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Article

Evaluation of two supplemental zilpaterol hydrochloride sources on meat quality and carcass traits of crossbred *Bos indicus* bulls in the tropics

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Abstract:

It was studied the effect of two zilpaterol hydrochloride (ZH) brands on carcass and meat quality traits of crossbred *Bos indicus* young bulls under tropical conditions. The patented ZH formulation (Zilmax®, ZHp) and a generic brand (Zipamix®, ZHg) were added to the feed (6 ppm) for 30 d before slaughter. Animals (n= 288) were randomly assigned to 1 of 3 diets, with 32 animals per pen and 3 replicates, for a total of 96 bulls per treatment: 1) basal diet without ZH (Control), 2) basal diet supplemented with Zipamix® at 6 ppm in the diet, as fed-basis (ZHg), and 3) basal diet supplemented with Zilmax® at the same concentration in the feed (ZHp). Carcass yield traits were significantly improved by ZH supplementation. Carcasses of ZH-treated bulls were 6-9 kg heavier (P=0.0023) and produced about 8-10 kg more of lean tissue (P<0.0001) as compared to the Control group. Carcass quality traits were less affected by ZH supplementation. Among meat quality attributes, ultimate pH of ZHg (5.81) and ZHp (5.89) was higher (P=0.0022) than that of the Control (5.78). Results showed both ZH brands, when administered for 30 d before slaughter, as recommended by the manufacturer, improve most carcass yield traits without compromising carcass or meat quality attributes. Hence, tropical beef producers may use the ZH formulation of lowest cost to improve their productivity.

Key words: Zilpaterol hydrochloride, Generic, Yield grade, Quality grade, Carcass, Beef, *Bos indicus*.

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Introduction

The food demand is predicted to increase 70 % by the year 2050[1]. This imposes a significant challenge on food production, particularly meats, which represent a significant proportion of the human diet[1]. Consequently, meat producers have adopted different technologies aimed at maximizing productivity. Among these, growth promoters have been shown to improve animal performance and carcass traits in several livestock species, including beef cattle[2].

Zilpaterol hydrochloride (ZH) is approved as a growth promoter for beef cattle in Mexico, North America and South Africa. It has been reported that steers supplemented with ZH improve their carcass weight between 5 and 7 % and their dressing percentage between 3 % and 3.5 %, as compared to untreated animals[3,4]. Moreover, feed supplementation with ZH has been shown to increase the *longissimus* muscle area[5], which is positively correlated with meat yield.

While positive effects of ZH on carcass traits are well documented in *Bos taurus* cattle, studies on *Bos indicus* are very limited. This information is relevant in several countries, such as Mexico, where 90 % of the slaughter population have a strong *B. indicus* genetic background[6], which is associated with poorer growth performance, carcass traits, and meat quality characteristics. Moreover, although ZH supplementation is known to increase utility per animal[7], the cost per kilogram of meat produced with ZH has been estimated at 1.53 to 1.62 USD[8], which represents around 35 to 40 % of the average market price per kg of beef carcasses in Mexico[9]. After the patent for ZH formulation expired, several generic ZH brands (ZHg) have become available. Since ZHg may represent a cheaper alternative as compared to the patented product (ZHp), ZHg brands have been recently studied. Avendaño-Reyes *et al*[2] observed no differences in slaughter weight or carcass traits of crossbred cattle (75 % *B. indicus*, 25 % *B. taurus*) treated with either a ZHg or the ZHp. However, this study was conducted with a limited number of animals per treatment (n=15). A former publication by the same research group of this study[10] also reports no differences in feedlot performance, beef proximate composition or consumer acceptability of meat from crossbred cattle (75 % *B. indicus*, 25 % *B. taurus*) treated with either a ZHg or the ZHp. Nonetheless, data on their effects on carcass and meat quality traits are limited. This information is necessary for a better assessment of the cost-benefit ratio of ZH use in feedlot cattle of *B. indicus* genotypes under commercial conditions. Therefore, the objective of this study is to assess differences in carcass traits and meat quality of *B. indicus* young bulls supplemented with either a ZHg (Zipamix®, PiSA agropecuaria, Mexico) or ZHp (Zilmax, MSD, Summit, NJ, USA) under tropical conditions.

