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EFFECT OF CLENBUTEROL ON II. CARCASS JOINTS AND MUSCLE COMPOSITION IN LAMBS

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SUMMARY

Eight wether Japanese Corriadale lambs weighing 36 kg each were randomly assigned to receive 0 (control) or 80 µg clenbuterol /kg body weight per day to study the effects of long term (8 weeks) administration of clenbuterol on body composition and skeletal muscle growth in lambs.

Leg joint weight was significantly increased by 28 % in clenbuterol fed than control lambs. The hindquarter and high priced cuts were significantly heavier in treated lambs. Clenbuterol fed-lambs had higher proportion of lean tissue and lower proportion of fat. Individual hind leg skeletal muscle weights were significantly heavier in treated lambs. In contrast, fore leg skeletal muscle weight increased but not significantly. *Longissimus dorsi* muscle of clenbuterol fed-lambs had significantly higher total amount of protein, protein percentage and lower fat percentage. Chemical analysis of *Triceps brachii* muscle (from the fore leg) was not significantly affected by clenbuterol treatment except for total amount of crude protein. Muscle glycogen concentration was significantly lower in treated lambs.

Keywords: Lambs, clenbuterol, joints, carcass, muscle composition

INTRODUCTION

A major aim in meat production by ruminants is to achieve maximum lean tissue growth on one hand and to avoiding excess fat deposition on the other hand, for several reasons. First, the energy required and hence the costs to produce 1 kg of adipose fat is considerably greater than that required to produce the same quantity of muscle (Van Es, 1977). Second, excess fat intake by human is one of the major causes of cardiovascular disease. This

dilemma has led reasearchers to seek for a way to alter the body composition in heavy market lambs through different methods.

In the last decade, there has been considerable interests for using β-adrenergic agonists (β-agonists) such as clenbuterol or cimaterol which resembles adrenalin and noradrenalin to repartition growth in animals raised for meat production. The first studies on the repartitioning of tissue growth by β-agonists of compound centered on the effects of clenbuterol in lambs (Baker *et al.*, 1984), in poultry (Dalrymple *et al.*, 1984a), in cattle (Ricks *et al.*, 1984) and in swine (Dalrymple *et al.*, 1984b).

Clenbuterol is one of the β-agonists group which is known as repartitioning agent, due to their effect on lipid and protein metabolisms and animal composition.

The objective of the present study was to investigate the effects of clenbuterol administration for long term (8 weeks) on carcass composition and individual skeletal muscle and the chronic effects of clenbuterol on metabolite profiles and glycogen level in lambs.

MATERIALS AND METHODS

Animals and experimental design: This experiment was carried out at the Animal Experimental Farm, Animal Science Deartment, Faculty of Agriculture, Kyoto University, Japan. Eight wether Japanese Corriadale lambs with an average weight of 36 kg (6 months old) were randomly assigned to two treatment groups, control and clenbuterol groups. Lambs were individually housed in metabolic crates. All lambs were fed *ad libitum* a high concentrate diet (Table 1) containing 120 g crude protein/kg diet for 8 weeks, every morning feed residue was weighed and subtracted from the amount offered to calculate the actual feed intake. Clenbuterol group was given orally 80 µg clenbuterol [benzyl alcohol, 4- Amino-a-(t-butylamino) methyl-3,5-dichloro] /kg BW / day in gelatin capsules with glucose.

Table 1. Constituents of feed mixture

Ingredient	%
Timothy hay	22.0
Corn	37.0
Barley grain	15.7
Wheat bran	15.0
Soybean meal	9.0
$CaCo_3$	0.8
NaCL	0.5

Slaughter technique: At the end of the experimental period (8 weeks) all animals were slaughtered. Animals were left fasting for 12 hours prior

slaughter and fasted body weight (FBW) was recorded. The weight of empty body (EBW) was calculated as the difference between the weight of the fasted body and gut contents.

