

MODULATION OF PLASMA, BRAIN AND LIVER CHOLINESTRASES ACTIVITY; FATTY ACIDS PROFILE AND PERFORMANCE OF JAPANESE QUAIL BY A MEDICAL DRUG MIXTURE

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ABSTRACT

A total of 120 one day old Japanese quail chicks were used in this experiment. During the first two weeks, all chicks were fed a diet formulated to meet the recommended requirements for the growing quails. Thereafter, chicks were randomly classified into three equal groups (each of 40 chicks). The first and second groups, the treated groups were fed on the basal diet, supplemented with 3.3 and 6.6 mg/ kg /diet of a mixture, containing some chemical drugs i.e. Clidinum, Almitrine, Raubasine and Trazodone (CART), while the third group received only the basal diet without any addition (control). At four and six weeks post of groups, five quails from each of groups were randomly chosen and slaughtered. Plasma samples were collected and frozen until biochemical analysis. Liver and brain were frozen until estrases enzymes activities were determined.

The achieved results indicated that body weight, feed consumption and feed efficiency and relative weight of some organs and plasma Cholinestrase activity during both of the two experimental periods of feeding have not been significantly affected by the CART supplementation. Lipogenic activity in liver slices (*in vitro*) was significantly affected by 6.6 mg CART and relatively by other groups, (9.33%) (control and 3.3 mg CART). Feeding CART increased liver ChE activity especially in quails that fed on 6.6 mg than the other groups (47.7%) . Also, age of birds had a significant effect on liver fat content (3.7%). Brain AChE activity increased significantly especially in quails fed on 3.3 mg CART than other groups (22%). The CART feeding especially at the level of 3.3 mg increased unsaturated than saturated fatty acid in the liver fat. This study revealed that diets supplemented with CART possessed the ability to control the appetite center through different ways in regulating feeding intake and growth in quail birds.

Keywords: CART-Performances - Liver Lipogenic activity – Liver ChE activity - Fatty acids profiles- Brain AchE activity – Liver fat content

INTRODUCTION

Japanese quail (*Coturnix coturnix japonica*) is becoming more popular as a source of meat and eggs in various parts of the world including Egypt. Moreover, it has worldwide importance as a laboratory animal in the research studies involving different avian species. (Baumgartner, 1994).

However, the aggressive and nervous behaviour of quail birds are found to be the main problem that affect the productive efficacy of these birds. Evidence from published studies and clinical experience indicates that some tranquilizing and beta adrenergic agonists drugs possess varying abilities to control and (or) modulate brain and liver physiological functions which consequently can increase appetite, stimulate carbohydrate craving, and enhance live body weight over a prolonged period of administration (Jacques and Patricia, 1984; Bernstein, 1987; Schwartz, *et al.*, 2004 and Ruetsch, *et al.*, 2005). The endocrine and metabolic aspects of such drugs as well as the mode of action for these chemical compounds were found to contribute directly on the appetite centers and muscular growth (Baker, *et al.*, 1979; Allain and Bentne – Ferrer, 1998 ; Benzi, 1998; Small and Gimmonna, 2000; and De Meester, *et al*, 2001). Numerous studies indicated that a wide variety of compounds are capable of decreasing carbohydrate-hunger, reducing consumption of carbohydrate-rich foods and inhibiting fat deposition in humans and animals (Sullivan, *et al.*, 1978; Benzi, *et al.* 1987; and Allain and Bentue – Ferrer, 2002 and Aufy, 2006). Since, the recent advances in understanding the physiological control of feed intake, the metabolism of Lipids and carbohydrate (Schwartz, *et al.*, 2004) has led to identifying the potential role of different medical drugs in regulating some metabolic functions of chickens (Benzi, 1998 and El-Wardany and El-Gendi, 2000). Among these drugs are Clidinium (C) Almitrine (A); Raubasine (R) and Trazodone (T). The first one was known as anticholinergic drug, which may help in regulating the proventriculus (stomach) secretions (HCl, enzymes, hormones), and also as a mitochondrial ATP synthetase inhibitor which was used for the treatment of hypoxiemia (Ruetsch, *et al.*, 2005). Regarding the other three drugs which preferentially blocks α_1 -adrenoceptors, and also, strong lipase inhibitors, they could help in the prevention of fatty liver syndrome in laying hens (Cristian, *et al.* 2006) or in the therapy of lipase related diseases. The effect of using a mixture of these previous drugs in poultry production was not studied, either separately or in combination.

