Alternative strategies to reduce liver abscess incidence and severity in feedlot cattle.

by

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Abstract

Since the 1960's liver abscess incidence and severity have been identified as a problem associated with feeding high concentrate finishing rations to feedlot cattle. Liver abscesses lead to decreased feedlot performance and decreased carcass value. Tylosin phosphate is a macrolide antibiotic commonly used by feedlots throughout the United States and has been shown to successfully control liver abscesses. In 2013, the FDA issued Guidance for Industry #213, which encourages reduced usage of medically important classes of antibiotics, such as macrolides, in animal feed. This will be achieved by implementing veterinary oversight of these drugs via Veterinary Feed Directives (VFD). Thus, it is of importance to find alternative strategies to reduce usage of tylosin in finishing rations to control liver abscesses. One strategy that has been suggested is increasing dietary roughage concentration. However, this isn't a viable option as increasing dietary roughage concentration not only leads to a decline in feedlot performance, hot carcass weight, and dressing percentage, but also has an environmental impact. Available research has also indicated that increasing dietary roughage has no impact on liver abscess incidence or severity. Our research objective was therefore to identify alternative strategies to reduce liver abscess incidence. Our first trial evaluated the impact of antioxidants on liver abscess incidence and severity. Treatments consisted of a control treatment (basal diet containing 200 IU/d α-tocopherol acetate), and an antioxidant treatment (basal diet containing 2000 IU/d α-tocopherol acetate and 500 mg/d crystalline ascorbate). Treatments were randomly assigned to 390 crossbred heifers. No differences in feedlot performance were detected; however, there was a tendency for improved feed intake ($P = 0.075$) and feed efficiency ($P = 0.066$) for heifers that received the antioxidant treatment. An increased number of yield grade 3 carcasses ($P = 0.03$) and fewer yield grade 1 carcasses ($P < 0.01$) was observed in the antioxidant treatment group. No differences were detected between treatments for other carcass characteristics or liver abscess incidence and severity. Another trial evaluated intermittent tylosin feeding and its impact on liver abscess incidence and antimicrobial resistant *Enterococcus* spp. when compared to continuous tylosin feeding. One of 3 treatments were randomly assigned to 312 crossbred steers: negative control (no tylosin fed throughout the feeding period); positive control (tylosin fed throughout the feeding period); or intermittent treatment (tylosin fed intermittently throughout the feeding period: 1week on, 2 weeks off). Fecal samples were collected on day 0, 20, and 118 to characterize antimicrobial resistant *Enterococcus* spp. By design, the intermittent treatment consumed 60% less tylosin than the positive control group. No differences were detected between treatments for feedlot performance. Liver abscess incidence was greatest for the negative control, and least for the positive control and intermittent treatments, with no difference being detected between the latter two treatments (P = 0.716). Antimicrobial resistance was unaffected by treatment, but was affected by sampling time. We concluded that supplementing antioxidants is not a viable option to reduce liver abscess incidence and severity, and that tylosin usage can be decreased without adversely affecting performance or liver abscess incidence.

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Dedication

I would like to dedicate this thesis to my grandmother and grandfather, Isabel Scholtz and Zipp Scholtz. Thank you for the remarkable people that you were and for the inspiration that you were not only to me, but to our entire family. You have instilled a passion into each and every one of us, to live life to the fullest. I love you and miss you.

“Die lewe is ‘n tuin.”

-Isabel Scholtz-

Chapter 1 - Literature Review: Liver abscess incidence and severity in feedlot cattle and increased roughage as an alternative for prevention of liver abscess incidence.

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Liver abscesses in feedlot cattle

The objective of feeding cattle in feedlots is to optimize growth and performance of finishing cattle and to produce carcasses that grade well. The majority of feedlots across the world achieve these goals by feeding high concentrate diets. Wise *et al.* (1965) indicated that cattle receiving a basal diet containing only ground shelled corn had improved feed efficiency, better marbling and thus also tended to grade better than cattle who received 2.5 lb/d of either ground or long-stemmed Bermuda grass added to the diet. In the 1960's, when researchers discovered the benefits of feeding high concentrate diets to finishing beef cattle, they also discovered that high concentrate diets caused an increase in the incidence of liver abscesses (Jensen *et al.*, 1954; McCartor *et al.*, 1964; Thrasher *et al.*, 1964). Brown *et al.* (1975) found that 55% of livers from cattle that received a basal diet (90%, 88%, 80% DM ground corn or 83% flaked sorghum) without chlortetracycline, were abscessed. Recent analysis from various processing facilities across the United States concluded that 16% of all livers were condemned due to major or minor abscesses (McKeith *et al.*, 2011).

Liver abscesses are classified in commercial abattoirs as follows; 0 indicates livers that have no abscesses, A- indicates livers that have between 1 and 2 small abscesses, A indicates livers that have 2 to 4 well developed abscesses that are no bigger than 1 inch in diameter, and A+ indicates livers that have 1 or more large abscesses and often a portion of diaphragm adhering to the abscessed liver (Brown *et al.*, 1975). Smith (1944) observed a correlation between occurrence of lesions in the rumen and incidence of liver abscesses. This observation was supported by Jensen *et al.* (1954) who consequently coined the term "rumenitis-liver abscess complex". The theory behind the complex is that an acidic environment, or foreign objects, can cause lesions in the rumen wall that will allow pathogenic bacteria to gain access to the liver via the portal vein. These bacteria then establish in the liver and lead to the formation of abscesses. Multiple studies have been performed over the years to identify the pathogenic bacteria that are associated for liver abscesses. Lechtenberg *et al.* (1988) isolated anaerobic bacteria from 49 abscesses that were obtained from 29 abscessed livers from feedlot cattle. *Fusobacterium necrophorum* was present in all of the abscesses present on the livers, followed by *Actinomyces pyogenes* that was present in 35% of all abscesses. *Fusobacterium necrophorum* is a lactate utilizing organism that occurs naturally in low numbers in the rumen (Amachawadi & Nagaraja, 2016). *Fusobacterium necrophorum* numbers in the rumen increase significantly in the rumen when cattle fed a 100% roughage diet are switched over to a high concentrate diet (Tan *et al.*, 1994a). This is due to the increased production of lactic acid from organisms that grow well in acidic environments (Nagaraja & Titgemeyer, 2007; Nagaraja *et al.*, 1985). Presence of increased levels of lactic acid leads to an increased number of *Fusobacterium necrophorum*, as the lactic acid is a substrate for *Fusobacterium necrophorum*.

The liver is a metabolically essential organ that plays a major role in nutrient digestion and absorption, detoxification, immune function, and production of hormones (Huntington, 1990). We can therefore hypothesize that a loss in liver function, due to presence of liver abscesses, could have a negative effect on feedlot cattle performance. Brink *et al.* (1990) gathered data from 12 independent experiments where 566 cattle were individually fed at the University of Nebraska Agricultural Research and Development Center. Their objective was to determine how severity of liver abscesses affected performance of finishing cattle. The different treatments in each of the individual experiments did not affect the incidence and severity of liver abscesses. Liver abscess scores were obtained from the processing facility where the animals were harvested. They found that live weight gain did not differ between cattle that had abscessed livers and those that did not have abscessed livers; however, when they determined daily gain from HCW and dressing percentage they found that animals with abscessed livers had lower daily gains compared with animals without abscessed livers. This might be attributed to carcass trim loss associated with liver abscesses which is discussed in the next paragraph. There was also a tendency for a decrease in feed intake and a decline in feed efficiency in cattle that had abscessed livers. Similarly, Brown *et al.* (1973) and Potter *et al.* (1985) found that cattle that received metaphylactic treatment for liver abscesses in the diet had greater gains and improved feed efficiency. In contrast to the previous studies, Heinemann *et al.* (1978) found no difference in DMI, ADG, or feed efficiency in yearling steers fed a 90% concentrate diet which contained either no tylosin or else 11 mg/kg tylosin.

The most significant impact of liver abscesses is a loss of carcass value. Data from 76,191 carcasses collected from 1998 to 2009 were analyzed by Brown & Lawrence (2010) to determine impact of liver abscesses on carcass grading and value. Compared to livers that weren't abscessed, carcasses with abscessed livers had lower hot carcass weights and dressing percentages. Hot carcass weight and dressing percentage also decreased as the severity of abscessed livers increased from A- to A+, which the authors concluded was due to greater trim loss associated with more severely abscessed livers. Livers with severe abscesses tend to adhere to portions of the diaphragm, which must be removed at processing. These findings were supported by Brink *et al.* (1990), who also found that trim loss associated with the presence of liver abscesses can have a significant impact on dressing percentage and HCW.

Various studies have identified chlortetracycline and tylosin phosphate as effective antibiotics to control liver abscesses. Harvey *et al.* (1965) conducted 2 experiments to evaluate influence of roughages and chlortetracycline on performance, rumen epithelial structure and integrity, and liver abscess incidence and severity. The basal diet contained 90% DM cracked corn, with or without chlortetracycline. They found that only 3% of animals that received chlortetracycline had abscessed livers, whereas 43% of livers from animals that did not receive chlortetracycline were abscessed. Bolsen *et al.* (1968) conducted 4 trials and assessed influence of nitrogen source, mineral supplementation, different moisture levels of corn, and chlortetracycline on performance of cattle fed all-concentrate diets. Two of these trials specifically evaluated effects of chlortetracycline concentration on cattle performance and incidence of liver abscesses in finishing cattle. They found that chlortetracycline in the diet decreased occurrence of liver abscesses, compared to trials where chlortetracycline was not added to the diet. Similar results were observed by Albin & Dunham (1967) for diets containing 89% DM cracked milo and chlortetracycline.

Tylosin phosphate is a macrolide antibiotic that is the most common preventative treatment for liver abscess incidence in feedlot cattle (Nagaraja & Lechtenberg, 2007; Reinhardt & Hubbert, 2015; Amachawadi & Nagaraja, 2016). Tylosin decreases incidence of liver abscesses by inhibiting growth of *Fusobacterium necrophorum* (Tan *et al.*, 1994b; Mateos *et al.*, 1997; Lechtenberg *et al.*, 1998). Vogel & Laudert (1994) summarized 40 trials and found that tylosin reduced liver abscess incidence by 73%, increased daily gain by 2.3% and improved feed efficiency by 2.6%. In support of these results, Brown *et al.* (1975) performed 4 feedlot studies to compare effectiveness of tylosin in controlling liver abscess incidence when compared to chlortetracycline. Compared to the control (no antibiotics in the diet), tylosin showed a 66.9% improvement in controlling incidence of liver abscesses. Chlortetracycline only showed a 21.3% improvement compared to the control group. Various other studies have shown the efficacy of tylosin for prevention of liver abscess incidence and severity (Brown *et al.*, 1973; Pendlum *et al.*, 1978; Heinemann *et al.*, 1978; Potter *et al.*, 1985; Meyer *et al.*, 2013).

Tylosin is approved by the U.S. FDA for in-feed application, and is used widely in feedlots across the United States of America. Guidance for Industry #213 was issued by the FDA in 2013 which established a timeline for implementation of Veterinary Feed Directives (VFD) (FDA Guidance for Industry #213, 2013). The objective of VFDs is to reduce use of medically important antibiotics in animal production by implementing judicious use of these antibiotics under veterinary supervision. This implies that to use in-feed antibiotics that are medically important, a producer will have to have a prescription from a veterinarian with whom they have a Veterinary-Client Relationship (VCR). As previously mentioned, tylosin is part of the macrolide family of antibiotics and the FDA classifies macrolides as medically important due to extensive use of macrolides in human medicine (e.g. erythromycin). Jackson *et al.* (2004) indicated a 30.5% increase in *Enterococcus* spp. isolates being resistant to erythromycin when a swine farm that used tylosin for growth promotion was compared to a farm that used no tylosin. Therefore, there is interest in the beef industry to find alternatives for prevention of liver abscesses. One of the suggested alternative strategies for reducing incidence of liver abscesses is by increasing the level of roughage in finishing rations. In the next section we will look at the hypothesis behind why increased roughage may reduce liver abscess incidence and analyze its effectiveness as an alternative preventative strategy.

The Role of Roughages in Feedlot Finishing Rations

The most distinguishing feature of the Bovidae family is their ability to convert non-structural carbohydrates to energy that can be used by the animal for maintenance and growth. This conversion of non-structural carbohydrates to usable energy occurs in the rumen via a symbiotic relationship between the animal and microorganisms, where microorganisms convert the non-structural carbohydrates to a usable energy source and in return the rumen provides a suitable habitat for the microorganisms. The rumen therefore has evolved into an organ that requires non-structural carbohydrates (in the form of roughage) for optimum health and performance. Roughage in diets are crucial to the health and function of ruminal papillae, which are the main structures responsible for absorption of volatile fatty acids produced through microbial fermentation. A recent study by Devant *et al.* (2016) found increased ruminal papillae clumping and vacuole grading in Holstein steers that didn't receive straw supplementation. Vacuole grading is used as a measure of papillae integrity, with increased vacuole grading indicating reduced papillae integrity. These results are consistent with the findings of Weigand *et al.* (1975) who observed that Holstein steers fed an *ad libitum* alfalfa diet had papillae that were more uniform than their counterparts that received an 80% coarsely ground corn diet.

Furthermore, Vance *et al.* (1972) observed extensive ruminal papillae degeneration in all-concentrate rations containing crimped corn. Physical and chemical characteristics of roughages contribute to its effects on the ruminal environment.