Samples of liver, *longissimus dorsi, Semitendinosus* and *triceps brachii* muscle which were dissected within 15 min. of slaughter were frozen in liquid nitrogen then, stored at -85°C for analysis of glycogen. Glycogen was estimated using the methods as described by Sugden *et al.* (1976).

Immediately after the animal being dressed, the carcass was weighed (hot carcass weight, HCW) and stored in the chilling room at 2°C.

After 24 hours of cooling, the carcass was re-weighed (chilled carcass weight CCW) and halved into right and left sides. The two halves were weighted. The right side was separated into commercial joints (neck, shoulder, brisket, bestend, breast, loin, flank and leg) according to the methods of Brown and Williams (1979). Each joint was dissected into lean tissue, fat including nervous tissue and blood vessels, and bone including associated connective tissue, as described by Sulieman *et al.* (1986).

Nine muscles were dissected from the left side of carcass (Table 2). Each muscle was trimmed until it was free of fat and weighed. Samples of *longissimus dorsi* and *triceps brachii* muscles were excised, minced and stored at -24 °C for chemical analysis. Crude protein, crude fat and moisture content were determined by methods of AOAC (1980).

Table 2. The nine largest muscles of the left side carcass of lambs and their anatomical location

Muscle	Anatomical location
1- *M. Longissimus dorsi*	neck and thorax/ lumbar abdominal
2- *M. Semitendinosus,*	pelvic limb
3- *M. Semimembranosus*	pelvic limb
4- *M. Vastus lateralis*	pelvic limb
5- M. *Quadriceps femoris*	pelvic limb
6- *M. Gluteus medius*	pelvic limb
7- *M. Supraspinatus*	thoracic limb
8- *M. Infraspinatus*	thoracic limb
9- *M. Triceps brachii*	thoracic limb

Statistical analysis: The data were analyzed using Student's t-test of Statistical Analysis System (SAS, 1988).

RESULTS

Joint weights and carcass composition: The results show that leg joint weight significantly (P 0.05) increased by 28.4 percentage in lambs fed clenbuterol relative to control lambs. Also, the clenbuterol- fed lambs had heavier neck, shoulder, brisket, breast, loin and flank cut weights than control lambs, but the differences were not statistically significant.

The hindquarter and high priced cuts (leg, loin and bestend) were significantly (P<0.05) heavier by 27.8% and 31.2%, respectively in lambs fed clenbuterol than control lambs.

Table 3. Effect of clenbuterol treatment on carcass joint weights in lambs (mean ± SE)

Weight	Control	Clenbuterol
Neck, g	680±60	860±0.71
Shoulder, g	1920±245	2150±74
Brisket, g	1500±98	1763±149
Best end, g	850±104	792±82
Breast, g	542±53	563±33
Loin, g	934±152	1175±127
Flank, g	374±48	489±65
Leg, g	3038±178	3900±230*
Fore quarter, kg	5.29±0.36	6.07±0.31
Hind quarter, kg	4.35±0.22	5.56±0.32*
Low priced cuts[1], kg	5.21±0.29	5.83±0.33
High priced cuts[2] kg	4.43±0.39	5.81±0.36*

[1] Flank, breast, shoulder, brisket and neck cuts, [2] Leg, loin and best end cuts

* P <0.05

The effects of clenbuterol administration on dissected joints from fore quarter, hind quarter and middle of the right side carcass are shown in Table (4). Clenbuterol had a significant effect on dissected joints from hind quarter and middle of right side carcass. Clenbuterol fed-lambs had higher proportion of lean tissue by 9.1, 13.7, 24.0 and 9.4% and lower proportion of fat by 11.3, 23.0, 30.4 and 27.4% for neck, shoulder, loin and leg cuts, respectively than control lambs. These data revealed that dissected loin and leg joints were more affected by clenbuterol than of neck and shoulder.

