동안 실험을 수행하였으며, 하루에 농후사료를 체중의 1.6 %, 볏짚을 1.5kg 급이하였다. P1 (75.02)와 P2 (77.50)의 평균 온습도 지수 (THI) ($P < 0.001$)는 P3 (67.63)보다 높았다. P1과 P2의 평균 THI는 alert heat stress 범위 내에 있었고 P3의 평균 THI는 thermo-neutral 범위였다. 온도는 일당증체량과 사료효율에는 영향을 미치지 않았으나, 글리세롤 첨가는 일당증체량 ($P = 0.056$)와 사료효율 ($P <0.05$)을 증진시켰다. 반추위 pH와 NH3-N은 온도 및 글리세롤 첨가에 의해 변하지 않았다. 반추위 C2 : C3 비율은 더운 달에 일반적으로 높았고 ($P = 0.051$), C5 농도는 더운 달에 더 낮았다 ($P < 0.001$). 반추위 C2 : C3 비율은 글리세롤

첨가에 의해 증가했지만 (P < 0.001), C5 농도는 글리세롤 첨가에 의해 감소했다 (P < 0.05). 반추위 C2, C3, C4, C5 및 총 VFA의 농도는 보다 더운 기간에서 낮았다 (P < 0.05). C5는 글리세롤 첨가에 의해 증가하였다 (P < 0.05). 더운 기간에 혈중 콜레스테롤은 다른 기간에 비해 낮았다 (P < 0.05). 글리세롤 첨가는 혈중 콜레스테롤과 고밀도 지단백질을 증가시켰다 (P <0.05). 실험 기간 동안 혈액 성상의 변화가 관찰되었지만 소의 성장은 변함이 없었으므로 한우 거세우는 alert heat stress로 유의미한 영향을 받지 않은 것으로 볼 수 있다. 그러나 글리세롤 첨가는 혈중 콜레스테롤 및 HDL 농도 변화와, 반추위 C2 : C3 비율 변화와 함께 소의 성장을 증진시켰다.