Various volatile fatty acids (VFA) are produced during fermentation of both structural and non-structural carbohydrates with the majority of the volatile fatty acids produced being acetate, propionate, and butyrate (Steven & Marshall, 1969). The proportions in which these VFA are produced is primarily dependent on the diet (Davis, 1967; Siciliano-Cortes & Murphy, 1989; Coe *et al.*, 1999). Calderon-Cortes & Zinn (1996) compared VFA profiles from ruminal fluid collected from cannulated steers and found that increasing the forage level from 8% to 16% led to a greater proportion of butyrate being either absorbed across the ruminal wall or utilized by ruminal papillae. Weigand *et al.* (1979) indicated that ruminal papillae had a much larger metabolic affinity for butyrate compared to acetate and propionate, leading to the formation of ketone bodies β-hydroxybutyrate and acetoacetate, therefore indicating that ruminal papillae rapidly utilize butyrate. Baldwin & Jesse (1992) observed a positive correlation between the amount of β-hydroxybutyrate observed in the portal blood (from ketogenesis of butyrate in ruminal papillae) to rumen weights in 42-d to 56-d old calves, confirming the hypothesis that butyrate is utilized by ruminal papillae and is an important substrate for ruminal papillae growth and integrity. Therefore, we can conclude that an increase in utilization of butyrate by ruminal papillae (Calderon-Cortes & Zinn, 1996; Bannink *et al.*, 2008) when there is an increase in roughage content in the diet, leads to improved ruminal papillae integrity.

The characteristic of roughage that has the largest impact on ruminal epithelial health is its ability for tactile stimulation of motility. Ruminal motility is positively correlated with roughage level in the diet (Sissons *et al.*, 1989), which allows for the removal of gases produced through fermentation via eructation, mixes ruminal contents to allow microbes access to feedstuffs, and allows for the passage of digesta through the reticulo-omasal orifice (McDonald *et al.*, 2011). A very interesting study that illustrates the importance of physical stimulation was performed by Loerch (1991). He observed that steers fed a 100% high-moisture corn diet and that had 4 or 8 pot scrubbers placed in their rumens had similar growth rates and feed intakes compared to steers that received 15% corn silage in their diet. Ørskov *et al.* (1978) infused ten lambs with only a mixture of VFA's and casein and found a high quantity of sloughed epithelial cells in the ventral sac of the rumen, which is an indication of rumenitis and parakeratosis. Physical form of roughage stimulates rumination activity by the animal, which decreases particle size, increases digestion, and increases saliva production, thus helping to maintain ruminal pH. Weiss *et al.* (2017) found that ruminal pH of steers fed 10% roughage spent more time ruminating compared to those fed 5% roughage which contributed to maintaining rumen pH above 5.6 for a longer period of time. Devant *et al.* (2016) found that adding 0.7 kg/d (DM basis) straw to a high concentrate diet maintained ruminal pH at a higher pH than a diet that contained no straw. Similar results were also seen by Nocek & Kesler (1980) and Calderon-Cortes & Zinn (1996).

Galyean & Hubbert (2014) suggested that feedlot finishing diets should be formulated on a physical NDF basis, thus accounting for both chemical and physical characteristics of roughages when formulating finishing rations. It is important to include roughages in high concentrate rations to maintain optimum ruminal health and to optimize energy intake (Galyean & Defoor, 2003). Samuelson *et al*. (2016) indicated that the majority of feedlot consulting nutritionists include roughage at a rate of 8% to 10% in finishing rations. The question arises as to why feedlots feed such a low level of roughage in the diet. In the next section we will consider four main aspects as to why feeding low levels of roughage in feedlot diets is optimal and why increasing roughage level in feedlot rations may not be a viable option to reduce liver abscess incidence.

Increasing dietary roughage and its impact on feedlot cattle performance and the environment.

1.Increasing roughage leads to a decline in feedlot performance.

As mentioned previously, the goal of the feedlot industry is to optimize animal performance while maintaining a low input cost (i.e. low feed, processing, labor and transport costs). The largest cost associated with beef production is for feed purchases. Feedlot nutritionist therefore strive to optimize cattle performance with the least amount of feed (i.e. obtain an optimum feed efficiency or gain:feed ratio). To achieve this optimum relationship, the diet needs to provide correct proportions of nutrients and energy to meet animal requirements for maintenance and growth (NRC, 2016). Samuelson *et al.* (2015) reported that 91% of consulting nutritionists surveyed recommended finishing rations that provide between 1.41 Mcal/kg to 1.59 Mcal/kg net energy for gain. Corn is the most common grain source used by feedlots throughout the United States, with alfalfa hay and corn silage being the primary roughage sources in feedlots (Samuelson *et al.*, 2016). Steam-flaked corn provides 1.67 Mcal/kg NEg and high moisture corn provides 1.56 Mcal/kg NEg, while alfalfa and corn silage only provide 0.59 and 0.96 Mcal/kg NEg, respectively (NRC, 2016). It is therefore clear that relatively more roughage will be required to supply enough energy for growth compared to grain.

A key study that evaluated optimum ratios of roughage to grain in feedlot rations was performed by Gill *et al.* (1981) at the Oklahoma Agricultural Experiment Station. They fed 5 different levels of roughage, which consisted of corn silage and alfalfa hay (8%, 12%, 16%, 20% and 24% on a ration DM basis), added finishing rations containing steam-flaked corn, high-moisture corn, or a combination thereof. They found that dry matter intake increased as roughage inclusion rate increased; however, average daily gain did not differ among treatments. This led to a decline in feed efficiency as the roughage inclusion rate increased. They also found that the diet containing only 8% roughage had the highest metabolic energy content (3.38 Kcal/g) and that this value decreased by 0.35% with every 1% increase in roughage concentration within the diet. Defoor *et al.* (2002) investigated how roughage source and concentration affect intake and performance in feedlot heifers. They determined that there was a strong positive correlation between NDF supplied from the roughage and the NEg intake. This indicates that cattle will increase their intake to try and maintain a constant NE intake when energy density of the diet is decreased by adding roughages. To further illustrate this concept, Defoor *et al.* (2002) designed a finishing study where the control treatment consisted of a diet containing chopped alfalfa hay (12.5% DM) and the other 2 treatments consisted of either sudan silage or cottonseed hulls added to the ration to provide the same amount of dietary NDF as the control treatment (5.2% NDF). Sudan silage was added at 7.1 % of dietary DM (SUD7.1) and cottonseed hulls at 5.9% of dietary DM (CSH5.9) and these values were derived from tabular values (NRC, 1996) and laboratory-determined values (Goering & Van Soest, 1970), respectively. No differences between treatments were detected for daily gain, feed intake, or feed efficiency therefore, further confirming that diets formulated to similar NDF levels have similar NEg intakes and performance.

Increasing NDF content of the diet, by increasing the level of roughage in the diet, will increase feed intake of cattle as they try to maintain a constant level of energy intake. Increasing roughage concentration beyond 10% DM in finishing rations leads to a decline in feed efficiency (Kreikemeier *et al.*, 1990; Bartle *et al.*, 1994). Calderon-Cortes & Zinn (1996) compared roughage concentrations of sudan grass (16% vs. 8% DM basis) and coarseness of grind (2.5 cm vs. 7.6 cm) in a finishing ration containing steam-flaked corn. Cattle receiving 8% roughage in the diet had improved average daily gain and feed efficiency when compared to cattle who received 16% roughage in the diet. The authors attributed this improvement to an increase in energy intake when a diet containing less roughage and more energy dense concentrate was fed. Hales *et al.* (2010) analyzed the effect of varying bulk densities of steam-flaked corn and roughage concentration on feedlot performance. They included roughage in the diet at 6% and 10% on a DM basis. They detected no difference in average daily gain, dry matter intake and feed efficiency between steers fed a diet containing 6% or 10% ground alfalfa hay. There were no interaction effects between bulk densities of the corn and the concentration of roughage in the diet. The above mentioned studies suggest that the optimum roughage inclusion rate is between 6% and 10% DM basis, which is in agreement with what Gill *et al.* (1981) found, in which 8% roughage was ideal for diets that contained steam-flaked corn.

As mentioned previously, some level of roughage is required in the diet to stimulate rumination and therefore maintain a healthy and stable ruminal environment. Feeding a roughage free diet can lead to decreases in intake and daily gain, and therefore result in poor feed efficiency (Woods *et al.*, 1969; Brandt *et al.*, 1987). Kreikemeier *et al.* (1990) fed steam-rolled wheat based finishing diets containing 50:50 alfalfa and corn silage as roughage source. The 50:50 roughage was added at 4 concentration levels i.e. 0%, 5%, 10%, and 15% of dietary DM. They observed a quadratic roughage effect with 0% roughage concentration having the lowest daily gain and feed intake, and poorest feed efficiency. No difference was detected in feedlot performance for cattle that received 5%, 10%, and 15% roughage. Farran *et al.* (2006) found similar results when they fed dry-rolled corn and wet corn gluten feed based diets and compared 0% alfalfa inclusion to 3.75% and 7.5% alfalfa inclusion. The authors didn't provide a reason as to why there was depressed performance when no roughage was added to the diet; however, this depression in performance might be due to decreased ruminal pH and less time that the animal spent ruminating (Gentry *et al.*, 2016; Weiss *et al.*, 2017). When cattle spend less time ruminating it leads to a decrease in salivary buffer production; saliva plays an important role in buffering ruminal contents and maintaining a stable ruminal pH (Allen, 1997). This decrease in ruminal pH may then lead to ruminal acidosis, which decreases feed intake and growth performance (Coe *et al.*, 1999; Brown *et al.*, 2006).

Various studies have also analyzed whether different corn processing methods and roughage concentrations affect performance. May *et al.* (2010) found no interaction effect between grain processing (steam-flaked corn vs. dry-rolled corn) and different corn silage concentrations (5% DM vs. 15% DM) for daily gain, dry matter intake, and feed efficiency. They did find that intake decreased and efficiency improved when corn silage concentration in the diet was decreased from 15% to 5%, which follows the same trend as seen in research mentioned earlier (Gill *et al.*, 1981; Kreikemeier *et al.*, 1990; Bartle *et al.*, 1994). Stock *et al.* (1990) found dissimilar results when they compared 0% and 7.5% roughage inclusion (50:50 corn silage and alfalfa hay) within diets containing either dry-rolled corn, dry-rolled grain sorghum, or dry-rolled wheat, and found that there was a grain type by roughage level interaction when they compared feed efficiency. They found that when dry-rolled wheat was fed, an increase in roughage level lead to an improvement in efficiency and the opposite was true that efficiency declined as roughage inclusion increased when cattle were fed a dry-rolled corn or dry-rolled sorghum based diets. The authors mentioned that decline in efficiency when 0% roughage was included in the dry-rolled wheat diet compared to 7% roughage inclusion, was due to increased starch digestibility of wheat which might lead to ruminal acidosis when no roughage is added to the diet. This is consistent with observation of Gill *et al.* (1981), who found that increasing roughage concentration from 8% to 24% had a larger negative effect in steam-flaked corn based diets compared to high-moisture corn diets, suggesting that the extent of reduction in performance from roughage inclusion is dependent on the extent of corn processing. Hales *et al.* (2010) observed no differences in daily gain or feed efficiency when roughage inclusion was increased from 6% to 10% in steam-flaked corn based diets that were flaked to different densities (335 g/L vs. 386 g/L). Differences in flake density, resulted in different starch availability percentages (67.3% for corn flaked to a density of 335 g/L, and 52.9% for corn flaked to a density of 386 g/L). Turgeon *et al.* (2010) similarly found no difference in feed efficiency when steam-flaked wheat diets containing either 0% or 6.9% alfalfa were compared to each other. Various other studies have detected no interaction effect between grain type or processing and roughage concentration for feed efficiency, when the inclusion rate is below 10% DM (Vance *et al.*, 1972; Bartle & Preston, 1992; Loerch & Fluharty, 1998; May *et al.*, 2010).

Increasing roughage concentration past 10% has negative effects on feedlot performance as the energy density of the diets decreases and fails to provide enough energy for optimal growth; however, absence of roughage in finishing rations also has negative effects on feedlot performance. From the above mentioned research we can then conclude that it is important to include roughage in finishing rations at an optimal inclusion rate to maintain optimal ruminal conditions.

2. Increasing roughage concentration has adverse effects on carcass quality

It is important to consider effects that increased roughage concentrations may have on carcass characteristics, as the main focus of feedlots is to produce carcasses that will receive premium payments. Bartle *et al.* (1994) performed 3 trials to determine the effect of dietary roughage concentration (10%, 20%, and 30% DM basis), roughage source, tallow level, and steer type on feedlot performance and carcass characteristics. In all of the trials they found that there was no interaction effect between roughage source, tallow level, or steer type on carcass characteristics. They found that increasing roughage concentration above 10% DM caused a decrease in hot carcass weight, while no difference was detected for dressing percentage. This indicates that increasing roughage concentration beyond 10% DM leads to carcasses that don't provide as much meat. Woods *et al.* (1969), Brandt *et al.* (1987), and Kreikemeier *et al.* (1990) found that hot carcass weight decreased when roughage concentration increased from 10% DM to 15% DM. All three studies indicated that roughage inclusion concentration of 5% to 10% provided an optimum hot carcass weight. Research done by Hales *et al.* (2014) provides a possible explanation for this decrease in hot carcass weight. They indicated that, as a percentage of gross energy, retained energy decreased when alfalfa inclusion in dry-rolled corn diets increased from 2% to 14% dietary DM. Therefore, less energy is being retained in the animal as muscle or fat tissue and thus contributing to carcasses that weigh less. Adding no roughage to a ration also decreases hot carcass weight (Woods *et al.*,1969; Brandt *et al.*,1987; Kreikemeier *et al.*,1990; Farran *et al.*, 2006; Turgeon *et al.*, 2010), this may be due to poorer performance and increased incidence of digestive disturbances.