Table 4. Effect of clenbuterol treatment on carcass joints composition in lambs (mean ± SE)

Carcass joints	Control	Clenbuterol	% change
Neck			
Muscle %	54.30±2.15	59.20±1.05	+9.07
Fat %	19.50±1.50	17.30±2.70	-11.28
Bone %	24.80±1.55	23.10±1.70	-6.85
Shoulder			
Muscle %	55.50±2.50	63.10±2.50	+13.69
fat %	20.40±1.40	15.70±1.75	-23.04
Bone %	21.80±1.05	19.50±0.25	-10.55
Loin			
Muscle %	54.90±3.30	68.10±3.75*	+24.04
Fat %	32.60±1.20	22.70±2.80**	-30.37
Bone %	10.40±3.05	8.10±1.90	-22.11
Leg			
Muscle %	66.01±0.54	72.19±1.77*	+9.36
fat %	16.80±0.30	12.20±1.45**	-27.38
Bone %	15.78±1.00	15.30±1.10	-3.04

* P <0.05 ; ** P<0.01

Individual muscle weight: Individual hind leg skeletal muscle weights; *longissimus dorsi, semitendinosus, quadriceps femoris, semimembranosus, vastus lateralis* and *gluteus medius* muscle were significantly increased by (P<0.05)45, 41, 40, (P<0.01) 38, 36, 48 and % ,respectively, relative to control lambs. In contrast, weight of fore leg skeletal muscle, *supraspinatus, infraspinatus* and *triceps brachii* muscle increased only by 23, 23 and 36%, respectively, but these differences were not statistically significant. These results revealed that individual hind leg skeletal muscles were more affected by clenbuterol treatment than fore leg muscles.

Chemical composition of *longissimus dorsi* and *triceps brachii* muscle: *Longissimus dorsi* muscle of clenbuterol fed- lambs contained significantly higher total protein amount (136.7 vs 87.5 g, P <0.01), protein percentage (22 vs 20.2, P <0.05) and lower fat percentage (3.1 vs 4.2, P <0.05) than control lambs (Table 6). The chemical analysis of *triceps brachii* muscle from the fore leg was not significantly affected by clenbuterol treatment except the total amount of crude protein was higher in lambs fed clenbuterol than control lambs (53.8 vs 37.9 g, P <0.05) which on the other hand lipid percentage was lower and crude protein percentage was higher in lambs fed clenbuterol. There was no difference between treatments in the moisture percentages of both muscles .

Table 5. Effect of clenbuterol treatment on individual muscles weight (g) in lambs (mean ± SE)

Name of muscle	Control	Clenbuterol	% Change
Longissimus dorsi m.	433±36	626±74*	+44.57
Semitendinosus m.	93±7	131±14*	+40.86
Semimembranosus m.	298±33	411±11**	+37.92
Vastus lateralis m.	311±11	423±30**	+36.01
Quadriceps femoris m.	272±22	382±40*	+40.44
Gluteus medius m.	178±14	263±19**	+47.75
Supraspinatus m.	116±6	143±17	+23.27
Infraspinatus m.	131±20	161±20	+22.90
Triceps brachii m.	192±21	262±25	+36.46

* P <0.05; ** P<0.01

Table 6. Effect of clenbuterol treatment on chemical analysis of *Longissimus dorsi* and *Triceps brachii* muscle in lambs (mean ± SE)

Trait	Control	Clenbuterol
Longissimus dorsi m.		
Moisture %	74.14±0.15	74.00±0.28
Protein %	20.24±0.48	21.95±0.43*
Fat %	4.23±0.31	3.06±0.09**
Total protein, g	87.54±7.18	136.66±13.51**
Triceps brachii m.		
Moisture %	75.93±0.16	76.14±0.26
Protein %	19.99±0.70	20.63±0.54
Fat %	2.82±0.21	2.27±0.17
Total protein, g	37.94±3.44	53.84±4.14*

* P <0.05; ** P<0.01

Liver and muscle glycogen: Glycogen concentration in liver and three muscles; *longissimus dorsi, semitendinosus* and *triceps brachii* are given in Table (7). The results show that liver glycogen concentration was decreased by clenbuterol treatment, but this reduction in glycogen was not statistically significant. Muscle glycogen concentration was significantly lower (P <0.01) in clenbuterol fed lambs than control lambs for the three muscles; *longissimus dorsi* (3.96 *vs* 0.37 mg/g), *semitendinosus* (4.02 *vs* 1.12 mg/g) and *triceps brachii* (3.04 *vs* 0.23 mg/g).