Material and methods

Animals and treatments

The study was conducted during the summer of 2016 in a company from San Luis Potosi, Mexico, which integrates a commercial feedlot and a beef slaughterhouse operation. All animals were managed according to official Mexican standards for the care and management of animals during transport and slaughter[11,12].

A total of 810 crossbred young bulls were selected for the experiment, based on the following criteria: 1) Only healthy animals were admitted, 2) A minimum of 50 % *B. indicus* genetic background, 3) Not older than 24 mo of age, and 4) Not less than 430 kg live weight. Animals meeting these requirements were distributed in nine pens of 90 animals each. Pens were 40 x 45 m and had 16 % of shade covering mainly the feeders. The animals had *ad libitum* access to water by means of automated water systems (two per pen), which were located at the side of each pen.

Upon selection, all bulls received an ivermectin injection (Dectiver®, Lapisa, Mexico) at a dose of 200 µg/kg, to control ectoparasites, and were vaccinated for clostridial diseases (Ultrabac/somubac®; Zoetis, Mexico). They also received an anabolic implant (200 mg of trenbolone acetate and 28 mg of estradiol benzoate, Synovex-plus®, Zoetis, Mexico) in the left ear. Animals were subjected to an adaptation period of 2 mo before beginning the test. Bulls were monitored daily and animals with evident signs of disease or injuries were removed from the trial. Finally, to conduct the experiment, animals were randomly assigned to three groups (n= 32) with three replicates each, as follows: 1) Basal diet without ZH (control), 2) Basal diet supplemented with the generic ZH brand Zipamix® at 6 ppm in the diet, as fed-basis (ZHg), per manufacturer's instructions, and 3) Basal diet supplemented with the patented ZH brand Zilmax® at the same concentration in the feed (ZHp), as recommended by the manufacturer. Both ZH commercial brands contain 48 g of the active ingredient per kilo of product, and the amount of commercial preparation added was 125 g/kg of feed in both cases.

All groups received the same corn-based basal diet (Table 1). Both ZH brands were included in the vitamin-mineral premix before it was incorporated into the basal diet. For that purpose, we weighed supplemental ZH to the nearest 0.001 g and mixed it thoroughly for about 5 min with the other premix ingredients in a paddle mixer. To prevent cross-contamination, the mixer was cleaned before preparing each experimental diet. The premix was prepared weekly, and the feed was prepared with and without ZH twice daily. We tested the uniformity of ZH mixing in batches of 5, 6, and 7 tons of ZH-supplemented feed (12 samples from each batch), with the aid of micro-tracers (Micro-Tracers Inc., San Francisco, USA), as previously described[13]. Feed was served twice daily (0700 and 1300) using Rotomix® automated trucks (International Trucks®, TX, USA), with an integrated weighing machine to verify the quantity. A 3 % food excess was delivered based on previous food consumption records per body weight. Unconsumed feed was removed, weighed and recorded daily.

Table 1: Dietary ingredients and chemical composition of the basal diet on dry matter (DM) basis

Ingredient	**%**
Dry-rolled corn	61.0
Dry distillers grains	14.0
Barley straw	8.0
Sugar cane molasses	6.0
Corn silage	5.0
Tallow	3.0
Elit-f (vitamin-mineral premix)	2.5
Soybean flour	0.5
Chemical composition[1]	
DM, %	80.9
Crude protein, %	14.0
Crude fat (ether extract), %	6.6
Carbohydrates (excluding fiber), %	56.4
Neutral-detergent fiber, %	18.4
Acid-detergent fiber, %	11.5
Ash, %	4.6
Calcium, %	0.9
Phosphorus, %	0.3
NE_m, Mcal/kg	2.2
NE_g, Mcal/kg	1.5

NE_m and NE_g calculations using equations proposed by NRC (2000).

The experimental feeding period lasted 30 d, followed by a 3-d withdrawal period of ZHg and ZHp, when all animals received the non-supplemented basal diet. On the third ZH withdrawal day, the bulls received only 40 % of their regular daily ration. Subsequently, 32 animals from each treatment were randomly selected and ship to the slaughterhouse for three consecutive days. Hence, a total of 96 bulls per treatment were actually evaluated. Transportation to the slaughterhouse was done early in the morning (at around 0500 h). The trip took about 10 min since the slaughterhouse is only 1 km off the feedlot.