Dressing percentage has been shown to be at an optimal level when around 5% DM roughage is included in finishing rations (Parsons *et al.*, 2007; Gentry *et al.*, 2016). A lower dressing percentage at lower roughage concentrations may be due to the occurrence of severe liver abscesses, however Gentry *et al.* (2016) did not observe any differences between treatment in liver abscess incidence while Parsons *et al.* (2007) didn't measure liver abscess incidence. Another hypothesis is that improved feed efficiency leads to a greater lean weight proportion and therefore a greater dressing percentage. Mader *et al.* (2009) indicated that gain:feed was positively correlated to lean weight and bone weight proportion.

Hales *et al.* (2010) found that KPH (kidney, pelvic, heart fat) decreased as roughage concentration increased from 6% to 10% DM basis in steam-flaked corn based diets. Vance *et al.* (1972) and Bartle *et al.* (1994) found also found that KPH decreased when roughage concentration exceeded 9% DM basis in diets comprised of dry-rolled corn and steam-flaked grain sorghum based diets.

No explanation for this occurrence was provided by either of the authors. Bartle *et al.* (1994) decreased roughage equivalent from 20% to 10% and observed an increase in marbling score and therefore also an increase in percentage carcasses grading USDA Choice. Research by Krehbiel *et al.* (2006) indicates that finishing cattle that receive high energy dense diets (low roughage), are more likely to deposit fat compared to cattle that received low energy dense diets, which serves as an explanation to the observations of Vance *et al.* (1972), Bartle *et al.* (1994), and Hales *et al.* (2010).

A majority of research shows no difference in carcass composition traits when roughage concentration is below 10% inclusion rate in finishing rations (Woods *et al.*, 1969; Utley & McCormick, 1980; Brandt *et al.*, 1987; Gill *et al.*, 1981; Kreikemeier *et al.*, 1990; Bartle & Preston, 1992; Traxler *et al.*, 1995; Calderon-Cortes & Zinn, 1996; Loerch & Fluharty, 1998; Parsons *et al.*, 2007; May *et al.*, 2010; Gentry *et al*, 2016). We can therefore conclude from the research that the optimal roughage inclusion rate to maintain carcasses of high quality and optimum hot carcass weight and dressing percentage, is between 5% and 10% DM.

3.Liver abscess incidence and severity

Although multiple studies have been conducted to investigate the impact of dietary roughage inclusion on performance and carcass characteristics, relatively few of these have investigated the impact that increasing roughage concentration has on liver abscess incidence and severity especially when no metaphylactic treatment is added to the diet (i.e. tylosin or chlortetracycline). In the majority of dietary roughage inclusion research, tylosin was used as a prophylactic against liver abscesses. No differences in liver abscess incidence and severity are evident among cattle fed dietary roughage inclusion rates that range from 0% to 30% DM (Albin & Durham, 1967; Vance *et al.*, 1972; Gill *et al.*, 1981; Stock *et al.*, 1990; Bartle & Preston, 1992; Bartle *et al.*, 1994; Traxler *et al.*, 1995; Farran *et al.*, 2006; Crawford *et al.*, 2008; Hales *et al.*, 2010; May *et al.*, 2010; Quinn *et al.*, 2011; Benton *et al.*, 2015; Gentry *et al.*, 2016). However, these observations are most probably due to the effectiveness of tylosin to reduce liver abscess incidence and severity (Brown *et al.*, 1975; Vogel & Laudert, 1994).

Woods *et al.* (1969) found that liver abscess incidence decreased when roughage inclusion increased from 0% to 15% (DM basis) in dry-rolled corn based finishing rations that contained no tylosin. Loerch & Fluharty (1998) saw similar results when they compared high moisture corn based diets containing no tylosin and either 0% DM or 15% DM corn silage. Contrary to these observations, Brandt *et al.* (1987) and Kreikemeier *et al.* (1990) fed steam-flaked wheat and steam-rolled wheat based diets that contained no tylosin and found high liver abscess incidences but these did not differ between different roughage inclusion rates. They had only 30 animals per treatment, however and therefore had high standard errors. Calderon-Cortes & Zinn (1996) also observed no difference in liver abscess incidence in steam-flaked corn based diets containing no tylosin, but they only had 8 animals per treatment. Maxwell *et al.* (2014) used more animals in their study (n ≈ 77 steers/treatment) and also found no difference in liver abscess incidence and severity between 2 different roughage inclusion rates (7% DM vs. 12% DM) that were added to a diet that was classified as natural beef production diet (containing no antibiotics).

It is therefore evident that more research, using a larger number of animals, is required to investigate the effect that increased dietary roughage, without tylosin in the diet, has on liver incidence and severity. From the research presented it seems that increasing roughage concentration beyond 10% DM likely has no effect on liver abscess incidence or severity.

4.Increasing roughage inclusion rate and its impact on the environment

i. Methane Emissions

Enteric fermentation by ruminants is responsible for production of 80 million tons of methane a year (Moss *et al.*, 2000; Beauchemin *et al.*, 2008). The United States Environmental

Protection Agency (USEPA, 2011) estimated that this production accounts for approximately 22% of all methane production, with 75% of enteric methane production originating from cattle. Enteric fermentation is a process where sugars and carbohydrates (both structural and non-structural) are fermented by anaerobic microorganisms in the rumen, through the Embden-Meyerhof-Parnas pathway to produce VFAs, CO_2 and H_2. During anaerobic fermentation, NAD^+ is reduced to NADH and NADH needs to be re-oxidized to NAD^+ via a hydrogenase enzyme to allow for complete fermentation of carbohydrates. Hydrogen (H_2) inhibits hydrogenase, therefore inhibiting re-oxidation of NADH. Re-oxidation of NADH is essential to allow for complete fermentation of carbohydrates (McAllister *et al.*, 1996; Moss *et al.*, 2000). It is thus essential for H_2 to be removed from the ruminal environment, as a buildup can lead to inhibited microbial growth, decreased fermentation, and decreased VFA production (McAllister & Newbold, 2008). Methane is produced by anaerobic methanogenic microorganisms which are from the domain *Archea* that produce methane from CO_2 and H_2 (Noll, 1992). Methane is therefore referred to as a hydrogen sink. Another key hydrogen sink product is propionate. Moss *et al.* (2000) indicated a strong positive correlation between the ratio of acetate:propionate and amount of methane produced, indicating that an increase in propionate production leads to a decrease in methane production (Johnson & Johnson, 1995; McAllister & Newbold, 2008). It is thus conceivable that a diet where propionate is produced as the primary VFA will yield decreased amounts of methane compared to a diet where acetate is produced as primary VFA. It is well known that diets high in concentrate produce more propionate while diets high in roughage produce more acetate (Davis, 1967; Siciliano-Cortes & Murphy, 1989; Coe *et al.*, 1999). Johnson & Johnson (1995) performed a regression analysis from literature data and found that fermentation of cell wall components is more methanogenic than fermentation of soluble carbohydrates.

High concentrate diets produce less methane compared to high roughage diets. Increasing dietary concentrate levels in dairy production systems are known to decrease methane emissions (Lovett *et al.*, 2005; Lovett *et al.*, 2006; Aguerre *et al.*, 2010). McGeough *et al.* (2010) fed high-roughage rations that consisted of whole-crop wheat (WCW) silage and straw + chaff fed at different ratios (i.e., 11:89, 21:79, 31:69 and 47:53). All of the diets received supplemental concentrate at a rate of 2.6 kg on a DM basis. The authors observed that diets containing WCW and straw + chaff at ratios of 21:79 and 31:69, had the highest NDF values and also the highest methane produced in g/d. They also compared the WCW silage diets to a diet containing *ad libitum* concentrate and found that the *ad libitum* concentrate diet produced significantly less methane than the WCW silage diets. In a study they did earlier that year where they compared finishing diets containing maize silage harvested at different stages of maturity and 2.57 kg DM basis supplemental concentrate with a diet containing *ad libitum* concentrate and 1.27 kg grass silage DM, they once again observed that diets with high NDF values had the highest methane production (g/d). Studies done by Zinn *et al.* (1994), Lovett *et al.* (2003), and Beauchemin &

McGinn (2006) all indicate that increases in roughage inclusion rate in feedlot rations lead to an increase in methane production. Calderon-Cortes & Zinn (1996); however, found no change in methane emissions when roughage inclusion was decreased from 16% to 8% (DM basis) in steam-flaked corn based diets. The authors didn’t provide any theories as to why no difference were observed. Molar proportions of propionate in the same study remained the same regardless of roughage inclusion rate, suggesting that there was no extra hydrogen sink to replace methane as a hydrogen sink product (Johnson & Johnson, 1995).

It has also been proposed that animals that are more efficient produce less methane. When animals increase their energy intake above what they require for maintenance, methane emissions decrease as apparent digestibility of the diet increases (Blaxter & Clapperton, 1965). Metabolic energy is energy that is available to the animal for use and is defined as the remaining energy after subtraction of energy loss from feces, urine and combustible gases (methane) (McDonald *et al.*, 2011). Therefore, a decrease in energy lost as methane leads to more energy being available to the animal for growth. Hales *et al.* (2014) made similar observations when they compared effects of increasing roughage concentrations on energy metabolism in steers fed a dry-rolled corn based diet. They found that as alfalfa hay inclusion increased from 2% to 14% (DM basis), energy lost as methane gas increased and energy retained by steers decreased. These observations from McDonald *et al.* (2011) and Hales *et al.* (2014) indicate that a decrease in energy lost as methane leads to more energy being available to the animal for growth, which in turn improves animal efficiency. Nkrumah *et al.* (2006) found that when residual feed intake (RFI) increased from -1.18±0.16 kg/d to 1.25±0.13 kg/d, there was a negative effect on feed efficiency and increased methane production. An animal that is more efficient may have more energy available for growth and therefore less energy lost as methane (Freetly & Brown-Brandl, 2013). Cattle fed finishing rations containing high roughage concentrations are less efficient (as seen in the previous sections), and it is plausible that these animals may also have a large proportion of their energy that is released as methane.

Another proposed hypothesis as to the effect that decreased roughage inclusion rate has on methane production is its effect on pH. Decreasing forage:concentrate ratio leads to a decrease in ruminal pH (Zinn *et al.*, 1994; Calderon-Cortes & Zinn, 1996; Beauchemin & McGinn, 2005; Aguerre *et al.*, 2010). Finishing cattle fed a diet that is low in roughage have a ruminal pH that is below 5.6 for a longer period of time compared to finishing cattle fed a diet higher in roughage concentration (Morine *et al.* 2014; Weiss *et al.*, 2017). Van Kessel & Russell (1996) indicated that a pH below 6.0 is inhibitory to methanogens. Therefore, cattle fed high concentrate diets maintain a lower ruminal pH, which is suboptimal for methanogens bacteria and therefore those cattle thus produce less methane.

ii. Fecal production

Increasing amounts of feces excreted by feedlot cattle contribute to not only increasing labor and pen maintenance cost (Weiss *et al.*, 2017), but also have an environmental impact. These impacts include leaching of pathogens and nitrates into ground water, leading to eutrophication, buildup of salts and minerals in soil, and production of gases which are harmful to both humans and animals (USEPA, 2011). Gollehon *et al.* (2001) estimated that only about 40% of manure nitrogen and 30% of manure phosphorous that is produced by animal feeding operations (such as feedlots) are used on-farm; that is, when it is applied to cropland at rates set by regulation. Alternatives are to either transport excess manure greater distances to other farms, to change the manure to a product that has more value or that can be transported more easily, to reduce the number of animals, or to change dietary components to reduce the amount of manure produced. The first three options all have a cost associated with them, which may make them less attractive options to feedlots.

An option for decreasing manure production that is practical and cost effective is to decrease the amount of roughage fed in finishing rations. Calderon-Cortes & Zinn (1996) observed a 277 $g.d^{-1}.animal^{-1}$ increase in the amount of feces produced when roughage inclusion was increased from 8% to 16 % DM. Hales *et al.* (2014) found an increase in energy lost due to feces production when alfalfa hay inclusion was increased from 2% to 10% (DM basis) in dry-rolled corn based finishing rations. This implies that as the proportion of dietary roughage increases, production of feces also increases. Not only are cattle losing energy that could have been used for growth, and therefore making them less efficient (as mentioned previously), but costs increase for removal of manure from the pens, storage and application to cropland. These findings are supported by research indicating that decreasing roughage levels lead to a decrease in the amount of manure that is produced by cattle (Zinn *et al.*, 1994; Calderon-Cortes & Zinn, 1996; Crawford *et al.*, 2008; Weiss *et al.*, 2017).

Hales *et al.* (2014) also found a linear increase in nitrogen excreted in the feces when the proportion of alfalfa hay was increased in isonitrogenous diets. Less of the nitrogen consumed by the animal was thus utilized, which is evident by the linear decrease in apparent nitrogen digested as alfalfa inclusion increased. Similar results for dry-rolled corn isonitrogenous diets were observed by Bierman *et al.* (1999). This decrease may be due to the amount of digestible energy available to the microbes in the rumen, which will allow them to utilize the nitrogen to produce amino-acids, peptides and proteins. Zinn *et al.* (1994), Calderon-Cortes & Zinn (1996), and Weiss *et al.* (2017) fed steam-flaked corn based isonitrogenous finishing rations and found no differences in the amount of nitrogen excreted in the feces when roughage inclusion rates were increased. Yan *et al.* (2007) observed that roughage inclusion plays a small role in the amount of nitrogen excreted in feces. In their prediction equation they found that nitrogen intake as a sole predictor provided a correlation coefficient to nitrogen excreted in feces of 0.90; conversely, while adding bodyweight and dietary roughage proportion to the equation only increased the correlation coefficient to 0.94.

