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Effect of Different Zilpaterol Hydrochloride Levels and Feeding Methods on the Growth Performance, Carcass Traits and Blood Parameters of Male Japanese Quails During Two Weeks of the Finishing Period

ABSTRACT

The objective of this study was to determine the effects of different zilpaterol hydrochloride (ZH) levels and feeding methods on the growth performance and carcass traits of Japanese quails. In this trial, 224 male quails with 34 days of age were distributed into seven treatments. A factorial arrangement with three ZH levels (0.2, 0.225, and 0.25 mg zilpaterol/kg of live weight⁻¹) \times two feeding methods (ZH supplementation daily or every two days) plus a control treatment (no ZH supplementation) was applied. The weight gain of birds fed ZH every two days was significantly higher than those fed ZH daily during the period of 41-47 days ($p=0.03$). During the period of 34-40 days, ZH-supplemented birds presented higher weight gain ($p<0.001$) and better feed conversion ratio ($p=0.005$) compared with the control group. Moisture, protein, ash, and cholesterol levels of the combination of breast and leg were not affected ($p\geq 0.10$) by zilpaterol supplementation. Leg muscle fat and protein contents of control birds were higher and lower compared with ZH-supplemented birds, respectively ($p=0.002$ and $p=0.05$). In conclusion, ZH supplementation in the diet of male Japanese quail improved growth performance during the finishing period (34-40d), and the most economical level of ZH is 0.2 (mg/kg of LW⁻¹ d⁻¹), when supplemented every two days.

INTRODUCTION

The β -adrenergic agonists (β -AA) improves the efficiency of gain and affects carcass characteristics and meat quality of several animal species (Avendano-Reyes *et al.*, 2006). These compounds reduce adipose tissue deposition and increase muscle mass in domestic species, such as pigs (Apple *et al.*, 2008; Carr *et al.*, 2005; Poletto *et al.*, 2009), beef cattle (Allen *et al.*, 2009; Gruber *et al.*, 2007) and poultry (Schiavone *et al.*, 2004). These compounds also stimulate adipocyte triacylglycerol degradation and inhibit fatty acid and triacylglycerol synthesis (Mersmann, 1998). Zilpaterol hydrochloride (ZH) is a pharmaceutical beta-adrenergic agonist (β -AA) commercially available in Mexico, U.S.A, Australia, New Zealand, Brazil, Indonesia, and the Republic of South Africa under the name of Zilmax (Intervet Inc.). ZH was approved in the United States by the Center for veterinary medicine in 2006 (FDA, 2006), because it is rapidly eliminated and safe when adequately used (Lopez-Carlos *et al.*, 2010). Synthetic β -AA are chemically and pharmacologically similar to the natural catecholamines dopamine, norepinephrine, and epinephrine (Vasconcelos *et al.*, 2008). Moody *et al.* (2000) mentioned that the β -AA compounds differ in potency, since cells and tissues vary in expression of β -adrenergic receptor types, and in consequence, their effects are also associated with the type of β agonist, species treated, and dose and duration of treatment. In this regard, Kim *et al.* (1987) reported that cimaterol, a β -AA, improved the



daily gain of lambs during the first 6 weeks of rearing, whereas Pringle *et al.* (1993) found that L644,969, a type of β -AA, improved daily gain of lambs only during the first 2 weeks, and dressing percentage during the first 4 weeks of feeding.

In our literature review, we did not find any published data on the effect of ZH on the growth performance of avian species. Therefore, this study aimed at evaluating the effects of three dietary levels of ZH and two methods ZH feeding methods on the growth performance, carcass traits, and blood parameters of Japanese quails.

MATERIALS AND METHODS

Facilities and birds

One thousand Japanese quails (1 d old) were reared in a temperature-controlled room on the floor until 34 days of age. Then, 224 male Japanese quails with similar average of live body weight were randomly assigned to 28 battery cages according to a completely randomized block design with seven treatments and four replicates (cage) of eight birds per experimental unit. Room temperature was maintained at 36-38°C for the first 3 d, and was then reduced by 3°C weekly until reaching 23-24°C. Continuous light was provided.

Diets and experimental design

The finisher diet was based on corn and soybean meal and its composition is shown in Table 1. Birds were fed diets with three different doses of zilpaterol (0.2 (D1), 0.225 (D2), or 0.25 (D3) mg zilpaterol/kg of live weight⁻¹) for 14 days of the finishing period by two methods: zilpaterol (M1) was fed daily or every two days (M2). A control group was established, in which the birds did not receive any ZH supplementation. Zilpaterol hydrochloride was withdrawn three days prior to slaughter.