To prevent bias, the trial was conducted as a randomized blind study. Thus, the investigators involved in carcass and meat quality evaluation did not know to which treatment the animals belonged. Moreover, animals from each treatment were slaughtered in a different order each of the 3 d. Slaughter and fabrication were carried out in a Federally Inspected slaughterhouse, following official regulations(11,12,14,15). It was recorded hot carcass weight (HCW) before carcass cooling at 2 °C for 24 h.

Carcass traits

Carcass traits were evaluated according to the USDA Beef Carcasses Grading System(16). Overall maturity was determined based on lean and skeletal maturity. Carcasses were assigned to one of the following overall maturity degrees: 100=USDA A^{100}/B^{00} or less, 200=USDA B^{00}-C^{00}, 300=USDA C^{00}-D^{00}, 400=USDA D^{00}-E^{00}, 500=USDA E^{00} or higher. It was also used USDA visual standards to determine the marbling degree of the m. *longissimus thoracis* (LM): 100=practically devoid^{00}, 200=traces^{00}, 300=slight^{00}, 400=small^{00}, 500=modest^{00}, 600=moderate^{00} and 700=slightly abundant^{00}. USDA quality grades were assigned based on marbling and maturity, as follows: Utility=300, Commercial=400, Standard=500, Select=600, Choice=700, Prime=800.

Kidney, pelvic and heart fat (KPH) was estimated as a percentage of hot carcass weight. It was also measured backfat thickness at the 12th rib, at ¾ of the top of the ribeye and perpendicular to the LM. Moreover, the lean area of the ribeye was drawn in an acetate and this was used to determined LM area with the aid of a planimeter (Digital type roller Placom KP-90N). These factors were used to assign carcasses to USDA yield grades 1 to 5(16).

Meat quality attributes

Beef color and ultimate pH (pHu) of LM were also determined at 24 h *post mortem*, after evaluating carcass traits. The pHu was determined as the average of two measures taken with a digital Hanna H199163 pH meter, with automatic temperature compensation and coupled with a penetration probe (Hanna Instruments, Woonsocket, Rhode Island, USA).Color measurements were performed following the American Meat Science Association Guidelines(17). The LM was allowed to bloom at 2 to 3 ºC for about 30 min before measuring instrumental color variables. It was used a HunterLab® MiniScan EZ 4500L (Hunter Associates Laboratory, Reston, Virginia) with a 10º observer and a 25-mm aperture size, set with illuminant A, the specular component excluded, and the CIELAB scale. The spectrophotomer was calibrated before conducting color measurements and at 100-reading intervals. It was taken a total of 3 to 4 readings of each LM, in a region free of fat deposits and/or connective tissue. The resulting color data (lightness, L*; redness, a*; yellowness, b*; hue, h*; chroma, C*) were averaged for statistical comparisons.

It was used pHu and L* values to estimate the incidence of dark-cutting beef for each treatment. The criteria used to identify a dark-cutter were pHu>6.0[18] and L*<35[19].For Warner-Bratzler shear force (WBSF) and cooking loss analyses, it was took a 2.5 cm thick steak from the LM between the 10th and the 12th ribs. The steak was vacuum-packed and aged for 11 d at 1±1 ºC. On d 12, it was frozen at -18 ºC for about 2 wk and slowly thawed at 4 ºC for 48 h before conducting the analyses. Both cooking loss and WBSF were determined according to the American Meat Science Association Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat[20], as previously described[21].

Statistical analysis

The effect of ZH supplementation on carcass and meat quality traits was tested for significance through a one-way analysis of variance. It was used the General Linear Model procedure of Statgraphics Centurion XV software, version 15.2.05 for Windows (Statpoint Technologies Inc., Warrenton, VA). Initial weight, degree of *B. indicus* genotype and slaughter age did not differ between treatments. Hence, these variables were not considered as sources of variation in the model. When significant (P<0.05) differences between treatments were detected, means were discriminated using the Tukey's range procedure. For proportion variables, it was conducted a chi-square test to determine if there was association between these variables and treatments.