We can therefore conclude that increasing dietary roughage increases the amount of feces produced which thus has to be removed, leading to greater costs and a negative influence on the environment. Although its impact on overall nitrogen excretion is small, increasing roughage in finishing rations also seems to decrease the efficiency with which nitrogen is utilized and therefore increases the amount of nitrogen which is excreted in the feces.

iii. Water Consumption

It is well known that there is an increased global demand for feed as global human population increases on a daily basis. This population growth places strain on all natural resources, most important of which is water. In a 2014 freshwater report from the United States Government Accountability Office (GAO), 40 out of 50 state water managers expect water shortages under current conditions in portions of their states in the coming years (GAO-14-430, 2014). Mekonnen & Hoekstra (2012) estimate that agriculture contributes 29% to the global human water footprint and that a third of that number is due to global beef production.

Meyer *et al.* (2006) conducted an experiment with 62 Holstein bulls to investigate the effect of various parameters on water consumption and found that the amount of roughage explained only 4% of the variation seen in water consumption. The top 2 contributors were ambient temperature and feed intake. Winchester & Morris (1956), and Brew *et al.* (2011) arrived at similar conclusions. Therefore, roughage inclusion by itself may not play a significant role in increased water consumption by beef cattle, but increased feed intake associated with increases in dietary roughage may lead to greater water consumption by beef cattle.

Mekonnen & Hoekstra (2012) indicated that the majority of the water footprint from animal agriculture comes from the feed they consume and not the animal itself. They found that approximately 12% of ground and freshwater is used for irrigation of crops and pasture intended for use as livestock feed. Beckett & Oltjen (1993) used government statistics and determined that water usage in feedlots from cattle drinking totaled 153,288 x 10^6 L per annum whereas water used in the production of feed totaled to 8,695,582 x 10^6 L per annum, indicating that 3,682 L of water was required to produce 1 kg of boneless beef in 1992. They also estimated that 257.1 L of water is required to produce 1 kg of alfalfa hay compared to 77.3 L to produce 1 kg corn and 27.3 L to produce 1 kg corn silage, and from their model determined that a 10% increase in area of irrigated pasture will lead to a 163.5 L increase in water required to produce 1 kg boneless beef. When their estimates of beef production in the United States for 1992 are taken into account, it means that a 10% increase in area of irrigated pasture will lead to an increase of approximately 640 000 x 10^6 L water consumed in the United States by feedlots alone. The authors concluded that irrigated pasture is a large contributor to water consumption by the beef industry, and that more sensitive irrigation control is required.

Therefore, increasing dietary roughage in finishing rations might not have a direct effect on water usage by increasing water consumption by the animal. However, it has an indirect effect in that by increasing dietary roughage more roughage will need to be produced and the production of this roughage (predominantly alfalfa in the United States) uses a lot of water and will therefore have a large negative effect on scarce water resources.

Conclusion

Roughage is an important part of the diet and is required to maintain optimum ruminal health and function. Diets that contain no roughage can lead to metabolic disorders such as ruminal acidosis, which can then lead to decreases in feedlot performance and carcass value. However, increasing dietary roughage beyond approximately 10% also has negative consequences on cattle performance without mitigating liver abscess incidence when no tylosin is added to the diet. Furthermore, increasing roughage in finishing rations also has negative environmental effects as it leads to not only greater fecal production by cattle but also increased methane emissions from cattle and creates a strain on water resources as more water is needed to supply an increased demand for roughage. We can conclude from the research presented that increasing dietary roughage inclusion is not a viable option to control liver abscess incidence and severity; therefore, other alternatives need to be investigated.

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Chapter 2 - Effect of alpha tocopherol acetate and ascorbic acid on performance, carcass traits and incidence and severity of liver abscesses in feedlot cattle.

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Abstract

Liver abscesses (LA) in cattle negatively affect feedlot performance by decreasing ADG, feed intake, and gain:feed ratio. Abscessed livers are condemned and abdominal adhesions associated with LA can result in extensive carcass trimming during harvest, further compounding adverse economic impact. Given regulatory changes pertaining to the use of in-feed antibiotics in cattle production, there is growing interest in alternatives to antibiotics for LA control. The objective of this study was to evaluate use of antioxidants, crystalline ascorbate and alpha tocopherol acetate, for mitigation of LA in feedlot cattle. Yearling crossbred heifers (n=392; initial BW 481 ± 9.4 kg) were blocked by previous treatment and allocated randomly 24 dirt-surfaced feedlot pens (10 m x 35 m) with 13 to 14 heifers/pen. Heifers were weighed, implanted with Component® TE-200 implants, and placed into feeding pens. Finishing diets consisted of 60% steam-flaked corn, 30% wet corn gluten feed, 8% alfalfa, and 2% supplement (DM basis) that provided 300 mg/d monensin, and either 200 IU/d alpha tocopherol acetate (CTL) or 2000 IU/d alpha tocopherol acetate plus 500 mg/day crystalline ascorbate (AOX). Heifers were fed once daily *ad libitum* for 94 days, then weighed and transported 450 km to a commercial abattoir for harvest. Hot carcass weight and incidence/severity of LA were determined the day of harvest, and carcass traits were evaluated following 36 h of refrigeration. Compared to CTL, feeding AOX tended to decrease DMI (10.66 vs. 10.31 kg/d; P = 0.08) and improve gain:feed ratio (0.1204 vs. 0.1254; P = 0.12), but did not impact ADG, incidence of LA (25.6 vs 23.5% for CTL and AOX, respectively), HCW (828.4 vs 830.5 kg for CTL and AOX, respectively), or other carcass traits (P>0.20). In conclusion, feeding antioxidants is not a viable alternative to decrease incidence of liver abscesses in finishing cattle.

Keywords: antioxidant, feedlot, liver abscess

Introduction

Liver abscesses are a significant source of economic loss in feedlot cattle. These losses arise due to a decline in feedlot performance and more notably due to a decline in carcass value. McKeith *et al.* (2012) reported that 13.7% of livers were condemned due to major and minor abscesses. Hot carcass weight decreases due to trim loss associated with abscessed livers, as portion of the diaphragm adheres to livers that have severe abscesses (Rezac *et al.*, 2014; Brown & Lawrence, 2014). Cattle with severe liver abscesses are observed to have ADG 0.17 kg/d less compared to animals that are healthy (Rezac *et al.*, 2014), poorer efficiency (Brink *et al.*, 1990), and decreased feed intake (Brink *et al.*, 1990).

Tylosin phosphate is a macrolide antibiotic that is used for prevention of liver abscesses (Tan *et al.*, 1994; Nagaraja & Lechtenberg, 2007; Reinhardt & Hubbert, 2015). Tylosin is effective in decreasing the occurrence of liver abscesses in cattle (Tan *et al.*, 1994; Vogel & Laudert, 1994; Meyer *et al.*, 2013). In 2013, the FDA issued Guidance For Industry (GFI) #213 which encourages reduced use of medically important antibiotics in animal feeding operations (FDA GFI 213, 2013).

Research on the effects of α-tocopherol acetate shows an increase in humoral immune response (Droke & Loerch, 1989; Peplowski *et al.*, 1981; Cusack *et al.*, 2005) and cattle receiving supplemental α-tocopherol acetate have less ruminal lesions in their ventral sac

(Arnold *et al.*, 1992). Tappel (1968) and Packer *et al.* (1979) showed a synergistic relationship between ascorbate and α-tocopherol acetate.

Therefore, the objective of this study was to determine the effect of antioxidants on feedlot performance, carcass characteristics and liver abscess incidence and severity in feedlot heifers fed a finishing ration that contained no tylosin.

Materials and Methods

Live animal procedures were approved by and conducted within regulations set forth by the Kansas State University Institutional Animal Care and Use Committee.

Experimental design

The study consisted out of 2 treatments and was designed as a randomized complete block design with 392 crossbred heifers (481.8 ± 9.45 kg) that were selected from a larger population. Heifers were blocked by previous treatment (different concentrations of lysine added to a receiving diet), and were randomly assigned within block to one of 2 treatments. Treatments consisted of a control treatment in which the diet contained 22 IU/kg supplemental α-tocopherol acetate (Control), and an antioxidant treatment in which the diet contained α-tocopherol acetate and crystalline ascorbate supplemented at a rate of 220 IU/kg and 550 mg/kg, respectively (Antioxidant).

Heifers were housed in 28 dirt surfaced pens that contained 14 animals per pen. Pens were designed to allow for approximately 20.44 m^2 space per animal. Each pen contained automatic waterers that were located between adjacent pens and approximately 6 m from feed bunks. Each pen had a concrete pad that extended 3 m from the feed bunk into the pen. Each feed bunk provided approximately 76 cm of bunk space per heifer.

Cattle Processing

Three hundred and ninety two heifers were selected from a group of 657 heifers that were already housed at the Kansas State University Beef Cattle Research Center (Manhattan, KS). Four hundred and forty eight of these heifers were on a receiving trial were the objective of the trial was to determine how different concentrations of ruminal bypass lysine affected growth performance on a roughage based diet. At the start of the receiving trial all heifers received individual identification in the form of an ear tag, a vaccine that contained clostridial antigens (Ultrabac®7/Somnubac®, Zoetis Animal Health, Florham Park, New Jersey), a 5-way respiratory vaccine (Bovishield Gold®5, Zoetis Animal Health), and an anti-parasitic pour-on treatment (Standguard™, Elanco Animal Health, Greenfield, IN). At the start of the finishing trial individual bodyweights were obtained, and heifers were implanted with Component® TE-200 with Tylan® (Elanco Animal Health). Heifers selected for the trial were then sorted into their respective experimental pens.

Average bodyweights were obtained at 28-d intervals and at the end of the 94-d feeding period. Average bodyweight was determined by dividing pen weight by the number of heifers in the pen. A large platform scale (Central City Scale, Central City, NE), set on 4 electronic load cells, was used to determine pen weights. Load cells were calibrated and verified with certified 1021 kg weights before every use. Average daily gain (ADG) and feed efficiency (gain:feed ratio) were determined from average bodyweight. Average daily gain was determined by subtracting the pen weight obtained on the day by the previous pen weight and dividing the answer by the days on feed (DOF). Feed efficiency was determined by dividing average daily gain by average daily dry matter intake (DMI).

Diet Preparation

Four step-up rations were fed for 21 days to transition heifers over to a high concentrate finishing ration. Ingredient and nutritional composition of the finishing ration is presented in Table 2.1. The control treatment diet had no tylosin and 22 IU/kg α-tocopherol acetate supplemented to the diet, whereas the antioxidant treatment diet had no tylosin, 220 IU/kg α-tocopherol acetate, and 550 mg/kg ascorbate supplemented to the diet.

Heifers were fed once daily starting at approximately 8:00 am using a truck-mounted feed mixer. To maintain *ad libitum* intake with minimal feed residues remaining, feed intakes were determined visually and adjusted accordingly. On days that pens were weighed and on days when there was excessive precipitation, unconsumed feed was removed from the pens to determine dry matter intake and feed efficiency. Dry matter (DM) of the unconsumed feed was determined by drying the sample in a 55^{o}C oven for 48-h. Dry matter intake (DMI) was determined as follows:

DMI = [(total feed delivered x DM %) – (unconsumed feed x DM %)] / number of heifers in pen

Harvest

After 94 DOF, final pen weights were obtained to determine average final bodyweight this was then multiplied by 0.96 to adjust for 4% shrink loss. Heifers were then transported approximately 450 km to a commercial abattoir. Hot carcass weight and liver abscess scores were obtained after heifers were slaughtered. Liver abscesses were scored according to the system set out by Brown *et al.* (1975) where: 0 = no abscess, A- = one or two small abscesses (mild), A = two to four well organized abscesses that are usually not larger than 2.5 cm in diameter (moderate), or A+ = 1 or more large abscess with inflammation surrounding the abscess and is usually characterized by a portion of the diaphragm being adhered to the liver (severe). USDA quality and yield grades, incidence of dark cutters, longissimus muscle (LM) area, 12th rib subcutaneous fat thickness, and marbling score were obtained after, carcasses were left to cool down for 24 hours, using camera imaging software (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany).

Statistical analysis

Feedlot performance (Initial and final BW, ADG, DMI and feed efficiency) was analyzed using the MIXED procedure of SAS version 9.4. The MIXED procedure was used to analyze non-categorical carcass data (HCW, dressing percentage, LM area, 12th rib subcutaneous fat thickness, marbling score, and overall yield grade) and the GLIMMIX procedure was used to analyze categorical carcass data (liver abscess incidence and severity, USDA quality and yield grades). The models contained pen as experimental unit, treatment as fixed effect and block as random effect. Differences in LS means were compared using the PDIFF function of SAS.

Treatment effects were declared significant at $P < 0.05$ and $0.05 > P \geq 0.01$ was declared as a tendency for an effect.

An adjustment for unequal variances using the Tukey method was made when we tested the effect that liver abscess severity has on hot carcass weight gain.

Results and Discussion

During the trial 4 animals were removed from the trial. One animal from the control group was removed due to a physical injury unrelated to the treatment. Three animals from the antioxidant treatment were removed due to reasons unrelated to the treatment. One heifer died due to respiratory disease, another was removed due to a leg injury, and one heifer died, however no cause for her death could not be found after a necropsy was done at the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS).

No interaction effects were detected between previous treatment received in the receiving trial and finishing study treatment.