Table 7. Effect of clenbuterol treatment on glycogen (mg/g) of liver, *Longissimus dorsi*, *Semitendinosus* and *Triceps brachii* muscle in lambs (mean ± SE)

Trait	Control	Clenbuterol
Liver glycogen	24.66±6.33	10.29±2.65
Muscle glycogen		
Longissimus dorsi m.	3.96±0.77	0.37±0.17**
Semitendinosus m.	4.02±0.44	1.12±0.58**
Triceps brachii m.	3.04±0.22	0.23±0.04**

** P<0.01, differs from control.

DISCUSSION

Joint weights and carcass composition: Clenbuterol treatment increased the value of carcasses not only by reducing fatness and increasing lean content but also by improving the proportions of hind quarter and high priced cuts in the carcass. In fact, the effect of clenbuterol was interesting, it increased leg joint weight by 28.4% (P <0.05) and hind quarter by 27.8% (P <.0.05) and high priced cuts by 31.2% (P <0.05) and there was no significant effect on fore quarter , low priced cuts, and fore quarter cuts (Table 3). Similar results were reported by Allen *et al.* (1987) in steers, that cimaterol improved hind quarter percentage and high price cuts.

Clenbuterol also had a significant effect on joints composition from hind quarter with not statistically significant on joints composition from fore quarter. Loin and leg lean increased by 24.0 and 9.4 % (P <0.05) and fat percentage decreased by 30.4 and 27.4% (P <0.01), respectively, while fat and lean percentage of neck and shoulder were not affected significantly by clenbuterol treatment. These results are in line with the significantly heavier weight of the individual skeletal muscles of hind quarter than fore quarter (Table 5), and with a significant reduction in the percentage of lipid and increase of crude protein percentage of *longissimus dorsi* muscle (Table 6). Similar results were reported by Sinclair *et al.* (1991) who found fromdissection of the loin and shoulder joints of lambs carcass that cimaterol significantly increased the proportion of lean tissue, whilst fat was reduced significantly in loin joint only.

Individual muscle weight: The most consistent response noted in β-agonist treated animals was an increase in the weight of various muscles (Yang and McElligott, 1989). Kim *et al.* (1987) reported that the gastrocnemius muscle increased by 40% in lambs treated with cimaterol compared to the control lambs. Similarly, lambs fed cimaterol for approximately 2 months showed a 25 to 30% increase in the weights of several muscles compared to control lambs (Beermann *et al.*, 1986; Beermann *et al.*, 1987 and O'Connor *et al.*, 1991). These findings are in agreement with the present study. Clenbuterol-treated