Results and discussion

Feed supplementation with both ZH brands significantly improved most carcass yield traits as compared to the control group (Table 2). In average, carcasses of ZH-supplemented bulls were 6 to 8 kg heavier than those of untreated animals. They also had higher LM areas and produced nearly 10 kg more of lean in relation to bulls fed the basal diet. Among the two carcass fatness variables, KPH was lowest in the ZHg treatment (P=0.0169). However, USDA yield grade was similar in both ZH treatments, and lower compared to the control group. This is consistent with the higher lean content of carcasses from ZH supplemented animals, which resulted in a higher proportion of USDA yield grade 1 in both ZH treatments (Figure 1). Overall, carcass yield traits across ZH brands were comparable.

Table 2: Effect of ZH supplementation on yield-related traits of bull carcasses

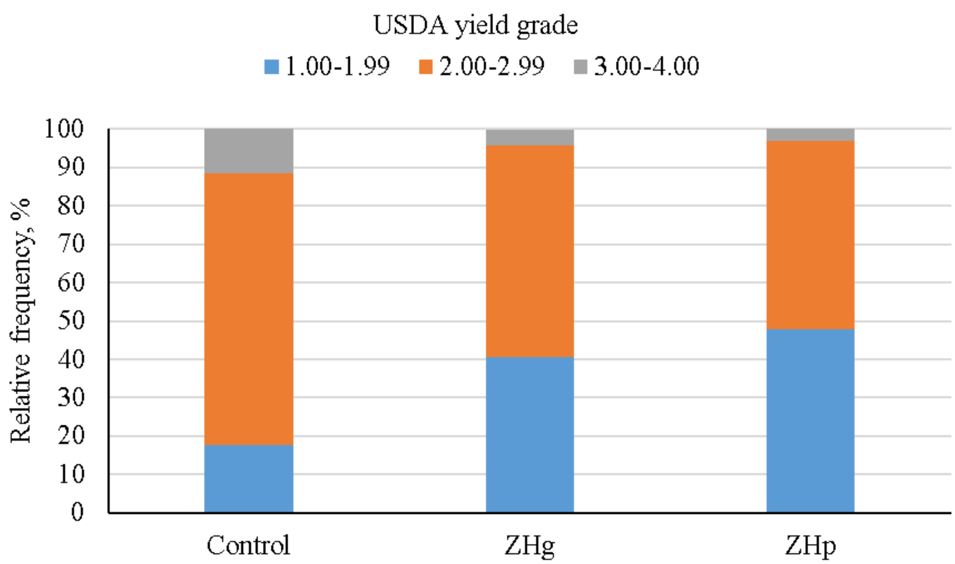
Variable	Treatments[1]				
	Control n=96	ZHg n=96	ZHp n=96	SEE[2]	*P*-value
Initial liveweight, kg	466.44	464.97	465.80	15.40	0.8032
Slaughter weight, kg	511.28	518.80	513.60	24.05	0.0872
Hot carcass weight, kg	311.48[a]	319.96[b]	317.22[b]	17.00	0.0023
Lean, kg	181.42[a]	191.42[b]	189.68[b]	12.98	<0.0001
Longissimus muscle area, cm^2	69.17[a]	75.53[b]	76.89[b]	10.63	<0.0001
Backfat thickness, cm	0.40	0.35	0.40	0.19	0.0562
Kidney, pelvic and heart fat, %	1.68[b]	1.49[a]	1.58[ab]	0.46	0.0169
USDA yield grade	2.42[b]	2.07[a]	2.06[a]	0.53	<0.0001

[1]Treatments, control: no ZH supplementation, ZHg: generic ZH (Zipamix®) at 6 ppm in the diet for 30 d, ZHp: patented ZH (Zilmax®) at 6 ppm in the diet for 30 d.

[2]Standard error of estimation.

[a,b] Means with different superscript within row are different ($P<0.05$).

Figure 1: Relative frequency of USDA yield grade in carcasses of young bulls with no ZH supplementation (control) or supplemented with either a generic ZH (ZHg, Zipamix ®) or a patented ZH (ZHp, Zilmax ®) formulation at 6 ppm in the diet for 30 d (n=96 per treatment)



These findings are consistent with previous studies documenting a positive effect of ZH supplementation on carcass traits of *B. taurus* cattle[22,23]. In general, results are also consistent with previous reports documenting a similar effect of different ZH brands on carcass traits of *B. indicus* bulls[10] and lambs[24]. Nonetheless, these results fail to support previous observations of a limited effect of ZH supplementation on carcass leanness of *B.*

indicus cattle(2). This could be partially explained by differences in sample size, composition of the basal diet, as well as animal selection criteria between experiments, among other factors. Moreover, bulls we subjected to a pre-trial adaptation period of 2 mo, instead of the 7-d period used by Avendaño-Reyes *et al*(2), which may have led to different outcomes.