Feedlot Performance

Feedlot performance is presented in Table 2.2. No differences were detected between treatments for any feedlot performance parameters (P > 0.05). There was a tendency for decreased DMI when the antioxidants were included in the ration (P = 0.075) which lead to a tendency for improved G:F ($P = 0.066$). Pogge & Hansen (2013) found no differences in feedlot performance when they compared high grain finishing rations that contained supplemental vitamin C with those that did not contain supplemental vitamin C. Arnold *et al.* (1992), Yang *et al.* (2002), and Burken *et al.* (2012) also saw similar results when supplemental vitamin E was added to high grain finishing rations. Cusack *et al.* (2009) did a meta-analysis to determine the effect of vitamin E supplementation on feedlot performance and found no effect of vitamin E supplementation on daily gain, feed intake, or feed efficiency.

Similar to our results, Burken *et al.* (2002) observed a tendency for improvement in feed efficiency that was driven by a tendency for a decrease in feed intake when finishing diets containing supplemental vitamin E were compared to a diet containing no vitamin E. This observation can be explained by the impacts that α-tocopherol acetate and ascorbate have on ruminal fermentation characteristics. α-Tocopherol acetate has been shown to increase ruminal bacteria activity and therefore total VFA production (Hidiroglou & Lessard, 1976; Hino *et al.*, 1993; Naziroglu *et al.*, 2002). Similarly, Tagliapietra *et al.* (2013) observed an increase in microbial activity when ascorbate was supplemented to a corn based diet *in vitro*.

Carcass Characteristics

Carcass characteristics are presented in Table 2.3. No differences were observed between control and antioxidant treatment for HCW, dressing percentage, LM area, 12^{th} rib subcutaneous fat thickness, marbling score, or USDA quality grades. These results are consistent with observations of Arnold *et al.* (1992), Burken *et al.* (2012), and Pogge & Hansen (2013).

More yield grade 1 carcasses were observed for heifers that received the control diet compared to those supplemented with α-tocopherol acetate and ascorbate (P = 0.007), whereas heifers that received supplemental α-tocopherol acetate and ascorbate had more yield grade 3 carcasses. Rivera *et al.* (2002) and Burken *et al.* (2012) found that with high α-tocopherol acetate supplementation rates, yield grades of carcasses increased compared to treatments where no α-tocopherol acetate was supplemented.

Response of hot carcass weight gain to different severities of liver abscesses are presented in Figure 2.1. No interaction effect of treatment and liver abscess severity on HCW gain was detected ($P = 0.27$), and no effect of liver abscess on hot carcass weight was detected for any of the treatments ($P > 0.6$). Our data was restricted to the number of livers that had a particular severity score per treatment and therefore, we have high standard errors within the treatment groups and severity groups. Brink *et al.* (1990) found that animals that had mild liver abscesses had an increase in hot carcass weight gain compared to animals that had no liver abscesses.

Liver abscess incidence and severity

Liver abscess incidence and severity is presented in Table 2.4. No differences in liver abscess incidence or severity were detected between the control and antioxidant treatment. Our incidence of liver abscess observed for both treatments is similar to those observed by Vogel & Laudert (1994), Meyer *et al.* (2013) and Maxwell *et al.* (2014) when no tylosin was added to the diet, indicating that the increased amount of α-tocopherol acetate and crystalline ascorbate likely has no effect on incidence of abscesses. Concurrently, Arnold *et al.* (1992) reported no difference when steers weighing approximately 227 kg received finishing rations that were supplemented with 110 IU/kg α-tocopherol acetate and contained no tylosin. They also observed fewer ruminal lesions in the ventral sack of the rumen, indicating that even though α-tocopherol acetate decreased the occurrence of lesions in the ruminal wall, the incidence of liver abscess was still high. This suggests that *Fusobacterium necrophorum* is still getting access to the liver regardless of whether there are lesions in the ruminal wall or not; this leads us to hypothesize that *Fusobacterium necrophorum* may be infecting livers differently than previously thought.

Implications

Supplementing α-tocopherol acetate and ascorbate at high concentrations to finishing rations does not reduce incidence and severity of liver abscesses when compared to a control diet. These antioxidants tend to improve feed efficiency.

Table 2.1 Composition of diets to assess effect of supplemental α-tocopherol acetate and crystalline ascorbate on liver abscess incidence and severity.

Item	
Ingredient, % DM	
Steam-flaked corn	60.10
Corn gluten feed	30.00
Corn silage	8.00
Supplement[1]	1.90
Nutrient composition (DM basis), calculated[2]	
CP, %	13.40
Net energy maintenance, MJ/kg	2.11
Net energy gain, MJ/kg	1.46
NDF, %	19.58
Ca, %	0.69
P, %	0.48
Salt, %	0.30

[1]Contains limestone, salt, urea, trace mineral/vitamin premix to provide (on a total diet DM basis) 0.15 mg/kg cobalt, 10 mg/kg copper, 0.50 mg/kg iodine, 20 mg/kg manganese, 0.10 mg/kg selenium, 30 mg/kg zinc, 2200 IU/kg vitamin A, 22 IU/kg α-tocopherol acetate, and 33 mg/kg monensin (Rumensin®, Elanco Animal Health). α-Tocopherol acetate was added to the supplement to provide 200 IU/kg α-tocopherol acetate and crystalline ascorbic acid was added to the supplement at a rate of 550 mg/kg for the antioxidant treatment.

[2]Calculated based of Nutrient Requirements of Beef Cattle (7th Revised Edition) values

Table 2.2 Effect of supplemental α-tocopherol acetate and ascorbate on feedlot performance

Item	Control[1]	Antioxidant[2]	SEM	*P*-Value
Initial BW, kg	481.4	482.1	9.45	0.76
Final BW, kg	601.4	602.9	7.68	0.70
ADG, kg/d	1.28	1.31	0.036	0.70
DMI, kg/d	10.72	10.36	0.191	0.08
G:F	0.1201	0.1251	0.0023	0.07

[1]Diet contained 22 IU/kg α-tocopherol acetate

[2]Diet contained 200 IU/kg α-tocopherol acetate and 550 mg/kg crystalline ascorbate

Table 2.3 Effect of supplemental α-tocopherol acetate and ascorbate on carcass characteristics

Item	Control[1]	Antioxidant[2]	SEM	*P*-value
Hot carcass weight, kg	377.9	379.3	4.83	0.50
Dressed yield, %	62.82	62.91	0.001	0.64
12th rib fat thickness, cm	1.49	1.54	0.012	0.11
LM area, cm^2	94.1	94.5	0.79	0.59
Marbling score[3]	507	509	8	0.87
USDA Prime, %	9.6	7.4	2.17	0.30
USDA Choice, %	68.4	71.8	3.72	0.38
USDA Select, %	16.4	13.2	3.54	0.13
Sub-select[4], %	5.6	7.6	2.56	0.20
USDA yield grade	2.5	2.6	0.11	0.85
Yield grade 1, %	12.3	4.2	2.81	0.01
Yield grade 2, %	38.0	40.5	5.01	0.63
Yield grade 3, %	37.4	46.3	4.97	0.03
Yield grade 4, %	11.3	9.0	2.93	0.12
Yield grade 5, %	1.0	0.0	3.08	0.70

[1]Diet contained 22 IU/kg α-tocopherol acetate

[2]Diet contained 200 IU/kg α-tocopherol acetate and 550 mg/kg crystalline ascorbate

[3]Marbling score determined by computer imaging system (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany). Small (400-499).

[4]Consists of carcasses grading USDA Standard and USDA Commercial carcasses.

Table 2.4 Effect of supplemental α-tocopherol acetate and ascorbate on liver abscess incidence and severity.

Liver abscess[1], %	Control[2]	Antioxidant[3]	SEM	*P*-value
Total	25.7	23.6	4.37	0.63
Mild	18.0	19.0	3.97	0.82
Moderate	5.7	3.2	2.10	0.23
Severe	1.6	1.1	1.16	0.67

[1]Brown *et al.* (1975)

[2]Diet contained 22 IU/kg α-tocopherol acetate

[3]Diet contained 200 IU/kg α-tocopherol acetate and 550 mg/kg crystalline ascorbate

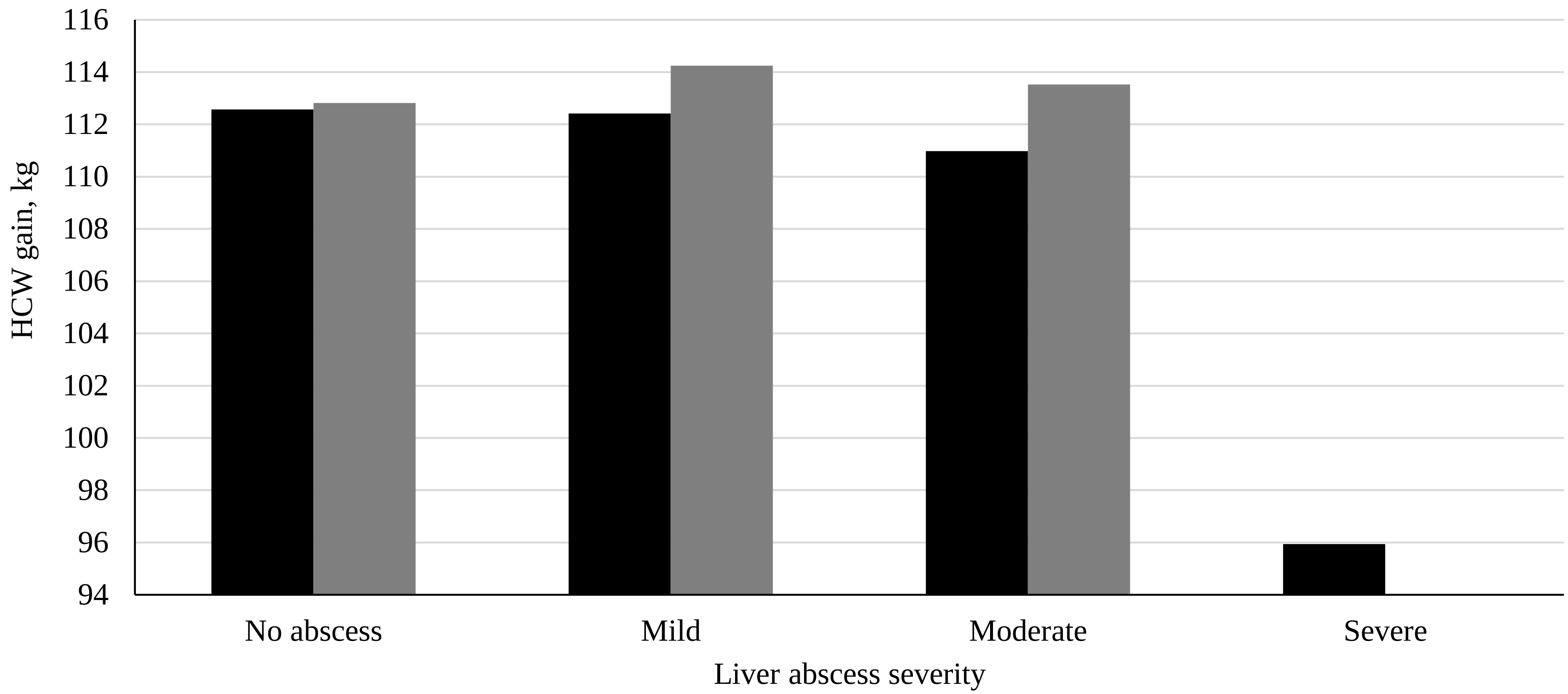

Figure 2.1 Effect of liver abscess severity on hot carcass weight gain. Control treatment (black) contained 22 IU/kg α-tocopherol acetate. Antioxidant (grey) treatment contained 200 IU/kg α-tocopherol acetate and 550 mg/kg crystalline ascorbate. Liver abscesses scored as described by Brown *et al*. (1975).

[1]HCW gain = HCW – (initial BW x 0.56)

No treatment x liver abscess effect, $P = 0.27$

No effect of treatment or liver abscess severity, $P > 0.6$

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Chapter 3 - Effects of intermittent feeding of tylosin phosphate during the finishing period on feedlot performance, carcass characteristics, antimicrobial resistance, and incidence and severity of liver abscesses in steers.

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Abstract

Liver abscesses (LA) are a source of economic loss for beef cattle feedlots, and the 2017 veterinary feed directive has restricted further use of tylosin phosphate to prevention and control of LA. Our objective was to evaluate effects of intermittent tylosin phosphate feeding on incidence and severity of liver abscesses in feedlot cattle and presence of antimicrobial resistant *Enterococcus* spp. Steers (n=312, 411.4± 6.71 kg) were blocked by initial bodyweight and randomly assigned to a treatment group. Treatments included a negative control group (no tylosin throughout the finishing period); positive control group (tylosin fed continuously throughout the finishing period); and a group that received tylosin on a repeated intermittent basis (1 week on, 2 weeks off). Steers were housed in 24 dirt-surfaced pens with 13 steers per pen. Bodyweights of cattle were obtained every 28 days and at the end of 119 d the steers were weighed and harvested at a commercial abattoir. Fecal samples were collected on day 0, 20, and 118 to characterize antimicrobial resistant *Enterococcus* spp. No difference was observed among treatments for ADG ($P = 0.21$), DMI ($P = 0.28$), or feed efficiency ($P = 0.75$). Marbling score was lower ($P = 0.022$) for positive control treatment when compared both to intermittent treatment and negative control treatment. No other differences were detected for carcass characteristics among treatments ($P > 0.10$). *Enterococcus* spp. bacterial counts on plain and antibiotic selective media (m-*Enterococcus* agar) did not differ by treatment group over time ($\mathrm{P} > 0.05$); however, there was a strong period effect for macrolide resistance among all groups ($\mathrm{P} < 0.01$), suggesting an important environmental component as cattle were first placed in pens and then progressed through the feeding period. Total LA count was higher ($P = 0.012$) for the negative control treatment compared to the other treatments, but did not differ between the positive control treatment and the intermittently fed tylosin treatment ($P = 0.716$). We conclude

that feeding tylosin intermittently during the finishing phase decreases the count of LA and maintains feedlot performance and carcass characteristics to the same extent as feeding tylosin throughout the finishing phase, and that antimicrobial resistance is a factor of antibiotic usage in a particular environment for an extended period of time rather than a factor of the treatment during any given feeding period.