lambs had higher values of nine individual muscle weight which was associated with increased crude protein content in different muscles (Tables 5&6). This muscle accretion could occur from increased protein synthesis, as reported in pigs fed ractopamine (Helferich *et al.*, 1990) and rats fed clenbuterol and fenterol (Emery *et al.*, 1984) or from decreased protein degradation as reported in lambs (Bohorov *et al.*, 1987). Anderson *et al.* (1991) showed a biphasic response of muscle to β-agonist treatment, whereby protein degradation was initially reduced followed by increased protein synthesis with chronic treatment. The response to clenbuterol treatment varied between nine muscles, hind leg being higher than fore leg skeletal muscles. The difference in response between muscles may be due to variations in the proportion of fast and slow twitch fibers in the muscle. Yang and McElligott (1989) from histochemical observations noted that the anabolic effect of β-agonist may be specific to certain fibre types. Muscles are composed of various ratio of type I (slow-contracting, oxidative) and type II (fast-contracting, mixed glucolytic/ oxidative) fibers. In this context, Beermann *et al.* (1987) reported that treatment of lambs with cimaterol reduced the proportion of slow-contracting oxidative (type I) fibers in the *semitendinosus* muscles and increased the cross section area of both type I and the fast-contracting, type II, fibers. They suggested that the most prominent increase in muscles was dominated by type II fibers. Other researchers (Kim *et al.*, 1987) reported that cimaterol increased type II fibers in *longissimus* and *semitendinosus* muscle by approximately 50% ($P < 0.01$), although no significant difference was found in type I fibers. This indicates that the increase of muscle mass in cimaterol lambs group was due to the hypertrophy type II only. An other reason for the differences in response between the nine muscles to clenbuterol treatment may be related to growth patternes of the muscles. Fore leg muscles (*supraspinatus*, *infraspinatus* and *triceps brachii*) have lower growth potential coefficients (.86 to .88, Beermann *et al.*, 1986) than those of hind leg muscles (*semitendinosus*, *semimembranosus*, *vastus lateralis*, *quadriceps* and *gluteus medius*) which ranged from 0.99 to 1.03 (Firth *et al.*, 1982; Lohse *et al.*, 1971). Similar results were reported by Beermann *et al.* (1986) who found that cimaterol had more pronounced effect on hind leg muscles; *biceps femoris*, *semimembranosus* and *semitendinosus* than fore leg muscles; *supraspinatus*, *infraspinatus* and *triceps brachii*.

Chemical composition of *longissimus dorsi* and *triceps brachii* muscle of lambs: The increase of individual muscle weight was confirmed by significantly increased protein content of two muscles; *longissimus dorsi* and *triceps brachii* muscle. Similar results were reported in lambs carcass by Baker *et al.* (1984) and Bohorov *et al.* (1987) with clenbuterol treatment, Hanrahan *et al.* (1987); Kim et al. (1989); Sinclair *et al.* (1991) and O'Connor *et al.* (1991) with cimaterol and Duquette *et al.* (1987) with β- agonist,

644,969. The data in the present study revealed that total amount of protein and protein percentage significantly increased by 56 and 8.4% ,respectively while fat percentage decreased by 27.7% ($P < 0.01$) in *longissimus dorsi* muscle treated with clenbuterol. In contrast, the chemical analysis of *triceps brachii* muscle from the fore leg was not significantly affected by clenbuterol treatment except the total amount of protein was higher (41.9%,$P < .05$)) in lambs fed clenbuterol than control lambs. Although protein percentage was increased by 3.2% and fat percentage decreased by 19.5% with clenbuterol administration, the increases of protein content as shown in present study by clenbuterol may be attributed to decreased protein degradation. Kim *et al.* (1987) concluded that β-agonists decreased protein degradation without affecting protein synthesis. Also, Reeds *et al.* (1986) reported that although clenbuterol increased the rate of protein and RNA accretion in gastrocnemius and sileus muscles, protein synthesis was not increased. More recently, Pringle *et al.* (1993) showed that the β- agonists may act on the calpain - calpastation system to stimulate muscle growth through a decrease in protein degradation. Decreased protein degradation has been suggested as the most efficient means of increasing muscle protein accretion. Also, our recent study (Abbas *et al., 1998*) revealed that 3-methylhistidine excretion was reduced by clenbuterol treated in lambs when expressed as per day or per kilogram body weight. The reduction in 3-methylhistidine suggest decreased myofibillar protein degradation.

The decrease of fat content determined by chemical analysis confirmed with the elevation of free fatty acids concentrations by chronic administration of clenbuterol (unpublished data) which may be due to enhanced lipolysis and/or reduce lipogenesis. The different responses between two muscles for protein accretion and decreased fat content may be due to fibre type of muscle. The reduction of fat determined by chemical analysis in muscle by clenbuterol indicates that reduction in fat content of the animals occurred not only in subcutaneous adipose depots but also in internal depots. Differencial response of various fat deposits to β-agonist has been reported in fat-tailed sheep by Aboul-Ela *et al.* (1988). Reduction in intermuscular lipids with increase in muscle nitrogen content was reported by Allen *et al.* (1987) in Friesin treated with cimaterol, Boucque *et al.* (1987) in bull treated with cimaterol and Berge *et al.* (1993) in calves after clenbuterol administration.

