Journal of Animal and Veterinary Advances 10 (17): 2263-2266, 2011
ISSN: 1680-5593

The Effects of Apilarnil (Drone Bee Larvae) Administration on Growth Performance and Secondary Sex Characteristics of Male Broilers

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Abstract: This study examined the anabolic and androgenic effects of apilarnil, a bee product on broilers. The study included 40 Ross-308 genotype broilers aged 21 days. The broilers were separated equally into two groups; an experimental group that was administered apilarnil and a control group. Between the 22 and 42 days, apilarnil was administered to the apilarnil group (4 g/broiler/day) and the same amount of water was given to broilers in the control group orally. In the apilarnil group, it was determined that body weight gain and feed intake showed significant decreases between the 29 and 35 days and body weight gain appeared a significant increase between the 36 and 42 days. From 22-28 and 36-42 days, feed conversion of the apilarnil-administered male broilers was found to be better than in the control group. However, apilarnil administration during the growth period had no affect on the male broilers' body weight on 42nd day. Apilarnil administration stimulated length of comb and length and width of wattle in male broilers. The results indicate that apilarnil has an androgenic effect rather than an anabolic effect.

Key words: Broiler, apilarnil, performance, secondary sex characteristics, male broilers, Turkey

INTRODUCTION

Gonadal steroids affect development either by increasing protein synthesis through bounding directly to special intracellular receptors or by indirectly stimulating the excretion of growth hormone and other anabolic hormones (Fennell *et al*., 1996; Lawrence and Fowler, 2002). For this reason, gonadal hormones have been used for long years in mammals, especially in cattle and sheep in order to increase meat yield (Lawrence and Fowler, 2002; Scanes, 2003). However, the use of anabolic compounds in livestock is now banned or restricted in the EU countries (although, not in the USA and Canada) in order to protect animal welfare and consumer health (Scanes, 2003). Despite these bans, the illegal use of anabolic compounds is still in question due to its positive effects on growth rate, feed conversion and meat quality.

It has been reported that synthetic or natural androgens stimulate breeding performance, sexual behaviors and secondary sex characteristics as well as muscle development in mammals (Lawrence and Fowler, 2002; Frandson *et al*., 2009). In the limited number of previous studies carried out on poultry, some androgens have been reported to have mainly anabolic effects (stimulating muscle development) and that some androgens have mainly androgenic effects (stimulating male breeding performance) (Scanes, 2003; Fennell and Scanes, 1992a). Testosterone is a major androgen (Scanes, 2003) and has equal anabolic and androgen effects on chickens. Fennell and Scanes (1992a) determined that androgen administration (testosterone, 5 α-dihydrotes tosterone, 19-nortestesterone) increased body and muscle development; reduced feed conversion rate and abdominal a dipose tissue weight, yet did not affect shank-toe length in female and male turkeys. Similarly, Maruyama *et al*. (1996) determined that growth rate increased when testosterone pellets were implanted in castrated and intact male turkeys.

These positive effects of androgen administration on turkeys could not be detected in chickens (Fennell and Scanes, 1992a). Fennell and Scanes (1992b) on the other hand, determined that androgen administration in chickens did not stimulate growth, yet increased length of comb and length and width of wattle. Similarly in another study carried out by Fennell *et al*. (1996), it was determined that body development (average daily gain, body weight, shank-toe length and breast muscle weight) and Bursa Fabricius weight showed a decrease; however comb weight increased in roosters that were administered testosterone in 2-6 weeks period. These results point out that androgens have an androgenic

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effect rather than an anabolic effect on chickens (Fennell *et al*., 1992b). Apilarnil is a natural bee product obtained from drone bee larvae. Apilarnil is deemed a unused product in circumstances where the number of drone bees is not wanted to outnumber the other bees in honeybee colonies. Due to the reason that particularly honeybee pest Varroa (*Varroa jacobsoni* Q.) completes its growth cycle comfortably in the honeycomb cells of drone bee larvae, these honeycombs are cut and discarded by beekeepers. By this way, a biological fight is made against varroa destructor.

It was reported that apilarnil contains 25-35% dry matter, 9-12% proteins, 6-10% carbohydrates, 5-8% lipids, 2% ash and 3% unidentified substances (Matsuka *et al*., 1973; Stangaciu, 1999). In addition, apilarnil is rich in male type hormones so, it has many male strengthening effects (Iliescu, 1993). It was suggested that apilarnil is a natural anabolism stimulator in males since, it increases the muscular body weight (Stangaciu, 1999).

This study investigated the potential for using apilarnil, a natural bee product, instead of banned anabolic compounds. In addition, the study examined whether or not apilarnil has an androgenic and anabolic effects on chicken broilers. Thus, the study examined the possibility of using apilarnil which is not usually utilized by beekeepers in the animal production cycle.

MATERIALS AND METHODS

Experimental design and measurements: Forty male broilers (Ross-308) aged 17 days were housed in individual cages of 30 cm wide ×30 long ×36 high. After 5 days adaptation period, birds were individually weighed and randomly allocated to 2 treatment groups (control and apilarnil) of equal mean body weight (883.11±11.45 g and 882.53±16.03 g in the control and apilarnil groups, respectively).