Key words: beef cattle, tylosin, feedlot, liver abscess, intermittent feeding

Introduction

Liver abscesses (LA) are a source of economic loss in feedlot cattle. McKeith *et al.* (2012) reported that 5.4% of livers were condemned due to major abscesses and 8.3% of livers were condemned due to minor abscesses. Carcass value decreases due to the trim loss associated with LA (Rezac *et al.*, 2014; Brown & Lawrence, 2010). Cattle with severe LA have an ADG that is 0.17 kg/d lower compared to animals that are healthy (Rezac *et al.*, 2014), a significant decrease in feed efficiency (Brink *et al.*, 1990), and a decrease in feed intake (Brink *et al.*, 1990).

Tylosin phosphate is a macrolide antibiotic that is used by U.S. feedlots (Berg & Scanlan, 1982; Nagaraja & Lechtenberg, 2007; Reinhardt & Hubbert, 2015). Various studies have shown that tylosin is effective in decreasing the incidence of liver abscesses (Tan *et al.*, 1994; Vogel & Laudert, 1994; Meyer *et al.*, 2013). In 2013 the FDA issued Guidance for Industry (GFI) # 213 which established a timetable for the reduction of medically important antimicrobials, such as macrolides, for performance enhancement in animal feeding operations. Guidance for Industry # 213 also laid the framework for implementation of veterinary supervision when using these antimicrobials (FDA GFI # 213, 2013).

It is therefore important to look at alternative approaches to control LA in feedlot cattle. We hypothesized that feeding tylosin intermittently will effectively control the incidence and severity of LA, while reducing total use. Our objectives for this experiment were to evaluate the effect of repeated intermittent feeding of tylosin on feedlot performance, carcass characteristics, incidence and severity of LA in feedlot cattle, and the prevalence of antibiotic resistant *Enterococcus* spp.

Materials and Methods

Procedures involving live animals were conducted and approved within the guidelines of the Kansas State University Institutional Animal Care and Use Committee.

Experimental Design

The trial was conducted from 30 March 2016 to 26 July 2016, and was designed as a randomized block design with 3 treatments using 312 crossbred steers (411.4 ± 6.71 kg). Steers were selected from a larger population and were selected based on their bodyweight and temperament. Steers were then blocked based on their initial bodyweight and were assigned within block to one of 3 treatments. The treatments consisted of a positive control in which steers received a basal diet that contained tylosin throughout the feeding period, a negative control in which steers received a basal diet that contained no tylosin throughout the feeding period, and a treatment where steers received a basal diet with tylosin added to the diet during the step-up phase and then subsequently on a repeated intermittent basis (1 week on, 2 weeks off) (Figure 3.1). Tylosin was fed at a rate of 9.9 mg/kg.

Animals were housed in 24 pens with each pen containing 13 animals/pen. Pens had a 10.1 m × 30.5 m area with pipe fencing and dirt surfaces. Each pen allowed approximately 20.44 m^2 of space per animal. Automatic waterers were located approximately 6 - 9 m from feed bunks and shared by animals in adjacent pens. Each pen had concrete pads extending 3 m into the pen from the feed bunk and each feed bunk allowed for approximately 76 cm of bunk space per steer.

Cattle Processing

Three hundred and eighty-five crossbred steers arrived at the Kansas State University Beef Cattle Research Centre (Manhattan, KS). Steers were placed in holding pens and provided *ad libitum* access to ground alfalfa and water. Twenty-four hours after each load was received, steers were individually weighed, received ear tags for individual identification, were vaccinated with clostridial antigens (Ultrabac®7 Somnubac®, Zoetis Animal Health, Florham Park, NJ), a 5-way respiratory vaccine (Bovishield Gold®5, Zoetis Animal Health), and received a pour-on treatment for parasites (Permectrin CDS, Bayer, Leverkusen, Germany). Fifty animals were given a tetanus toxoid vaccine (BarVac® CD/T, Boehringer Ingelheim, Ingelheim am Rhein, Germany), but were supposed to have received a clostridial antigen vaccine (Ultrabac®7 Somnubac®, Zoetis). These steers were closely monitored and the incident was recorded as a deviation in protocol. Three hundred and twelve steers (n = 312) were selected for use in the experiment. These steers were blocked according to their bodyweight and assigned to treatments. On the first day of the experiment the steers that were assigned to the experiment were individually weighed, implanted with Component® TE-200 with Tylan® (Elanco Animal Health, Greenfield, IN) and placed in their respective experimental pens.

Bodyweights were obtained for all animals in each pen every 28 d and then at the end of the 119-d feeding period. Bodyweights were determined by taking the pen weight and dividing that number by the number of animals in the pen. Cattle in pens were weighed using a large platform pen scale (Central City Scale; Central City, NE) set on 4 electronic load cells; each load cell was calibrated with 1,021 kg of certified weights before each use. These values were used to determine ADG by subtracting the previous BW from the current BW and then dividing the value by days on feed (DOF). Feed efficiency was determined by taking the ADG and dividing it by the DMI. Fecal samples were collected for each pen at the start of the experiment, after the 21-d step up period, and one day before the end of the experiment. These samples were then prepared and sent to researchers in the Department of Veterinary Pathobiology at Texas A&M University for microbiological analyses of antimicrobial resistance.

Diet Preparation

Steers were transitioned to their finishing diets using 4 step-up diets over a 21-d period. These diets were formulated to allow the steers to adapt gradually to the finishing diet that contained a high level of grain. The composition of the diets is presented in Table 3.1. For the positive control diet, tylosin was added to the supplement whereas no tylosin was added to the negative control diet supplement. For the intermittent treatment group, the diet contained tylosin during the 21-d transition period; thereafter, tylosin was fed in 1 week on, 2 weeks off cycle that repeated until the end of the trial. This pattern of tylosin feeding allowed for a 2-wk withdrawal period of tylosin imposed at the end of the 119-d feeding period. Monensin was fed at a rate of 33 mg/kg and ractopamine hydrochloride (Optaflexx®, Elanco Animal Health) was provided at a level of 200 mg/animal for the last 29-d of the feeding period.

Diets were mixed once daily and fed to the steers at approximately 8h00 am using a truck-mounted mixer. Feed intakes were visually determined and adjusted daily to allow for *ad libitum* intake with minimal feed residues remaining in the feed bunk. Unconsumed feed was removed on days that the pens were weighed (to determine feed efficiency) and after excessive precipitation events. To determine DM content a subsample of the unconsumed feed was dried for 48 h in a 55°C oven. DMI was determined for each 28-d interval and at the end of the experiment as follows: DMI = [(total feed accessible x %DM) – (unconsumed feed x %DM)]/ (number of steers x day). Tylosin consumption per head was determined as follows: [pen intake x 0.0041 (percentage tylosin in the diet)] / headcount in the pen. This equation was used to determine the tylosin consumption per head per day, these values were then totaled.

Harvest

After 119 DOF, the steers were harvested. Pen weights were obtained on the day that the steers were shipped, and after pen weights were recorded steers were loaded onto trucks and shipped to a commercial abattoir. Final BW was determined by taking the pen average and multiplying the value by 0.96 (pencil shrink). Liver abscess scores were assessed at the abattoir using the scoring system describe by Brown *et al.* (1975): 0 = no abscesses, A- = one or two abscesses, A = 2 to 4 abscesses which are in average under 1 inch in diameter, and A+ = 1 or more large abscesses. USDA quality grades, yield grades, LM area, 12^{th} subcutaneous rib fat thickness, marbling score and incidence of dark cutters were obtained from the abattoir through camera images (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany).

Isolation of fecal enterococci and estimation of colony forming units

Fecal samples were collected from 8 randomly selected animals per sampling day (0, 20 and 118). Day 0 samples were collected before animals began their feeding trial and were placed into the pens. One gram of thawed fecal sample and glycerol (50/50) mixture was added to 9 mL of sterile phosphate buffered saline (PBS). After thorough mixing, 50µl of this fecal suspension was spiral plated onto each of plain selective m-*Enterococcus* (ME) agar, erythromycin (8µg/mL) infused ME agar, and tetracycline (16µg/mL) infused ME agar using the Eddy Jet 2™ instrument (Neutec Group Inc., NY). The plates were then incubated at 42°C for a period of 48 h before estimating the number of typical *Enterococcus* spp. colonies formed per mL of the fecal sample suspension using the Flash & Go™ colony counter. Further calculations based on all dilutions resulted in colony forming units (CFU) estimates per gram of feces.

Statistical Analyses

Analysis for feedlot performance and carcass characteristics was performed using SAS version 9.4. PROC MIXED procedure was used for feedlot performance and non-categorical carcass data, whereas PROC GLIMMIX procedure was used for categorical carcass data. The model contained treatment as a fixed effect, weight block as a random effect and pen as the experimental unit. Treatment effects were declared significant at a level of $P < 0.05$. Least-squares means were compared between the 2 control groups and the treatment group using the PDIFF function of SAS. The Tukey method was used to accommodate for unequal variances when hot carcass weight gain was determined between animals that had differences in liver abscess severity.

Antimicrobial resistance data were recorded and tabulated by pen (n=24 divided into 3 treatments: 1) no tylosin fed, 2) tylosin fed continuously, or 3) tylosin fed intermittently), sample (n=8 per pen), and day (0, 20, 118) and these were further tabulated by selective medium (no antibiotics in ME agar, or with erythromycin (ERY) at 8 µg/mL or tetracycline (TET) at 16 µg/mL). Log_{10} colony forming units (CFU) were calculated with a single CFU added to each data point. The difference between Log_{10} CFU calculated for plain ME agar versus ME plus ERY or versus ME plus TET for each pen/sample/day was determined and used for multi-level mixed linear regression analysis (Stata® 12.1, Stata Corp., College Station, TX). A full factorial model of treatment by day, adjusted for the clustering effects of pen, was built. Marginal means were estimated and plotted with 95% confidence intervals to examine the interactive and main effects of the pen-level treatments over time. Smaller differences signified a higher proportion of total enterococci that were resistant to either antibiotic; conversely, larger differences represented a smaller proportion of antibiotic-resistant bacteria.

Results and Discussion

Two steers were removed from the positive control group. One steer was removed due to a back injury not related to treatment, and the other steer died due to a respiratory tract infection. One steer from the negative control group was euthanized due to an injury unrelated to the treatment.

Feedlot Performance

Overall feedlot performances for the 3 treatment groups are represented in Table 3.2. There were no treatment effects for ADG ($P \geq 0.207$), DMI ($P \geq 0.278$) and G:F ($P \geq 0.752$). Our results are consistent with what Brown *et al.* (1975), Heinemann *et al.* (1978) and Potter *et al.* (1985) found. However, Meyer *et al.* (2009) observed improved performance when tylosin was added to the diet. Their reported improved performance may be due to the fact that they saw a higher percentage of severe LA in their control group (34 % of total LA) compared to the amount of severe LA we observed (9.1 % of total LA). The larger difference in the number of severe LA between the control group and tylosin treatment group in their study likely led to the significant difference they saw in performance between the treatments. Larger abscesses could lead to a portion of the liver losing function, or more severe predisposing factors such as ruminal acidosis, and thus reflect a greater decrease in growth performance. Sides *et al.* (2009) similarly found no difference in feedlot performance when they performed a study with 4,000 heifers in a commercial feedlot setting. They compared feeding a combination of monensin and tylosin throughout the feeding period to a treatment where they withdrew the combination of monensin and tylosin for 35 days pre-slaughter. They found no difference for DMI, DOF, final BW, ADG or G:F. This is consistent with what we found in the treatment where steers were fed tylosin intermittently. Total tylosin consumption per head in our study is represented in Table 2. There was a 60% decrease ($P < 0.0001$) in the amount of tylosin that was consumed by the intermittent treatment group, when compared to the tylosin treatment group (positive control).

Carcass Characteristics

Carcass performance is represented in Table 3.3. No differences were observed between treatments for HCW ($P = 0.512$), dressed yield ($P = 0.257$), 12th rib fat thickness ($P = 0.860$), LM area ($P = 0.921$), quality grades ($P \geq 0.182$) and yield grades ($P \geq 0.847$). Brink *et al.* (1990) found similar results for HCW and dressed yield. Previous studies have indicated that carcasses with mostly severe LA had lower 12th rib fat thickness and LM area compared to carcasses with normal livers (Brown & Lawrence, 2010; Rezac *et al.*, 2014). This might explain why we did not see a difference in 12th rib fat thickness and LM area as only a few of the livers we saw had severe abscesses.

There was a decrease in marbling score when the continuously fed tylosin treatment was compared to the other 2 treatments ($P = 0.022$). Our findings on marbling scores differed with what Sides *et al.* (2009) and Davis *et al.* (2007) found, in that they found no differences observed in marbling score between cattle that had LA and those that did not have LA. We hypothesize that the decreased marbling score that we observed for the continuously fed tylosin treatment is due to the absence of carcasses that graded prime for the continuous tylosin treatment compared to the no tylosin and intermittent tylosin treatments that had a few carcasses that graded prime.

Differences in hot carcass weight between different liver abscess severities are represented in Figure 3.3. No differences were detected ($P > 0.15$). It does, however, seem that there is a numerical increase in hot carcass weight gain when animals that had a mild liver abscess score are compared to animals that had normal livers. Brink *et al.* (1990) found that animals that had livers with mild abscesses had higher daily gains, final bodyweights, hot carcass weights and were more efficient then animals that had no liver abscesses.

