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ORIGINAL ARTICLE

The effects of Feeding Regimens and duration of Zilpaterol hydrochloride on meat Characteristics of fattening lambs

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ABSTRACT

Fifty six Lori-Bakhtiari feedlot lambs (initial BW=44±4.7 kg) were used in a 2×3+1 factorial experimental design, with the objective of evaluating the effects of different methods supplementation of zilpaterol hydrochloride (ZH) (every day, 1day on, 1day off and 2days on, 2days off supplementation) for the last 28 and 42 days of feeding period on meat quality. Each group was consisted of 8 lambs. Lambs were fed a diet with %14 CP and 2.36 Mcal/kg of metabolizable energy supplemented with 0.2 mg/kg of live weight d^{-1} zilpaterol. The control group was fed basal diet without ZH. Different methods supplementation and feeding period of ZH had not significantly ($P > 0.05$) effect on the ultimate PH, cooking loss, drip loss and meat cholesterol. Duration of feeding ZH had no significant effect on shear force but Feeding regimen had a significant effect on shear force ($P = 0.013$) with the higher value in the daily regimen compared to intermittent regimens. Lambs fed ZH in group daily-42 had higher shear force compared to the control group ($P < 0. 05$). Feeding regimen had a significant effect on tenderness ($P = 0.025$) with the higher value in the intermittent regimens compared to daily regimen but, Regimen and duration did not affect other sensory characteristics of meat (Juiciness, Flavor, odor and Overall acceptability). Overall, feeding ZH in group daily-42d increased shear force value and tenderness of meat, while the other meat characteristics were not affected by treatments in Lori-Bakhtiari fat-tail lambs.

Key Words: *β-agonist zilpaterol hydrochloride; meat cholesterol, meat quality; sensory analysis; fat-tail lambs*

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INTRODUCTION

The compounds known as β-adrenergic agonists (β-AA) are organic molecules that have the ability to bind to β-adrenergic receptors (β-AR) and start biochemical reactions that will result in increase of accretion of skeletal muscle and reduction in accretion of fat [1, 2]. Zilpaterol hydrochloride (ZH) is an orally active members of a β-AA, which have generally been shown to improve average daily gain (ADG), gain: feed (G:F), carcass weight gain, dressing percentage, and *longissimus dorsi* (LD) muscle in beef cattle [3] and in lamb [4-8]. ZH is approved for use in the US, México and several other countries, and zilpaterol does not have the potential negative potency or pharmalogical activity of products like clenbuterol or cimaterol [28]. Lopez-Carlos et al. [9], in lamb and Kellermeier et al. [10] in beef cattle have been documented to cause a hypertrophic increase in skeletal muscle fiber diameter. These improvements in skeletal muscle hypertrophy are a result of changes in protein synthesis and degradation rates, whereas in adipose tissue they promote lipolysis [11].

Lamb quality is influenced by factors inherent to the animals from which the products are derived, such as chronological age, slaughter weight, breed and sex .The effect of metabolic modifiers such as beta agonist zilpaterol is also an important factor influencing lamb quality [12]. Also, generally there is an increase in shear force and a consumer panel assessed perception of toughness in meat obtained from animals that have been selected to be lean or leaner because of dietary manipulation [13]Karlsson et al., 1993). While some studies have suggested that meat from animals treated with β- agonists is tougher, there is still much controversy as to whether this is the case and if consumers can detect the differences.

In part, this is because there are differences in mode of action of the various metabolic modifiers, even within a class of modifier, dose responses, as well as species, genotype and individual muscle differences [12]. Because the consumer is the goal of production chain of lambs, production must be aimed at enhancing meat acceptability by the consumer. The factors that influence acceptability of lamb meat most are the color at the time of purchase and the favor intensity on consumption, plus consumer appraisal of other characteristics such as juiciness and tenderness. Unfortunately, due to the resulting hypertrophic increase in muscle fiber diameter, meat tenderness has been shown to be compromised with β-AA usage [3]. There were insufficient data obtained from ruminants to conduct a meta-analysis it seems reasonable to conclude that most β-agonists decrease intramuscular fat and increase shear force, drip loss and ultimate pH. Continually oral feeding zilpaterol for 45 days indicated negative effects on meat characteristics [14, 15]. Based on authors' best knowledge, there is not any information in the literature regarding the effect of ZH intermittent feeding on meat quality of feedlot lambs.