Data collection

Body weight and feed intake were recorded at weekly intervals. At the end of the finishing period, when broilers were 50 days of age, birds were submitted to 12 h of feed withdrawal, eight birds (two birds which body weight was similar to the average in each replicate) per treatment were sacrificed by severing the right carotid artery and jugular vein. After bleeding, birds were processed and dissected by a trained team. The heads and necks were removed by cutting through the last cervical and the first thoracic vertebrae, and the shank at the tibiotarsal-metatarsal joints Vidyadaran *et*

Table 1 – Ingredients and calculated composition of the basal diet (as-fed basis)

Item	Ingredient (%)
Corn	56.09
Soybean meal	32.43
Gluten meal	8.00
Calcium co3	1.29
Dicalcium phosphate	0.94
Salt	0.34
DL-Methionine	0.14
L-Lysine	0.27
Vitamin premix1	0.25
Mineral premix 2	0.25
Total	100.00
Calculated value	
ME (kcal/kg)	2900
CP (%)	24
Methionine (%)	0.5542
Met+Cys	0.75
Lysine (%)	1.3
Ca (%)	0.8024
Available P (%)	0.3017
Sodium	0.15

al. (1988). Carcass dressing percentage was calculated as carcass weight (without blood, feathers, head, neck, shank, or giblets) relative to live weight. Liver, abdominal fat, legs, and breast were weighed and kept at -20 °C for chemical composition determination.

Carcass chemical analyses

Leg and breast chemical composition was determined as described by the AOAC (1990). The skin and the subcutaneous fat were removed. The muscles of the right breast and leg were carefully dissected, homogenized using a blender, and the analyzed for total moisture, ash, crude protein, crude fat, and cholesterol contents. The left breast and leg muscles were separately analyzed for crude protein and crude fat contents. In order to determine total cholesterol content of the muscles, tissue samples were thawed and extracted with 2:1 chloroform: methanol (Bligh & Dey, 1959). Total cholesterol levels were then enzymatically determined by the method of Allain *et al.* (1974).

Blood samples collection and analysis

Individual blood samples were collected during bleeding, and immediately centrifuged at 3500 rpm for 15 min. Plasma was harvested after centrifugation of the clotted blood, stored at -20°C in a deep freezer until the time of chemical determinations. Blood biochemical parameters were enzymatically determined using commercial kits.



Statistical analysis

At first, data of all treatments were analyzed without control group according to a completely randomized block design in a 3 (levels of ZH) × 2 (feeding method) factorial arrangement, using cages as blocks. At the second step, in order to compare the averages of the treatments with the control treatment, data were analyzed according to a randomized block design using the GLM procedure of SAS. Means were compared using Duncan's test at significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The main effects of doses and methods (D & M) and their interactions on growth performance during the two last finishing weeks were shown in Table 2. Significant dose x method interactions were observed for feed intake during the period of days 34-40. The highest feed intake was obtained in group D2 x

M1. There were significant weight gain differences between M1 and M2 when birds were fed zilpaterol on days 41-47 ($p = 0.03$), with M2 birds presenting higher weight gain than M1 birds. In this regard, Moloney *et al.* (1991) stated that extended time of β -adrenergic use during the finishing period may cause β -adrenergic receptor desensitization and down regulation, thereby reducing growth rate responses. In addition, pig studies indicate that intermittent use of ractopamine may help reducing this negative effect on performance because of the resting time given to the receptors Dibi *et al.* (2010). It seems that in our trial, the M2 method reduced the down regulation of the β -adrenergic receptor. In fact, we suppose that the higher weight gain obtained with the M2 method on days 41-47 may be due to the up regulation of the β -adrenergic receptor during the two days the product was not fed after two days of feeding. The effect of zilpaterol supplementation on the growth performance of the treated and control groups is shown in Table 2.

Table 2 – Main effects and interactions of doses and methods of zilpaterol supplementation on the body weight (BW), feed intake (FI), and feed conversion ratio (FCR) of Japanese quails and comparison with the control group.