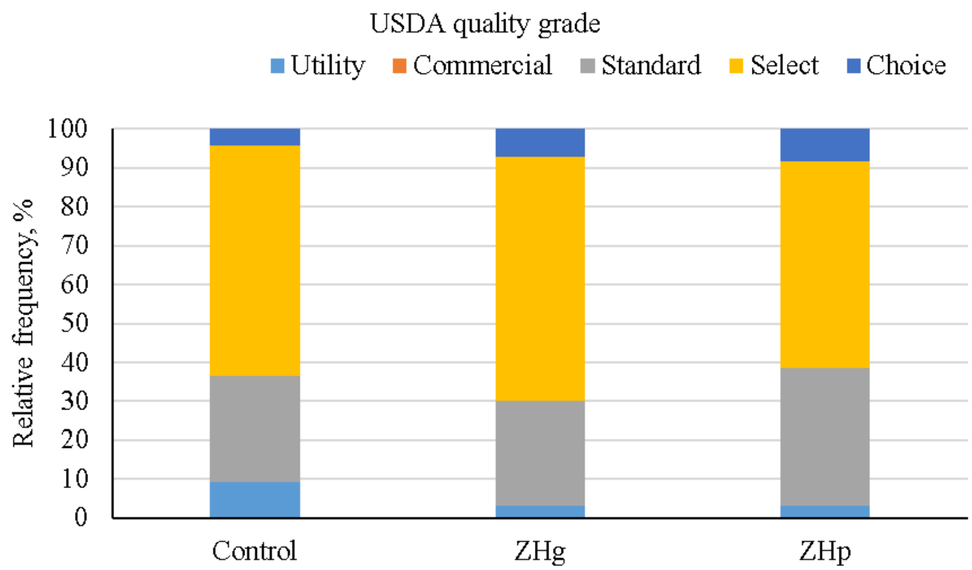
The changes induced by ZH supplementation on carcass quality traits were less pronounced (Table 3). For instance, dietary ZH did not affect marbling score (*P*=0.4991). In average, it remained around 300 (Slight category) across treatments, which is typical of bull carcasses from the tropics. In contrast, numeric values for overall maturity were significantly lower (*P*=0.0217) in carcasses from ZH-supplemented bulls as compared to the control group. These differences, however, lack of practical importance since the average overall maturity of all treatments corresponded to the A category, which is typical of young animals. Moreover, the average USDA quality grade for all treatments corresponded to a quality category between “Standard” and “Select”. In fact, around 90 % of carcasses from all treatments were graded as Standard or Select (Figure 2). Overall, as observed for yield-related traits, results for carcass quality traits were similar across ZH brands.

Table 3: Effect of ZH supplementation on quality-related traits of bull carcasses

	Treatment [1]				
Variable	**Control n=96**	**ZHg n=96**	**ZHr n=96**	**SEE[2]**	***P*-value**
Marbling score[3]	305.10	303.23	291.77	84.73	0.4991
Overall maturity[4]	136.26[b]	116.46[a]	116.17[a]	56.16	0.0217
Quality grade[5]	538.46	563.54	556.38	81.55	0.0993

[1]Treatments, control: no ZH supplementation, ZHg: generic ZH (Zipamix®) at 6 ppm in the diet for 30 d, ZHp: patented ZH (Zilmax®) at 6 ppm in the diet for 30 d.
[2]Standard error of estimation. Means with different superscript within row are different (*P*<0.05).
[3]200=traces, 300=Slight, 400=Small.
[4]100-199=A maturity; 200-299=B maturity; 300-399=C maturity.
[5]Utility=300, Commercial=400, Standard=500, Select=600, Choice=700, Prime=800.