From 22-42 days, all birds had free access to a commercial grower diet in pellet form which did not contain any antibiotics or growth promoters. During the growth phase each bird in the apilarnil group was given 0.8 mL apilarnil once daily, orally by injector whlie control birds were administered the same amount of water orally. From the age of 21 days, the feed intake and weight (per cage) of all birds were measured weekly. Body weight gain and feed conversion ratio were determined individually from 22-28; 29-35; 36-42 and 22-42 days. Feed efficiency ratio was calculated on the basis of unit feed consumed to unit body weight gain. Mortality was recorded daily. At 41 days of age, comb and wattle dimensions were measured in an individual bird using a caliper compass. Feed and water were available *ad libitum*. A fluorescent lighting schedule of 23 h light; 1 h darkness was used during the experimental period. The nutrient composition of the growth diet was as follows: 929 g kg^{-1} dry matter, 228 g kg^{-1} crude protein, 114 g kg^{-1} crude fat, 4.06 crude fibers, 7.2 g kg^{-1} total phosphorus, 10.5 calcium, 13.2 MJ kg^{-1} metabolisable energy. The diet was ground through a 1 mm screen in preparation for chemical analysis. The chemical composition was determined according to Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschung-sanstalten (VDLUFA). Metabolisable Energy (ME) content of the diet was calculated based on chemical composition (Turkish Standards Institute, 1991).

Preparation of apilarnil and determination of apilarnil usage level: The experiment used the comb of 3-7 days old (larvae period) drone bees as a source of apilarnil; all drone bees had open eyes and were obtained from a beehive during the spring period. After removed from the honeycomb via thin glass sticks, the harvested apilarnil was kept in deep freeze in plastic freezer bags in daily usage doses at -18°C.

The dose of apilarnil administered to broilers was determined by taking into consideration, the usage level of 1600 mg/kg/day, suggested by Stangaciu for mice and rats. The calculation was carried out according to estimated slaughtering weight of male broilers (2500 g). Therefore, the level of apilarnil to be administered daily was calculated as 1600×2.5 = 4000 mg (4 g).

Between 22 and 42 days of the experiment, a plastic freezer bag was taken from the deep-freeze each day, the content of the bag was filtered through cheesecloth and the filtrated substance was taken into 20 syringes (4 g $syringe^{-1}$). Due to the sensitivity of apilarnil to ambient temperature (it should be kept at 0-4°C after removal from deep-freeze), the syringes were carried to the poultry-house packed in ice and administered to broilers orally. This application was repeated at the same time each day (10:00 a.m.) for 21 days.

Data and statistical analysis: All data except for mortality were analyzed by Analysis of Variance (ANOVA) using General Linear Models (GLM) procedures of SAS (2000). When the effect of apilarnil was significant, the differences between group means were separated by Duncan's multiple range test. Mortality data were analyzed by Chi-square (χ^2) test.

RESULTS AND DISCUSSION

Table 1 shows the effects of oral administration of apilarnil on body weight, body weight gain, feed intake,

Table 1: Means and analysis of variance for the apilarnil effect on growth performance and secondary sexual characteristics

Variables	Control	Apilarnil	p-values
Body weight, g/bird (days)			
21	883.11±11.45	882.53±16.03	0.9767
28	1303.11±22.69	1317.26±24.21	0.6731
35	1806.00±21.82	1762.53±31.33	0.2675
42	2286.11±26.86	2330.11±32.45	0.3063
Body weight gain, g/bird (days)			
21-28	420.00±13.70	434.74±12.54	0.4320
29-35	502.89±12.57^{a}	445.26±15.82^{b}	0.0076
36-42	480.11±15.17^{b}	567.58±19.32^{a}	0.0012
22-42	1403.00±20.91	1447.58±24.94	0.1819
Feed intake, g /bird (days)			
21-28	698.11±17.99	675.99±17.81	0.3842
29-35	897.56±19.67^{a}	804.11±24.27^{b}	0.0053
36-42	1032.22±25.28	1081.26±25.00	0.1767
21-42	2627.89±46.47	2521.16±41.58	0.2907
Feed conversion ratio, g g^{-1} (days)			
21-28	1.68±0.04^{b}	1.53±0.03^{a}	0.0271
29-35	1.80±0.05	1.83±0.07	0.6905
36-42	2.18±0.07	1.94±0.09	0.0547
21-42	1.88±0.03	1.78±0.04	0.0861
Mortality, dead birds/total birds			
21-42 days	2/20	0/20	0.152
Comb (cm)			
Length	4.06±0.097^{b}	4.6±0.10^{a}	0.0006
Height	1.63±0.08	1.72±0.08	0.4244
Wattle (cm)			
Length	1.93±0.07^{b}	2.45±0.05^{a}	<0.0001
Height	1.27±0.05^{b}	1.67±0.07^{a}	<0.0001