Item	34-40 d			41-47 d		
	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR
ZH doses						
D ₁	47.91	174.18	3.66	25.78	198.95	7.81
D ₂	49.54	175.71	3.58	26.45	197.58	7.65
D ₃	47.35	172.04	3.6	24.56	193	8.01
SEM	0.89	1.59	0.08	1.45	3.67	0.35
P (value)	0.22	0.28	0.77	0.65	0.5	0.78
Feeding method						
M ₁	48.58	173.65	3.58	23.7	192.79	8.2
M ₂	47.96	174.31	3.65	27.48	200.22	7.44
SEM	0.72	1.29	0.06	1.19	3.0	0.29
P (value)	0.55	0.72	0.49	0.03	0.09	0.07
Doses x Method						
D ₁ M ₁	47.81 ^{ab}	171.53 ^B	3.59 ^b	23.91	197.87	8.37
D ₁ M ₂	48.02 ^{ab}	176.8 ^{AB}	3.73 ^b	27.66	200.03	7.25
D ₂ M ₁	51.47 ^a	179.43 ^A	3.55 ^b	23.81	193.45	8.19
D ₂ M ₂	47.62 ^{ab}	172 ^B	3.61 ^b	29.09	201.72	7.12
D ₃ M ₁	46.47 ^b	170 ^B	3.60 ^b	23.40	187.06	8.06
D ₃ M ₂	48.25 ^{ab}	174.1 ^{AB}	3.60 ^b	25.71	198.94	7.96
SEM	1.25	2.25	0.11	2.06	5.2	0.5
P (value)	0.09	0.02	0.85	0.77	0.64	0.5
Control	40.5 ^c	171.8	4.25 ^a	23.79	191.9	8.06
SEM	1.23	0.11	2.31	1.92	5.27	0.47
p (value)	<0.001	0.09	0.005	0.27	0.45	0.42

D₁, 2, 3: (0.2, 0.225, 0.25 mg/kg of LW-1 d-1 zilpaterol, respectively)

M₁: received zilpaterol daily; M₂: received zilpaterol every two days

Control: diet with no ZH supplementation

D₁, 2, 3 & M₁, 2,: effect of the interaction between D and M in the different treatments.

abc Means in the same column without a common superscript letter are different when groups receiving ZH were compared with the control group ($p < 0.05$).

AB Means in the same column without a common superscript letter are different, showing effects of the interaction between ZH dose and feeding method ($p < 0.05$).



Zilpaterol supplementation improved weight gain ($p=0.0003$) and feed conversion ratio ($p=0.005$) in all treatments compared with the control group, but it did not affect feed intake on days 34-40 ($p=0.09$). However, on days 41-47 days, zilpaterol did not significantly affect growth performance. There are some reports about different effects of β -agonists on the growth performance of animals. Ansari-Pirsaraei *et al.* (2007) demonstrated that terbutaline did not affect daily weight gain, but reduced feed conversion ratio (FCR) of male broilers when 5 and 10 mg/kg were fed, respectively. On the other hand, in some studies, ZH supplementation increased average daily gain and gain efficiency in feedlot lambs (Estrada-Angulo *et al.*, 2008), steers (Avendano-Reyes *et al.*, 2006) and cattle (Vasconcelos *et al.*, 2008). However, Felix *et al.* (2005) did not observe any effect of ZH supplementation on

the growth performance of Pelibuey lambs. The reasons why no performance differences were observed on days 41-47 days between birds receiving ZH and the control group may be bird maturity and hormonal changes. Another reason for this phenomenon is that the β -AA receptor in the target tissues may be rapidly inactivated, or a particular species may have a limited number of β -AA receptors in the target tissues, reducing the response to the agonist (Mersmann, 1998). Perhaps the Japanese quail is one the species that has a limited number of receptors.

Table 3 shows that, when the zilpaterol supplemented groups were compared, carcass traits were not influenced by the treatments or their interactions. However, leg weight was higher in ZH-fed chickens (D1 and D2 of M1, D1 and D3 of M2) than in control group ($p=0.01$), but the weights of the other parts

Table 3 – Main effects and interactions of doses and methods of zilpaterol supplementation on carcass and parts weight and yield of Japanese quails and comparison with the control group.