Liver and muscle glycogen: Glycogen is the most abundant carbohydrate, which is present in liver and muscle, generally the later contains only a small amount. The results in Table (7) show that treatment with clenbuterol tended to decrease glycogen stores. However this decrease in liver glycogen concentration was not statistically significant ($P < 0.08$), reflecting the greater residual variation for this measurement. This reduction was confirmed by a significant decrease in liver percentage (Abbas *et al., 1998)*. Also, treatment

with clenbuterol significantly (P <0.01) reduced glycogen levels in *longissimus dorsi, semitendinosus* and *triceps brachii*. Similar effects were reported in lambs treated with cimaterol (10 mg/ kg diet) or clenbuterol (2 mg/kg diet) for 48 days by Warriss *et al.* (1989) who noted that glycogen levels in the *semitendinosus* , the *longissimus dorsi* muscle and liver were lower in the treated groups than in control group. This reduction was only significant in the *semitendinosus* muscle (P <0.05). The authors postulated that muscle glycogen depletion resulted in less formation of lactate *post mortem* and this was reflected on a reduced extent of acidification of the muscles. Also, a decrease in muscle glycogen levels in sheep treated with cimaterol has been reported by McEwan *et al.* (1985). However, Allen *et al.* (1985) demonstrated that muscle glycogen decreased from 59.5 to 45.5 µ-moles glucose units per g wet tissue. The effect was greater after slaughter when muscle glycogen was depressed from 52.1 to 35.6 µ-moles glucose units per g wet tissue in finishing lambs treated with cimaterol. Recently, Garssen et al. (1995) reported that *longissimus* muscle glycogen concentration decreased by 19% (P<0.05) in calves treated with β-agonist (salbutamol) compared with control animals.

CONCLUSION

In the present study, clenbuterol administration increased the weight of leg joint, hind quarter and high priced cuts. Loin and leg lean percentages increased and fat percentage decreased in treated lambs. The weight of the individual skeletal muscles of hind quarter were heavier than those of fore quarter ones. Clenbuterol reduced lipid percentage and increased crude protein percentage in *longissimus dorsi* muscle .

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تأثير مركب Clenbuterol على قطعيات الذبيحة ومكونات العضلات فى الحملان

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قُسمت ٨ حملان من الكوريادیل اليابانية المخصية بمتوسط وزن ٣٦ كجم عشوائياً الى مجموعتين مجموعة لم تعامل والأخرى تناولت ٨٠ ميكروجرام Clenbuterol/ كجم وزن حى/ يوم لدراسة تأثير تناول Clenbuterol (لمدة ٨ أسابيع) على مكونات الجسم ونمو العضلات فى الحملان. زاد وزن الفخذ معنوياً (٢٨ ٪) فى الحملان المعاملة وكانت الأرباع الخلفية والقطعيات الممتازة أثقل وزناً فى الحملان المعاملة وبدرجة معنوية. وإحتوت ذبائح الحملان المعاملة على كمية إكبر من النسيج العضلى و أقل من الدهن. وأوزان عضلات الفخذ المختلفة كانت أثقل معنوياً فى الحملان المعاملة بينما بالنسبة لعضلات القوائم الأمامية كانت الزيادة غير معنوية. وكانت عضلة الظهر (العينية) فى الحملان المعاملة أعلى معنوياً بالنسبة لكمية ونسبة البروتين وأقل فى نسبة الدهن. و لم يكن هناك فرق معنوى فى التحليل الكيماوى للعضلة الثلاثية فى القائمة الأمامية عدا كمية البروتين كانت معنوية . أما تركيز جليكوجين العضلات كان أقل معنويا فى الحملان المعاملة.