The most important counterpart of the Iranian livestock industry belongs to sheep production. The largest fat-tail by circumference and weight between Iranian fat-tailed breeds belongs to Lori-Bakhtiari sheep breed and the fat deposition in the fat-tail is the major part of weighting in fattening period of this breed [16]. It is postulated that the use of ZH as a β-agonist in the fat-tailed sheep might lead to a decrease in fat-tail weight and an increase in lean meat. The objective of this study was therefore to ascertain the effects of continuous and intermittent use of ZH for a period of 28 d and 42 d on lamb meat quality.

MATERIALS AND METHODS

Location, animals, and management

Fifty six Lori-Bakhtiari male lambs with age of approximately 5-6 months were used in this experiment. Lambs were transported to the Research and Development Center of Lori-Bakhtiari breed sheep located near Shahrekord (Latitude: 32′17″N; Longitude 50′51″E; Altitude: 2049 m).The external treatment with albendazole and levamisole was employed against parasites as well as all lambs were vaccinated against Enterotoxaemia at the beginning of the trail. The fattening period was 80 days. In the first 10 days, lambs were fed with high quality alfalfa hay offered ad libitum and the ration were gradually acquainted for minimizing the risk of gastrointestinal disorders. TMR diets ad libitum were applied three times a day which contained 14% CP and 2.36 Mcal/kg dry matter (DM). Feed was provided in a quantity so that each lamb would have about 10% orts and water and salt licking blocks were free accessible. All lambs had an analogous ration including 40% of alfalfa hay and 60% of pelleted concentrate. The body weight was criteria for adjusting the amount of feed offered every two weeks. For determining DM, crude protein (CP), ether extract (EE) and ash, samples of the diets were grounded (1-mm screen) and were analyzed according to the Association for Official and Analytical Chemists [26] method. Table 1 shows the feed ingredients [27] and chemical composition. The 28 and 42 last days was considered for experimental period with 3 days withdrawal period before slaughter in the current study. Animals were weighed 45 days before slaughter (initial BW= 44±4.7 kg; mean±SD kg), were classified according to the weight, separated into seven groups including 8 lambs, and then specified to one of seven treatments (8 lambs/treatment). The treatments groups were included: lambs in group 1 (control) were fed a basal diet for 42 d without ZH. Those in group 2 (daily-28) were continuously fed the ZH (0.20 mg/kg of live weight d^{-1}) for 28 d. Those in group 3 (daily-42) were fed the ZH for 42 d, continuously. Those in a group 4 (1on 1off-28d) were intermittently fed the ZH 1 day on and 1 day off for 28 d. Those in a group 5 (1on 1off-42d) were fed the ZH 1 day on and 1 day off for 42 d, intermittently. Those in a group 6 (2on 2off-28d) were intermittently fed the ZH 2 days on and 2 days off for 28 d, and those in a group 7 (2on 2off-42d) were intermittently fed the ZH 2 days on and 2 days off for 42 d. To warrant a full consumption of ZH by lambs, it was mixed with dough wheat and shaped in a tablet form. Afterward, the tablet balling gun was employed for complete feeding of tablets to lambs. The lambs were kept in individual pen during the experiments. The cement-floors pens (1×2 m) which equipped with 1.5 m metallic fence-line feed bunk were used.

Meat quality measurements

Lambs were slaughtered at the slaughterhouse after 45 days feeding period. Feed and water were withdrawn 24 h before slaughter. After slaughter, all the abdominal and thoracic organs were eliminated after skinning. Carcass was stored in cold room for 24 h at 4 °C. The ultimate pH of muscle was determined using a TPS-MC80 pH meter with a combined electrode, by insertion into the *Semimembranosus* (SM) muscles, on the cooled carcasses. The pH meter was re-calibrated after every third reading and the electrode rinsed with distilled water between each measurement. Afterward, six samples of LD muscle from each treatment were randomly taken at the 12th and 13th Rib position of cold carcass and were frozen at −20 °C to be measure for the meat quality. In the laboratory, muscles were weighed prior to thawing and allowed to thaw for 18 h at 4°C. After thawing, muscles surface were dried