Item	Weight(g)					Yield			
	Carcass	Breast	Leg	Liver	AF*	Breast	Leg	Liver	AF*
ZHdoses									
D1	161	64.9	36.7	3.73	2.95	40.1	22.7	2.3	1.81
D2	163	65.1	36.5	4.05	3.05	39.8	22.3	2.48	1.89
D3	159	63	35.9	3.83	3.11	39.6	22.6	2.4	1.97
SEM	3.61	1.77	0.76	0.19	0.25	0.6	0.34	0.12	0.16
P(value)	0.68	0.66	0.74	0.48	0.89	0.84	0.76	0.59	0.78
Method									
M1	163	66.2	36.7	3.96	3.1	40.5	22.5	2.43	1.91
M2	159.7	62.53	36.1	3.78	2.98	39.1	22.6	2.37	1.87
SEM	2.95	1.45	0.62	0.15	0.21	0.49	0.28	0.1	0.13
p (value)	0.4	0.09	0.47	0.41	0.69	0.06	0.89	0.64	0.81
doses×Method									
D1M1	164.4	67.9	37.6a	3.68	3.31	41.37	22.94	2.23	2.00
D1M2	159.5	61.95	36.5a	3.78	2.58	48.83	22.89	2.38	1.61
D2M1	167.1	67.41	37.5a	4.25	2.90	40.31	22.52	2.56	1.76
D2M2	159.73	62.72	36.0ab	3.85	3.21	39.27	22.58	2.41	2.01
D3M1	158.2	63.13	36.1ab	3.96	3.08	39.93	22.89	2.50	1.96
D3M2	159.8	62.91	36.55a	3.7	3.30	31.15	22.88	2.31	1.97
SEM	5.1	2.5	1.08	0.27	0.36	0.85	0.49	0.17	0.23
p (value)	0.67	0.49	0.54	0.62	0.34	0.52	0.95	0.58	0.39
Control	157.5	61.4	34.45b	3.91	3.92	38.99	21.78	2.48	2.49
SEM	4.75	2.33	0.54	0.26	0.34	0.82	0.48	0.16	0.21
p (value)	0.76	0.3	0.01	0.76	0.23	0.34	0.71	0.83	0.2

D_{1,2,3}: (0.2, 0.225, 0.25 mg/kg of LW⁻¹ d⁻¹ zilpaterol, respectively)

M₁: received zilpaterol daily; M₂: received zilpaterol every two days

Control: diet with no ZH supplementation

D_{1,2,3} & M_{1,2}: effect of the interaction between D and M in the different treatments.

^{abc} Means in the same column without a common superscript letter are different when groups receiving ZH were compared with the control group ($p < 0.05$).

*Abdominal fat



were not significantly different ($p>0.05$). This finding is in agreement with the results of Morgan *et al.* (1989), which obtained heavier legs in chickens fed with cimaterol. However, in some cases, β -agonist supplementation did not affect carcass weight or dressing percentage (Koochmaraie *et al.*, 1991). This disparity in responses may be due to the fact that a given agonist may not activate the target tissue receptors as well in one species as in another. The possible mechanisms include agonist affinity for the receptor(s), coupling of the agonist-receptor complex to the signal transduction system, and factors that influence delivery of the compound to the receptor sites (Mersmann, 1998).

In this study, plasma glucose and cholesterol levels were not influenced ($p<0.05$) by the administration of β -AA (Table 4). These results are consistent with the previous report of Lopez-Carlos *et al.* (2010), but opposed to those found by Ansari-Pirsaraei *et al.* (2007). Plasma cholesterol decreased in chickens treated with

dopamine in another experiment (Buyse *et al.*, 1991). Responses to various β -AA vary with age, species, sex, diet, breed, dose and duration of treatment, which may be due to stimulation of different receptors (Beermann, 2002).

Table 5 shows the main effects and interactions between doses and methods of zilpaterol supplementation on the composition of breast and leg muscle in Japanese quails. There were no significant differences among ZH-supplementation treatments. However, as seen in Table 5, the fat and protein contents of the leg muscle in control group were higher and lower compared with the ZH treatments, respectively ($p=0.002$ and $p=0.05$). This finding is in agreement with the results of Morgan *et al.* (1989) with broiler chickens treated by cimaterol. Zilpaterol may affect muscle fat content by two mechanisms. The first is related with the activation of β -adrenergic receptors, resulting in an increase in the number of cAMP and higher lipolysis rate in the fat tissue, thereby reducing muscle fat content. The second mechanism is related with the phosphorylation of protein kinase A and then deactivation of acetyl COA carboxylase, reducing lipogenesis rate. On the other hand, ZH may increase muscle protein content by increasing the biosynthesis of intracellular protein. Watson-Wright & Wilkinson(1986)reported that skeletal muscle has beta-2 subclass receptors, but that receptor density is a function of muscle type. White fibers appear to have low beta-receptor density, whereas red fibers contain high beta-receptor density. The lack of response in the breast muscle to ZH treatment in the present experiment could be partially due to the higher proportion of white fibers in the breast muscle relative to leg muscle. The percentage of moisture, protein, ash, and cholesterol in the breast and leg muscles were not affected ($p\geq 0.10$) by zilpaterol. Our results are consistent with those of Dawson *et al.* (1997), who supplemented beef steers with other β -AA and did not find any effects on carcass ash percentage.