Liver abscess incidence and severity

LA incidence and severity are represented in Figure 3.2. There was an increase ($P = 0.012$) in the total number of LA when the no tylosin treatment was compared to the continuous tylosin and intermittent tylosin feeding treatments; however, no differences ($P = 0.716$) were observed between the two latter treatments. The no tylosin treatment group also had a higher ($P = 0.026$) number of moderate LA compared to the continuous tylosin feeding treatment. There were no further differences observed between treatments for mild LA or severe LA. Our overall results are consistent with what was found in various research projects in the late 1970's that looked at the LA mitigation properties of tylosin fed continuously (Brown *et al.*, 1975; Heinemann *et al*, 1978*;* Pendlum *et al.*, 1978; Vogel & Laudert, 1994); however, there are no published studies of intermittent feeding upon which to base comparisons.

It is of considerable interest to note that there were no treatment effects for total LA between steers that were fed tylosin intermittently and steers that were fed tylosin throughout the feeding period. Sides *et al.* (2009) and Bohrer *et al.* (2016) also noted that simply withdrawing tylosin from the diet for the last 33-d to 35-d on feed decreased the total amount of LA to the same extent as feeding tylosin for the entire time on feed. No explanation was provided by the authors; however, we hypothesize that it might be due to the post antibiotic effect (PAE) of tylosin. Macrolides cause prolonged PAE (which is the inhibition of regrowth of a microorganism after the initial exposure to the particular antibiotic) for gram-negative bacteria (Mathers *et al.*, 2011). Our results indicate the possibility that tylosin has a prolonged PAE which in turn causes the inhibition of *Fusobacterium necrophorum*, a gram-negative bacterium that is the most prominent cause of LA in feedlot cattle (Nagaraja & Lechtenberg, 2007). Alternatively, the most important effects of tylosin phosphate in preventing LA might occur during the periods of greatest risk; that is, during ‘step up’ periods of considerable dietary compositional change. During other constant feeding periods, the importance of the antibiotic may be diminished.

Antimicrobial resistant enterococci

Overall, there were highly significant period (*P* < 0.01) effects when examining the difference between Log_{10} CFU of enterococci grown on plain ME agar versus ME agar with 8 µg/mL of erythromycin (Figure 3.4). A large increase in the proportion of enterococci resistant to erythromycin is implied in the large reduction in differences in counts over the 118 day feeding trial. These period effects were much less pronounced with tetracycline, where levels of resistance were quite high initially (Figure 3.5); however, these differences did decrease over the 118 days. The main effects of both erythromycin and tetracycline were non-significant ($P > 0.05$) at each time period, suggesting that time accrued in the feeding pen environment during the feeding trial was more important than the tylosin regimen being fed.

Generally speaking, there was little difference among treatment groups at each time point; notably, as the amount of time spent in the feeding pen increased the total number of enterococci that were resistant to both erythromycin and tetracycline increased. This was further reflected in decreasing differences in the total CFU between plain ME agar and agar infused with the two antibiotics at breakpoint concentrations. This suggests that the major factor impacting the levels of resistance is not the use of the antibiotic; rather, it is likely the cumulative effect of continued exposure to an environment in which a macrolide such as tylosin has been fed for extended periods of time (e.g., years if not decades). Other authors have noted similar effects for other antibiotic-bacteria combinations (e.g., Agga et al., 2016). Unpublished work from members of our own group was notable for a similar dramatic step-up of resistance upon placement in a feed yard, regardless of treatments (H.M. Scott, personal communication). Thus, it seems probable that beneficial effects of reduced use of tylosin will not manifest immediately, or at least not in the same environment in which the product or other antibiotics have been used in the recent past. Reductions in antibiotic resistance among enterococci are likely to accrue slowly in the absence of major environmental interventions.

Implications

In conclusion, feeding tylosin intermittently during the finishing phase decreased the incidence of LA and maintained feedlot performance and carcass characteristics in steers to the same extent as when tylosin was fed continuously throughout the finishing phase. According to guidelines established by the VFD, if animals are fed intermittently, a new prescription from a licensed veterinarian will be required for every period that tylosin is fed (FDA, 2015). This warrants more research to determine when the optimum time to feed tylosin will be to reduce liver abscesses without having to feed tylosin multiple times.

Table 3.1 Diet composition to assess different tylosin feeding strategies

Item	
Ingredient (%, DM)	
Steam-flaked corn	57.68
Corn gluten feed	30.00
Corn silage	10.00
Supplement[1]	2.32
Nutrient composition (DM basis), calculated[2]	
CP, %	13.30
Net energy maintenance, MJ/kg	2.14
Net energy gain, MJ/kg	1.48
NDF, %	19.06
Ca, %	0.66
P, %	0.49
Salt, %	0.25

[1]Contains limestone, salt, urea, trace mineral/vitamin premix to provide (on a total diet DM basis) 0.15 mg/kg cobalt, 10 mg/kg copper, 0.50 mg/kg iodine, 20 mg/kg manganese, 0.10 mg/kg selenium, 30 mg/kg zinc, 2205 IU/kg vitamin A, 22 IU/kg vitamin E, and 33 mg/kg monensin (Rumensin®, Elanco Animal Health). Tylosin (Tylan®, Elanco Animal Health) was fed at 9.9 mg/kg.

[2]Calculated based on Nutrient Requirements of Beef Cattle (7th Revised Edition) values

Table 3.2 Effect of tylosin feeding strategy on feedlot performance

	Treatment[1]				
Item	No Tylosin	Tylosin	Intermittent Tylosin	SEM	*P*-Value
Initial BW, kg	410.2	411.9	411.9	6.71	0.40
Final BW, kg	628.7	635.3	626.9	4.86	0.23
ADG, kg/d	1.83	1.87	1.80	0.036	0.21
DMI, kg/d	10.89	11.23	10.86	0.235	0.28
Tylosin consumed, kg/steer	0.00[a]	13.23[b]	4.52[c]	0.07	<0.001
G:F	0.1678	0.1665	0.1657	0.0027	0.75

[1]No tylosin received no tylosin, Tylosin received tylosin continuously throughout the feeding period, and Intermittent tylosin received tylosin in a 1 week on, 2 weeks off pattern

[a,b,c] Means within a row and without a common superscript letter are different, $P < 0.05$.

Table 3.3 Effect of tylosin feeding strategy on carcass characteristics

	Treatment[1]				
Item	No Tylosin	Tylosin	Intermittent Tylosin	SEM	*P*-value
Hot carcass weight, kg	380	383	380	6.11	0.51
Dressed yield, %	65.4	64.9	65.8	0.21	0.26
12th rib fat thickness, cm	1.25	1.27	1.25	0.030	0.86
LM area, cm^2	88.7	86.9	89.1	1.30	0.92
Marbling score[2]	455[a]	429[b]	458[a]	12	0.02
USDA Prime, %	1.0	0	1.9	1.38	0.38
USDA Choice, %	75.0	73.5	76.7	6.01	0.85
USDA Select, %	24.0	26.6	18.5	5.82	0.39
Sub-select[3], %	0.0[a]	0.0[a]	2.9[b]	1.12	0.05
USDA yield grade	2.6	2.6	2.5	0.11	0.85
Yield grade 1, %	4.9	2.0	3.9	2.58	0.52
Yield grade 2, %	39.8	40.6	43.3	6.92	0.87
Yield grade 3, %	49.5	52.5	48.1	6.97	0.81
Yield grade 4, %	5.8	5.0	4.8	3.12	0.94

[1]No tylosin received no tylosin, Tylosin received tylosin continuously throughout the feeding period, and Intermittent tylosin received tylosin in a 1 week on, 2 weeks off pattern

[2]Marbling score determined by computer imaging system (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany). Small (400-499)

[3]Consists of carcasses grading USDA Standard and USDA Commercial carcasses

[a,b]Means within a row and without a common superscript letter are different, $P < 0.05$

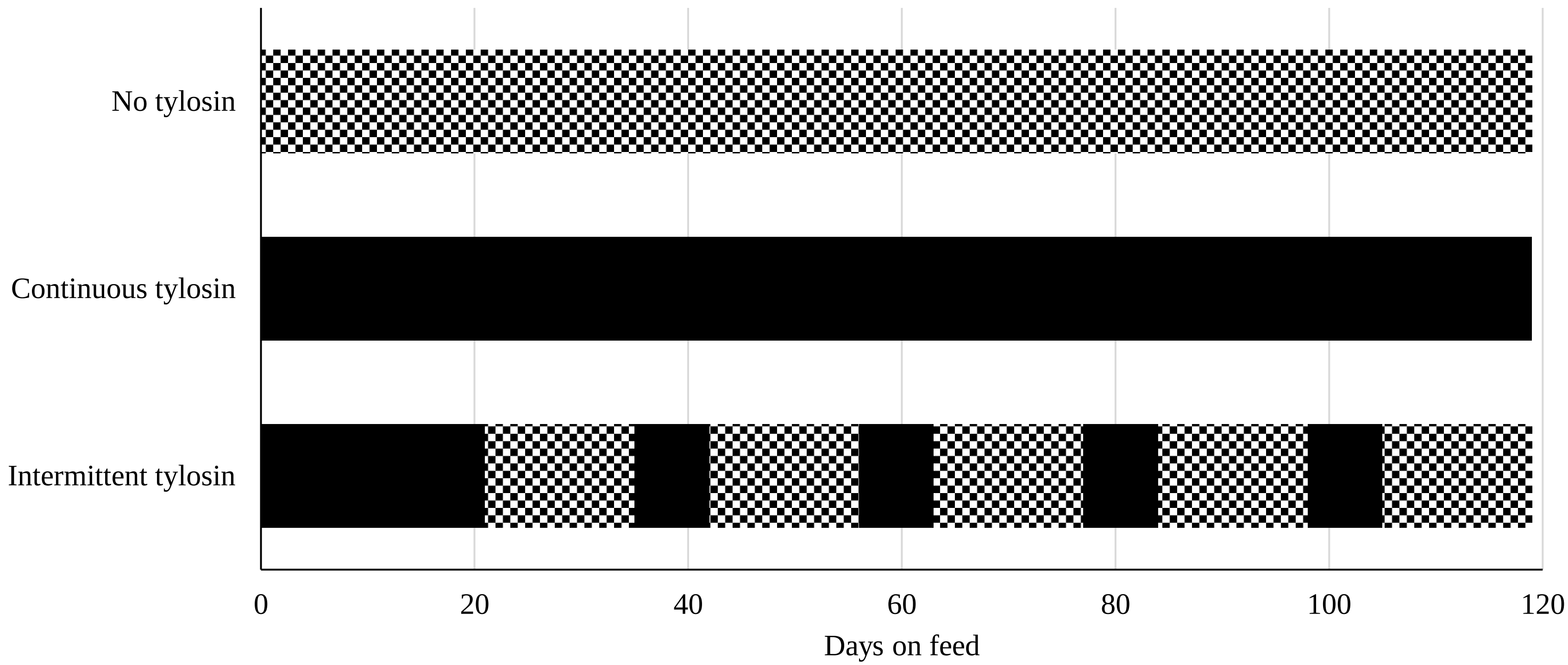

Figure 3.1 Treatment design to describe different tylosin feeding strategies**.** Diet containing no tylosin is represented by a checkered pattern, while a diet containing tylosin is represented by solid black. No tylosin treatment received no tylosin, Tylosin treatment received tylosin continuously throughout the feeding period, and Intermittent tylosin treatment received tylosin in a 1 week on, 2 weeks off pattern.

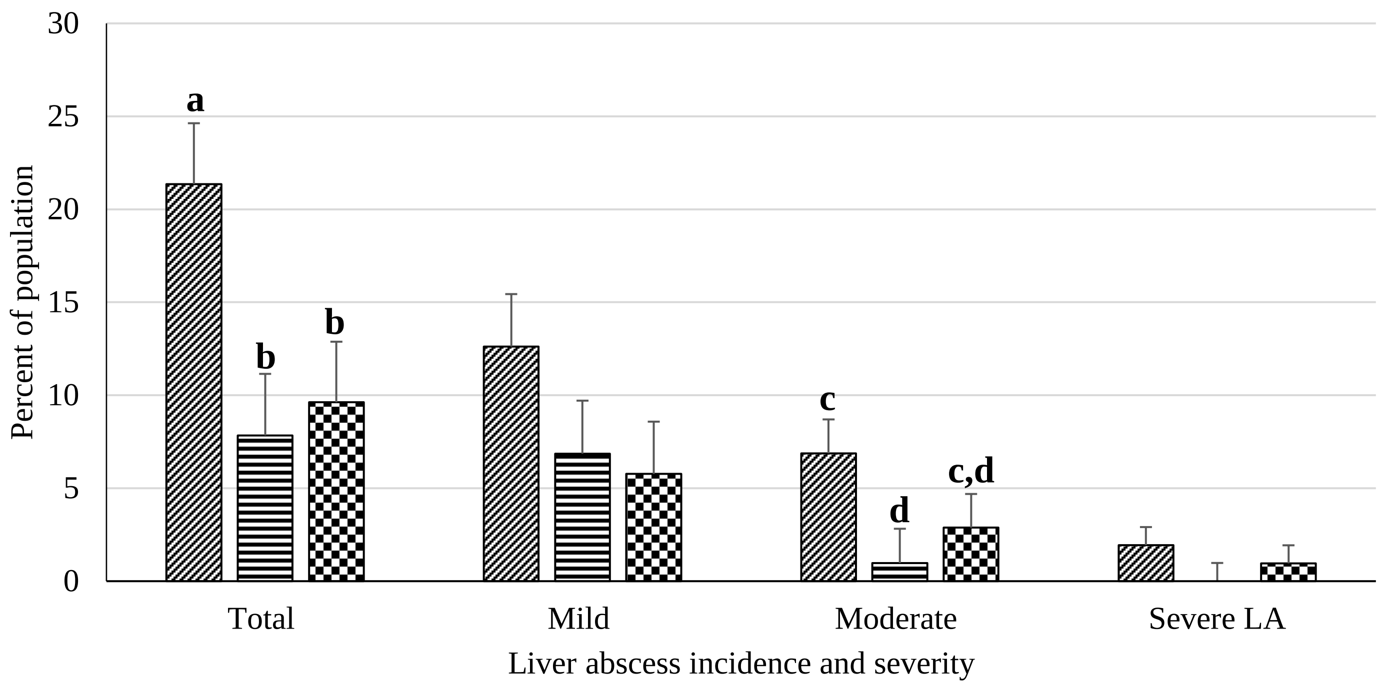

Figure 3.2 Effect of tylosin feeding strategy on the incidence and severity of liver abscesses (LA). Liver abscess scores were assessed using the scoring system described by Brown *et al.* (1975): 0 = no abscesses (mild), A- = one or two abscesses (mild), A = 2 to 4 abscesses which are in average under 1 inch in diameter (moderate), and A+ = 1 or more large abscesses (severe). No tylosin treatment (diagonal lines) received no tylosin, Tylosin treatment (horizontal lines) received tylosin throughout the feeding period, and Intermittent tylosin treatment (checkered) received tylosin in a 1 week on, 2 weeks off pattern.