with filter paper and thaw weights recorded to determine drip loss; drip loss was determined using the following equation: ((frozen – thawed) / frozen) × 100%. muscles were then cooked in a plastic bag in a water bath at 80 °C for 1 h, After cooking, samples were cooled at room temperature; surface dried with filter paper and reweighed. Cooking loss was calculated using the following equation: ((thawed – cooked) / thawed) × 100%. After collecting cooked weight, 3 sub samples with a cross section of 1 cm^2 and at least 3 cm long were cut parallel to the muscle fibers and shear force value of the LD muscle was measured using a Warner Bratzler shear blade fitted to an instron Model Testometric (M350-10CT, Rochdale, England) with a 100 mm/min crosshead speed. The average of the measured peak force values for each muscle was expressed in Newton.

Six samples of LD muscle from each treatment were used for measuring meat cholesterol. The samples were thawed at room temperature (~25°C) in the laboratory and the samples were then minced three times through a 2 mm sieve to ensure homogeneity of samples. The method described by Janssen and Meijer [17] with a slight modification was employed to determine the cholesterol content of samples. Cholesterol content of each LD muscle was measured in triplicate. Briefly, lipid was extracted from 1 g of homogenized sample [18], and then 0.5 ml of final aliquot was separated and evaporated by N2. After recording the weight of dried material from the last stage, a six fold of Triton-X100 chloroform solutions (1:1 volume ratio) based on obtained dried material weights was added. The final product was used to assess the amount of cholesterol using a Pars Azmoon kit (1500010, Iran).

Sensory analysis

Six samples of LD muscle of cold carcass from each treatment were randomly taken and were frozen at −20 °C until panel evaluation. One day prior to each panel session, the frozen samples were taken from the freezer and thawed at 4 °C for 24 h. Samples were cooked in an electric oven at 180 °C until the internal temperature reached 80 °C. Each cooked sample was cut into six sub-samples (1 cm3 dimensions). Sub-samples were then wrapped in pre-coded aluminum foil and stored at 60 °C until serving to panelists. Six-member panel evaluated the meat for the following sensory attributes: tenderness, juiciness, flavor intensity, odor intensity, and overall acceptability (scale 1 = extremely tough, extremely dry, no flavor, no odor, and dislike extremely; scale 8 = extremely tender, extremely juicy, very strong flavor, very strong lamb odor and like extremely) by means of an eight-point structured line scale [19]. Crackers and water were used to cleanse the palate between samples. The sensory panel was carried out in three sessions so that in each session two samples from each treatment was served.

Statistical Analysis

All collected data were analyzed by ANOVA appropriated as a 2×3+1 factorial experiment based on a completely randomized design using the General Linear Models procedure of SAS version 9.1 [20]. The cold carcass weight was used as covariate for analyses of data related to meat quality. The means of all traits were compared by using LSMeans test and $P<0.05$ was considered as the significant level. The same control group has been used for both of the duration and administration regimen.

RESULTS AND DISCUSSIONS

Table 2 shows the Effect of feeding regimens and duration of ZH supplementation on meat characteristics of lambs. Feeding regimen had a significant effect on shear force value ($P = 0.013$) with the higher value in the daily regimen compared to intermittent regimens. However main effects of Feeding regimen was not significant on the ultimate PH, drip loss, cooking loss ($P>0.05$). There were no significant differences ($P>0.05$) between the 28 and 42 days of ZH supplementation for all the meat characteristics as well. There was no period×regimen interaction for any of recorded meat quality parameters ($P>0.05$). Lambs fed ZH in group daily-42 had higher ($P<0.016$) shear force value compared to the control group (Table 2). There was no significant difference in cholesterol content between treatments ($P>0.05$). Means for sensory quality characteristics of the LD muscle samples are presented in Table 3. Lambs fed ZH in every second day regimen had more tender ($P = 0.025$) than the daily regimen. Different period of supplementation of ZH showed no significant difference in sensory analysis ($p>0.05$). There was no period×regimen interaction for any sensory analysis ($P>0.05$). Whereas, tenderness tended to decrease in daily-28 and daily-42 treatments compared to the control group ($P = 0.070$).