Figure 2: Relative frequency of USDA quality grade in carcasses of young bulls with no ZH supplementation (Control) or supplemented with either a generic ZH (ZHg, Zipamix ®) or a patented ZH (ZHp, Zilmax ®) formulation at 6 ppm in the diet for 30 d (n=96 per treatment)



It has been proposed that the slight decrease of marbling scores induced by ZH supplementation is not enough to modify carcass quality grade in *B. taurus* cattle[25]. This is also applicable to the present experiment, considering *B. indicus* bulls produce leaner, low-quality carcasses. Overall, these results support previous findings documenting a limited effect of ZH supplementation on carcass quality attributes[2,26-28].

Regarding meat quality attributes (Table 4), beef from all treatments had similar WBSF values (*P*=0.1507). Despite meat was aged for 11 d, WBSF remained quite above 45 N, which is typical of tough meat[29], a phenomenon that is frequently observed in ZH-supplemented cattle[30-32]. Moreover, this research involved young bulls with a strong *B. indicus* genetic background, which are known to produce tougher meat as compared to other sex and/or breed categories[33-35]. It should be noted, however, that differences in WBSF among muscles are well documented[36-38]. WBSF values reported here are limited to the LM muscle cooked to 70 ºC (well done) and subjected to 11 d of aging. It has been demonstrated that meat tenderness may differ if considering other muscles, longer aging times or a different endpoint cook temperature[32,39,40].

Table 4: Effect of ZH supplementation on meat quality attributes of bulls

	Treatments[1]				
	Control	ZHg	ZHr		
Variable	**n=96**	**n=95**	**n=93**	**SEE[2]**	***P*-value**
Cooking loss, %	25.10	25.48	25.51	5.99	0.8704
WB shear force, N	59.70	64.16	63.61	17.26	0.1507
L*	40.40	39.88	39.66	3.72	0.3654
a*	28.91[b]	28.03[a]	27.70[a]	2.84	0.0099
b*	20.65[b]	19.68[a]	19.02[a]	2.82	0.0003
C*	35.53[b]	34.27[a]	33.62[a]	3.85	0.0024
h*	35.43[b]	34.86[a]	34.40[a]	1.83	0.0006
pHu	5.78[a]	5.81[b]	5.89[b]	0.23	0.0022

[1]Treatments control: no ZH supplementation, ZHg: generic ZH (Zipamix®) at 6 ppm in the diet for 30 d, ZHp: patented ZH (Zilmax®) at 6 ppm in the diet for 30 d.

[2]Standard error of estimation.

[a,b] Means with different superscript within row are different (*P*<0.05).

Cooking loss was also similar across treatments (around 25 %), which is in the order of that observed in lean muscles(41,42). Again, these results may change if considering other cooking methods and targeted endpoint temperatures, as previously demonstrated(43,44). Ultimate pH was higher in meat from ZH-supplemented animals as compared to that from the untreated ones (*P*=0.0022). This may be an advantage from a meat processing standpoint since higher pH values are associated with better water holding capacity(45). However, the average pHu across treatments falls within the typical interval of “normal quality” beef(46).

Among instrumental color variables, only L* was not affected by ZH supplementation (*P*=0.3654). Conversely, both ZH brands reduced redness (a*) and yellowness (b*) of meat, which resulted in a less vivid red color, as shown by the lower C* and h* values. According to recent research(47), it is unlikely that these differences would have economic implications since Mexican consumers appreciate beef with a light red color.

The occurrence of dark-cutting beef does have a strong economic importance. While the frequency of dark cutters observed here is higher than that reported elsewhere(48,49), there is no evidence supporting it was due to ZH supplementation. In fact, the percentage of dark cutters was similar across treatments $(\chi^2 = 3.6; P = 0.1661)$, with a rate of 6.3, 7.4 and 8.3 %, for control, ZHg, and ZHp, respectively. Therefore, the higher rates in relation to other trials are likely associated with differences in production practices, pre-slaughter handling procedures, as well as criteria used to classify carcasses as dark cutters.

Conclusions and implications

In general, THE results showed dietary ZH supplementation of crossbred *B. indicus* young bulls, under tropical conditions, improves most carcass yield traits without compromising carcass or meat quality attributes. These effects are similar for the two ZH brands tested here when administered for 30 d before slaughter. Therefore, tropical beef producers may use the ZH formulation of lowest cost to improve their productivity.

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