[a,b,c,d]Bars without common subscripts differ, $P < 0.05$.

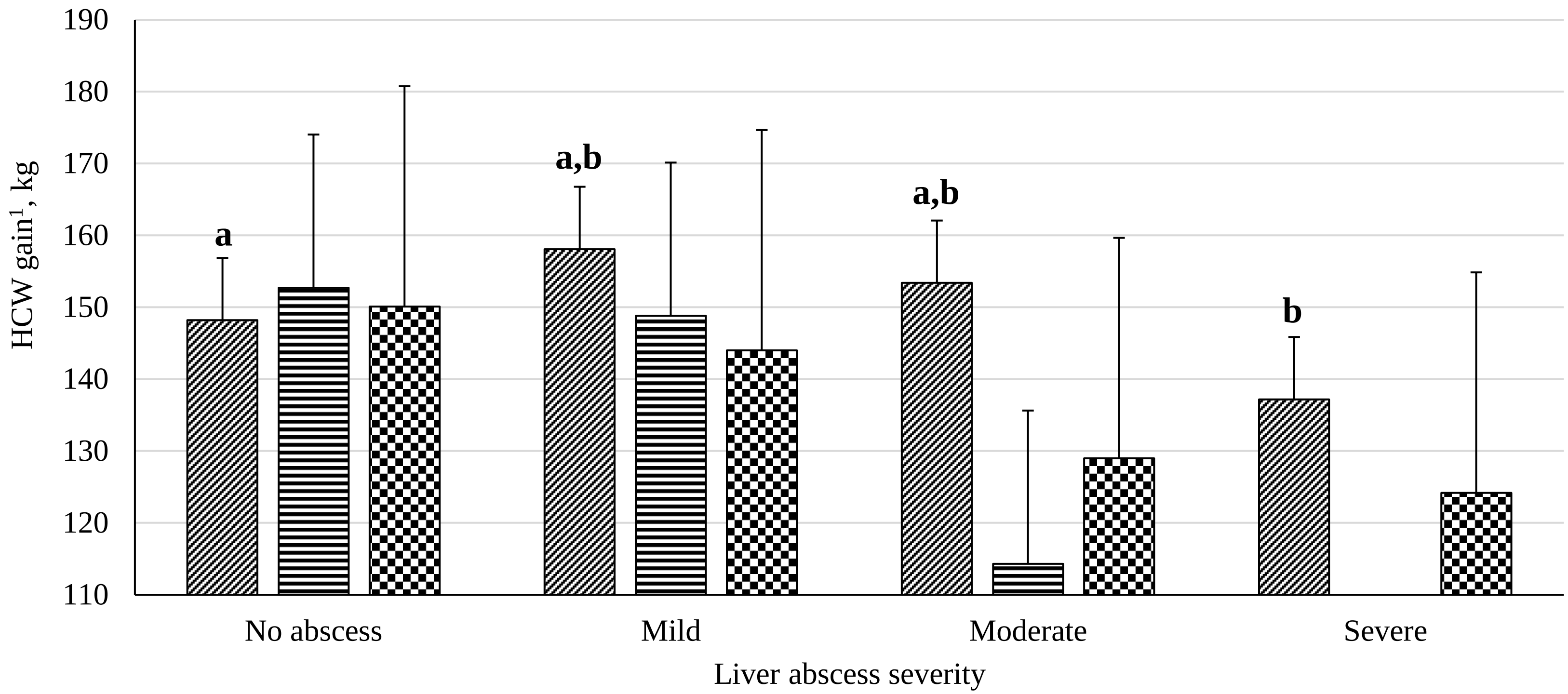

Figure 3.3 Impact of liver abscess severity on hot carcass weight gain. Liver abscess severity scored according to the system described by Brown *et al.* (1975). No tylosin treatment (solid black) received no tylosin, Tylosin treatment (horizontal lines) received tylosin throughout feeding period, and Intermittent tylosin treatment (diagonal lines) received tylosin in a 1 week on, 2 weeks off pattern.

[a,b,c,d]Bars without common subscripts differ, $P < 0.05$.

[1]HCW gain = HCW – (initial BW x 0.56)

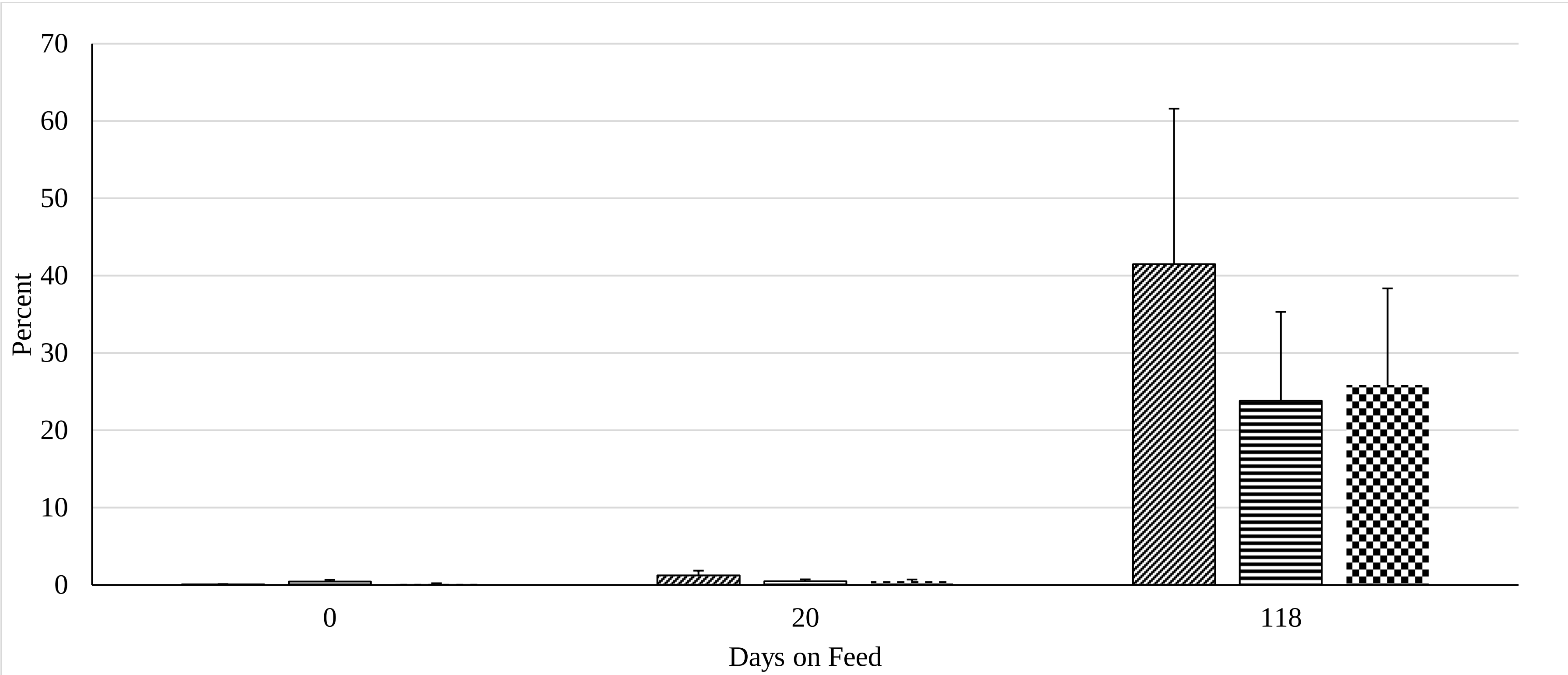

Figure 3.4 Effect of different tylosin feeding strategies on percent of *Enterococcus* population resistant to erythromycin. Adapted from a multi-level mixed linear regression analysis. No tylosin treatment (diagonal lines) received no tylosin, Tylosin treatment (horizontal lines) received tylosin throughout the feeding period, and Intermittent tylosin treatment (checkered) received tylosin in a 1 week on, 2 weeks off pattern.

Treatment x day, $P = 0.35$

Effect of sampling day, $P < 0.001$

Effect of treatment, $P = 0.63$

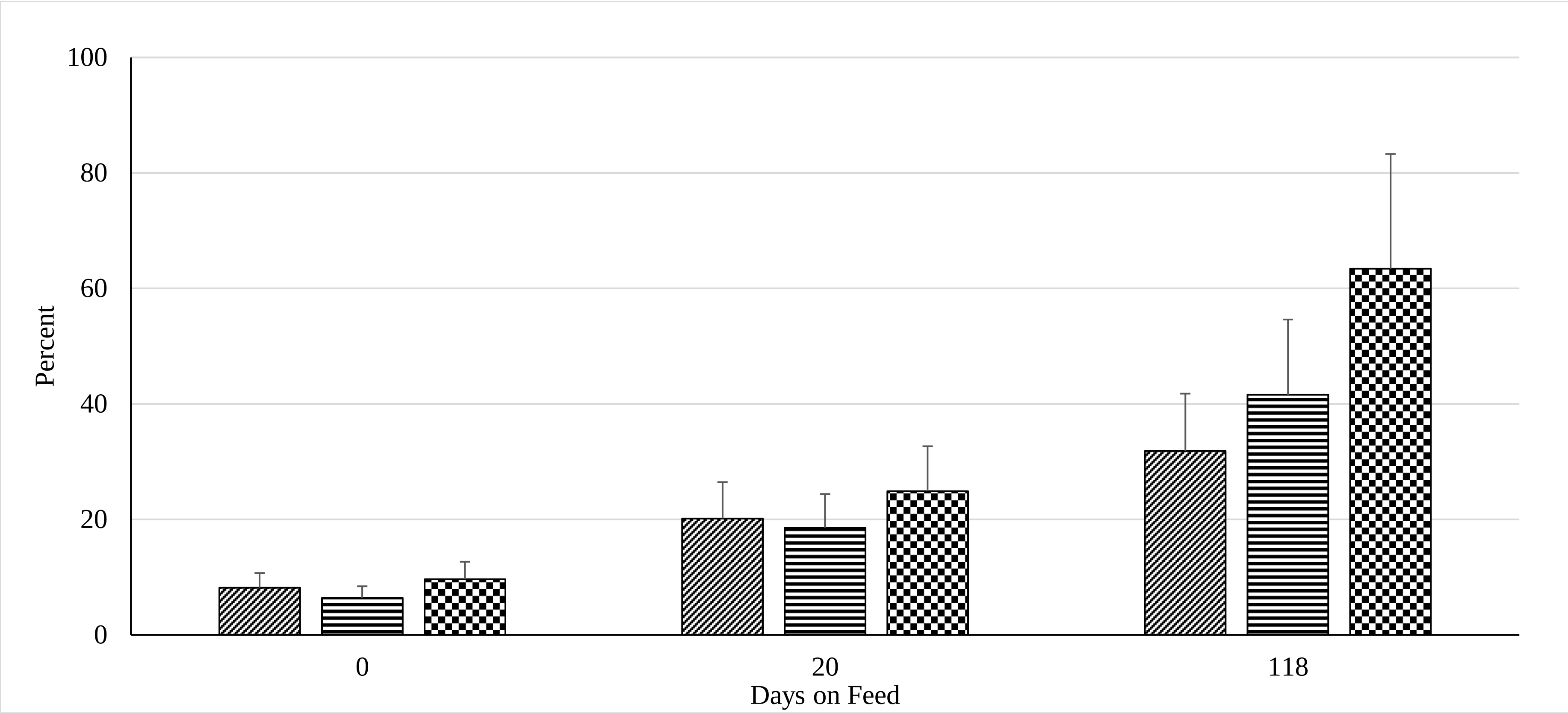

Figure 3.5 Effect of different tylosin feeding strategies on percent of *Enterococcus* population resistant to tetracycline. Adapted from a multi-level mixed linear regression analysis. No tylosin treatment (diagonal lines) received no tylosin, Tylosin treatment (horizontal lines) received tylosin throughout the feeding period, and Intermittent tylosin treatment (checkered) received tylosin in a 1 week on, 2 weeks off pattern.

Treatment x day, $P = 0.42$

Effect of sampling day, $P < 0.05$

Effect of treatment, $P = 0.71$

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