The shear force of LD muscle was greater ($P<0.019$) in daily treatment compared with intermittent. Effects of β-agonists on meat quality are equivocal. Our tenderness data agree with previous findings that the meat from βAA-treated animals is consistently tougher than untreated meat and increments in shear force value has been demonstrated with most β-agonists with both cattle and sheep [1, 21]. However Plascencia et al. [22], Macías-Cruz et al. [7] reported that ZH did not cause tougher meat compared with untreated animals. In other studies, zilpaterol had no negative effects on meat quality when fed for 15 or 30 days, whereas feeding it for 50 days resulted in lower sensory tenderness, juiciness ratings, and the shear force of the muscle also was negatively affected [23]. In the study feeding ZH for 35 days did not

influence significantly shear force in the LD muscle [24]. In agreement with results from the present study, lopez et al. [4] reported that there were no differences in drip loss, cooking loss, pH at 24 h postmortem, in LD muscle of lambs fed with ZH, independent of the feeding program applied.

Changes in the muscle fiber size and proportion and decrease intramuscular fat are considered responsible for increased shear force values [12]. Also, the mRNA for the calpain protease inhibitor-calpastatin increases after βAA treatment. Suppression of the calpain proteases reduces the rate of protein degradation, resulting in increased muscle growth. The negative effects of calpastatin on the rate and extent of post mortem proteolysis and meat tenderness are documented. The occurrence of the Callipyge gene in cattle and sheep as well as data from some βAA trials provide evidence to associate negative effects of increased muscle hypertrophy (via reduced protein degradation) with decreased meat tenderness [25].

Table 1: Ingredients and chemical analyses of the experimental diet offered to lambs

Item	Amount
Ingredient, % (as-fed basis)	
Alfalfa hay	40
Barley	20
Wheat grain	9
Corn	8
Soybean meal	5.4
Wheat bran	9
Beet pulp	3
Cane molasses	3
Sodium bicarbonate	0.48
Salt	0.3
Calcium carbonate	0.4
Zeolite	0.6
Vitamin–mineral premix [a]	1.2
Nutrient composition (dry matter basis)	
Dry matter (%)	89.9
Crude protein (%)	14
Metabolizable energy (Mcal/kg DM)	2.45
Ether extract (%)	1.9%
NDF (%)	35.4
Ash (%)	8.5
NFC (non-fiber carbohydrate)	41.7 %

[a] Each kg of the vitamin–mineral premix contained: vitamin A (50,000 IU), vitamin D3 (10,000 IU), vitamin E (0.1 g), calcium (196 g), phosphorus (96 g), sodium (71 g), magnesium (19 g), iron (3 g), copper (0.3 g), manganese (2 g), zinc (3 g), cobalt (0.1 g), iodine (0.1 g), selenium (0.001 g).

Table 2: Effect of feeding regimens and duration of zilpaterol hydrochloride (ZH) supplementation on meat characteristics of Lambs.

Traits	Control[1]	Treatment[2](treat)						SEM	Regimen(R)			SEM	Duration(D)		SEM	p-value			
		Daily 28	1on 1off 28	2on 2off 28	Daily 42	1on 1off 42	2on2off 42		Daily	1on 1off	2on 2off		28d	42d		Treat	R	D	R × D
Ultimate pH	5.76	5.71	5.60	5.68	5.59	5.79	5.64	0.08	5.65	5.70	5.66	0.06	5.67	5.68	0.05	0.51	0.87	0.91	0.81
Cooking loss (%)	26.3	29.5	26.4	27.6	28.3	28.5	29.5	1.52	28.8	27.3	28.7	1.06	27.8	28.6	0.87	0.60	0.54	0.54	0.42
Drip loss (%)	1.8	1.87	2.13	1.92	1.92	2.08	2.10	0.15	1.89	2.08	2.02	0.11	1.98	2.01	0.09	0.75	0.43	0.78	0.64
Shear force (N)	61.8b	74.5ab	67.0ab	64.7ab	79.6a	71.9ab	69.3ab	3.60	77.4a	69.9ab	66.8b	2.43	68.7	74.0	2.00	0.016	0.013	0.07	0.94
Cholesterol (mg/100 g)	57.7	51.9	55.5	54.4	55.4	53.8	49.5	2.00	54.0	54.9	52.3	1.38	54.3	53.2	1.13	0.17	0.42	0.49	0.14

[A,b,c] Means with different superscript letters in rows are significantly different, P < 0.05.