In conclusion, ZH supplementation in the diet of male Japanese quail improved growth performance during the finishing period (34-40d), and the most economical level of ZH is 0.2 (mg/kg of LW⁻¹ d⁻¹), when supplemented every two days.

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Table 4 – Main effects and interactions of doses and methods of zilpaterol supplementation on blood parameters of Japanese quails and comparison with the control group.

Item	Glucose(mg/dL)	Cholesterol(mg/dL)
ZH doses		
D ₁	354	183
D ₂	342	176
D ₃	345	184
SEM	7.85	4.69
p (value)	0.54	0.42
Method		
M ₁	351	183
M ₂	343	179
SEM	6.4	3.82
P (value)	0.37	0.47
Doses×Method		
D ₁ M ₁	369	191
D ₁ M ₂	335	174
D ₂ M ₁	344	175
D ₂ M ₂	341	176
D ₃ M ₁	339	181
D ₃ M ₂	351	186
SEM	11.01	6.63
p (value)	0.15	0.24
Control	344	176
SEM	10.44	6.64
p (value)	0.36	0.46

D_{1,2,3}: (0.2, 0.225, 0.25 mg/kg of LW⁻¹ d⁻¹ zilpaterol, respectively)

M₁: received zilpaterol daily; M₂: received zilpaterol every two days

Control: diet with no ZH supplementation

D_{1,2,3}&M_{1,2}: effect of the interaction between D and M in the different treatments.

^{abc} Means in the same column without a common superscript letter are different when groups receiving ZH were compared with the control group ($p<0.05$).



Table 5 – Main effects and interactions of doses and methods of zilpaterol supplementation on muscle composition (%) of Japanese quails and comparison with the control group.

Item	Leg and breast %					Breast muscle%		Leg muscle%	
	Moisture	Fat	CP	Ash	Cholesterol µg/ml	Fat	CP	Fat	CP
ZH doses									
D ₁	73.26	2.19	21.92	1.59	162	2.06	23.09	2.42	20.56
D ₂	73.25	2.42	22.08	1.6	151	2.03	23.09	2.56	20.66
D ₃	73.43	2.16	21.88	1.59	136	1.99	22.94	2.38	20.65
SEM	0.22	0.13	0.13	0.06	11.23	0.1	0.08	0.06	0.06
P (value)	0.82	0.33	0.49	0.99	0.29	0.86	0.3	0.09	0.47
Method									
M ₁	73.43	2.31	21.9	1.6	154	2.04	23.03	2.46	20.64
M ₂	73.21	2.20	22.01	1.6	145	2.02	23.04	2.44	20.62
SEM	0.18	0.11	0.11	0.49	9.2	0.08	0.06	0.05	0.05
p (value)	0.4	0.49	0.56	0.89	0.48	0.85	0.95	0.75	0.73
Doses×Method									
D ₁ M ₁	73.1	2.36	21.7	1.62	169	2.1	23.09	2.55 ^b	20.57 ^a
D ₁ M ₂	73.43	2.02	22.13	1.56	155	2.02	23.08	2.30 ^b	20.56 ^a
D ₂ M ₁	73.55	2.33	22.05	1.54	156	2.01	23.10	2.49 ^b	20.67 ^a
D ₂ M ₂	72.96	2.51	22.10	1.65	146	2.05	23.08	2.64 ^b	20.65 ^a
D ₃ M ₁	73.63	2.24	21.94	1.63	138	2.00	22.91	2.36 ^b	20.67 ^a
D ₃ M ₂	73.22	2.07	21.80	1.55	133	1.97	22.96	2.4 ^b	20.63 ^a
SEM	0.31	0.19	0.19	0.85	15.88	0.14	0.11	0.08	0.08
p (value)	0.32	0.41	0.35	0.53	0.96	0.92	0.94	0.06	0.98
Control	73.23	2.7	21.88	1.53	1.42	2.15	22.98	3.06 ^a	20.28 ^b
SEM	0.35	0.2	0.17	0.8	0.15	0.14	0.12	0.11	0.08
p (value)	0.69	0.26	0.6	0.91	0.7	0.97	0.89	0.002	0.05

D₁, 2, 3: (0.2, 0.225, 0.25 mg/kg of LW–1 d–1 zilpaterol, respectively)

M₁: received zilpaterol daily; M₂: received zilpaterol every two days

Control: diet with no ZH supplementation

D_{1,2,3} & M_{1,2}: effect of the interaction between D and M in the different treatments.

^{abc} Means in the same column without a common superscript letter are different when groups receiving ZH were compared with the control group (p< 0.05).

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