$\chi^2 = 2$; each value represents the mean±SEM (n = 20) ^{a,b}Means within a row with different superscripts differ significantly ($p<0.05$)

feed conversion ratio and dimensions of comb and wattle in male broiler chickens. No significant difference was detected between the average body weights of the experimental group (male broilers given apilarnil) and the control group (male broilers not given apilarnil) on the 28, 35 and 42 days. However, body weight gains in the 2nd and 3rd weeks of apilarnil administration were significantly different to those of the control group. While the body weight gain of the broilers receiving apilarnil reduced between the 29 and 35 days ($p<0.0076$), it increased between the 36 and 42 days ($p<0.0012$). During the 21 days experiment period, total body weight gain of broilers were calculated to be 1403.0±20.91 g and 1447.58±24.94 in the control and apilarnil groups, respectively.

Apilarnil administration did not affect feed intake significantly except on the 29-35 days. In this period (between the 29 and 35 days), feed intake in broilers receiving apilarnil reduced significantly ($p<0.0053$) in comparison to the control group. The decrease detected in the body weight gain of broilers received apilarnil may have resulted from broilers' not consuming enough feed. The effects of apilarnil administration on feed intake and body weight gain were expected to be more dramatic in the 1st day of the study (between the 21 and 28 days) when the broilers were in adaptation period. However, the expected effect was observed 2 weeks later than the date on which apilarnil was first given. The feed conversion of the male broilers in the apilarnil group was better between 22 and 28 days, 36 and 42 days than those in the control group. The feed conversion values determined in control and apilarnil groups were 1.68 and 1.53, respectively between the 21 and 28 days and 2.18 and 1.94 between 36 and 42 days. The feed conversion value for 21 days period of apilarnil administration was 1.88 for the control group and 1.78 for the apilarnil group. Mortality recorded from 21st day until the 42nd day is shown in Table 1. Two broilers died only in the control group on 35th day. According to the statistical evaluation, apilarnil administration does not seem to have had a significant effect on mortality.

Oral administration of apilarnil to male broilers during the growth period did not negatively affect final body weight and body weight gain, feed intake and feed conversion ratio. Moreover, although it was not statistically significant, feed conversion in the apilarnil group showed approximately 9% improvement. A previous study by Fennell and Scanes (1992a, b) and Holst-Schumacher *et al.* (2010) also determined that androgen administration to chickens either inhibited growth or did not affect it.

Similarly, Holst-Schumacher *et al.* (2010) suggested that steroid hormones do not constitute a good growth promoter in broilers. The most important reason for this suggestion is that steroid hormones are very short-body in the bloodstream of non-laying birds since, they have a higher metabolic clearance rate than in laying birds.

Comb and wattle are secondary sex characteristics in chickens. Androgens are required to induce growth of the comb and wattles in roosters (Etches, 1996). Comb growth has also been used as the basis of a relatively sensitive bioassay for androgens (Johnson *et al.*, 1996). In addition, McGary *et al.* (2002) stated that comb area was related to fertility and that the weights of testes and comb were reliable indicators of fertility in roosters. Comb size also determines the social position of a rooster or chicken among the flock. Animals with higher body weight and larger combs tend to be dominant in the flock (Cloutier and Newberry, 2000).

In this study, apilarnil administration during the growth period stimulated length of comb and height and length of wattle in male broilers. The length of comb and the height and length of wattle in the animals given apilarnil showed a significant increase. Yoshioka *et al.* (2010) determined that the combs of roosters (Single Comb White Leghorn) in a testosterone-treated group took on a red tinge and were longer, more elastic and thicker than in controls. They concluded that the capillary endothelial cells in the peripheral dermis layer of the comb

are androgen targets and that androgen might have induced comb growth via an increase in blood flow caused by vasodilation and surface neovascularization. Since, the experimental animals were housed in cages, behavioral assessments could not be carried out. However, it was observed that the broilers in the group receiving apilarnil were more aggressive than those in the control group. As a result of increased aggression, particularly during the last week of the experiment, it became problematic to open the beak of roosters in the experimental group to orally administer apilarnil. Increasing aggression, growth of comb and wattle indicate that apilarnil had a stimulating effect on breeding. In addition as reported by Fennell and Scanes (1992b) in reference to Dube and Trembley, the growth depression in chickens is associated with an exaggerated appearance of the male secondary sex characteristics (i.e., comb, wattle and ear lobe development).

CONCLUSION

In the present study, administration of 4 g day^{-1} oral apilarnil to male broilers during growth period stimulated the development of secondary sex characteristics without affecting the performance. These results indicate that apilarnil has mainly androgenic effects or testosterone-like effects (anabolic effect = androgen effect).

RECOMMENDATIONS

More detailed studies are required to determine potential androgenic effect of apilarnil. Carrying out such studies, especially with male broiler breeders will be more beneficial. Preparation of apilarnil preperats which can be added to feed will facilitate its commercial usability. On the other hand, the fact that apilarnil is a natural bee product will prevent the residue problem which negatively effects the health of consumers or even causes collective food poisoning.

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