[1]Control=basal diet without ZH.

[2]Zilpaterol hydrochloride (ZH; Zilmax, Intervet, Mexico City, Mexico) was added to the diet according to feeding regimens and duration. daily28= were fed the ZH diet (0.20 mg/kg of live weight d−1) for 28 d, continuously. daily42= were fed the ZH diet for 42 d, continuously. 1 on 1 off 28= were intermittently fed the

ZH diet 1 day on and 1 day off for 28 d. 1 on 1 off 42= were intermittently fed the ZH diet 1 day on and 1 day off for 42 d. 2 on 2 off 28 were intermittently fed the ZH diet 2 days on and 2 days off for 28 d and 2 on 2 off 42 = were intermittently fed the ZH diet 2 days on and 2 days off for 42 d.

Table 3 Effect of feeding regimens and duration of zilpaterol hydrochloride (ZH) supplementation on sensory analysis of LD muscle

Traits	Contro1[1]	Treatment[2](treat)						SEM	Regimen(R)			SEM	Duration(D)		SEM	p-value			
		Daily 28	1on 1off 28	2on 2off 28	Daily 42	1on 1off 42	2on2off 42		Daily	1on 1off	2on 2off		28d	42d		Treat	R	D	R × D
Tenderness	5.29	4.48	5.14	5.08	4.27	5.12	4.71	0.26	4.38b	5.14a	4.90ab	0.19	4.90	4.71	0.15	0.070	0.025	0.38	0.82
Juiciness	5.16	4.89	5.31	5.43	4.61	4.83	4.93	0.25	4.74	5.06	5.16	0.17	5.19	4.78	0.14	0.26	0.21	0.054	0.87
Flavor	4.75	5.10	5.02	5.20	4.98	4.60	5.02	0.25	5.02	4.78	5.07	0.15	5.07	4.84	0.12	0.51	0.40	0.21	0.79
odor	4.29	4.61	4.19	4.59	4.81	4.38	4.80	0.25	4.70	4.28	4.68	0.17	4.45	4.66	0.14	0.47	0.18	0.34	0.99
Overall acceptability	5.20	4.56	5.06	4.99	4.69	4.78	4.63	0.20	4.63	4.92	4.82	0.14	4.88	4.70	0.12	0.21	0.34	0.29	0.46

[A,b,c] Means with different superscript letters in rows are significantly different, P < 0.05.
[1]Control=basal diet without ZH.
[2]Zilpaterol hydrochloride (ZH; Zilmax, Intervet, Mexico City, Mexico) was added to the diet according to feeding regimens and duration. daily28= were fed the ZH diet (0.20 mg/kg of live weight d−1) for 28 d, continuously. daily42= were fed the ZH diet for 42 d, continuously. 1on1off 28= were intermittently fed the ZH diet 1 day on and 1 day off for 28 d. 1 on 1 off 42= were intermittently fed the ZH diet 1 day on and 1 day off for 42 d. 2 on 2 off 28 were intermittently fed the ZH diet 2 days on and 2 days off for 28 d and 2 on 2 off 42= were intermittently fed the ZH diet 2 days on and 2 days off for 42 d.

CONCLUSION

In Conclusion, administration of ZH during the last 28 seemed to have no negative impact on meat quality. However, in the present study, the higher values of shear force and the lower value of tenderness were obtained for daily supplementations of ZH compared intermittent regimen in the last 42 of the feeding period. It can be concluded that we could use intermittently ZH in feedlot lams to decrease fattening costs and shear force value and increase tenderness of meat instead of daily administration of ZH in the last 42 of the feeding period. Moreover, further study should be performed before making the definite recommendations on feedlot lambs

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