Under some circumstances these drugs inhibit serotonin reuptake mechanisms and may simultaneously block serotonin receptor sites. Thus, their effects on appetite and weight gain may represent a balance between serotonergic and antiserotonin activities (Dairman and Juhasz, 1978; Jacques and Patricia, 1984; Benzi, *et al* 1987 and Schwartz, *et al.*, 2004). Also, they could regulate digestive tract periodic motility by stimulating both sympathetic and parasympathetic nerves which regulate several metabolic and physiologic functions in different animal species including birds (Oyawaye and Krueger, 1990; Parikh and Pilo, 1995, and El-Wardany and El-Gendi, 2000). The neural control mechanisms of the gastrointestinal (GI) tract are thought to be partially impaired, in a number of diseases and medical conditions, e.g. spinal cord injury, as in case of cage layer fatigue syndrome in laying hens even though the colonic muscles remain intact and responding to acetylcholine. (Etches, 1996).

Recently, numerous researchs have focused on developing immunity drugs including Almitrine and Clidinium, to enhance birds immune response

or to decrease fat deposition by affecting adrenergic receptors (Aufy, 2006). Therefore, the purpose of this study was to elucidate the possible effects of a mixture containing the previous drugs, Clidinium, Almitrine, Raubasine and Trazdone (CART) on blood plasma, brain and liver esterases activities in Japanese quail birds. Besides, lipogenic activity in liver tissue slices and fatty acids profile of liver lipids concomitant with the productive performance of quails were also investigated.

MATERIALS AND METHODS

A total number of 120 one day old Japanese quail chicks were used in the present experiment. They were housed in 5-tier battery cages and subjected to the adequate and recommended. Water and feed were provided all the time. During the first two weeks, all quail chicks were fed a basal diet, formulated to meet the requirements of quails as recommended by NRC, (1994). The composition and calculated analysis of the basal diet is shown in Table (1).

Table (1): Composition and calculated analysis of the basal experimental diets of the growing quails.

Ingredients (%)	%
Yellow corn	54.65
Soybean meal 48%	32.0
Wheat bran	5.10
Corn gluten meals 62%	4.5
DL- Methionine	0.11
L- Lysine HCL	0.195
Vegetable oil	0.50
Mono - Ca phosphate	0.825
Premix*	0.30
Limestone	1.49
Salt	0.33
Calculated analysis	
CP %	24
ME (Kcal / Kg)	2900
Calcium %	0.81
Av. Phosphorus %	0.32
Methionine %	0.5
Methionine + Cystine %	0.89
Lysine %	1.32
EE %	3.16
CF %	3.07

*Each 3Kg contains: Vit. A 12000000 IU, Vit. D₃ 2500000 IU, Vit. E 10g, Vit. K₃ 2g, Vit. B₁ 1g, Vit. B₂ 5g, Vit. B₆ 1.5g, Vit. B₁₂ 0.01g, Niacin 30g, Folic 1g, Biotin 0.05g, Pantothenic acid 10g, Copper 10g, Iodine 1g, Selenium 0.1g, Iron 30g, Manganese 60g, Zinc 50g, Cobalt 0.1g.

After this period chicks were randomly classified into three equal groups, the first and the second groups were fed the basal ration supplemented with 3.3 and 6.6 mg/Kg diet of a mixture contained equal parts

of chemical drugs including the Clidinum, Alimtrine, Raubasine, and Trazodone (CART) which were replaced by similar amounts of yellow corn to control appetite centers and acts by different ways in regulating feed intake and growth, while the third group was fed on the basal diet as control.

Measurements:

Growth performance parameters: Live body weight of hatched chicks and at weekly intervals was measured to the nearest gram. Body weight gain was then calculated by subtracting the average live body weight of chicks in a previous period from a given period being recorded. Feed consumption was weekly recorded for each group and then the feed conversion ratio was calculated as gram body weight gain to gram feed.

Carcass traits: At the end of the experimental period, five birds from each treatment groups were randomly taken, weighed and slaughtered. Feathers were manually removed and birds were reweighed and eviscerated. Carcass weight and weights of head, liver, heart, spleen and gizzard were also recorded to the nearest 0.01 gram.

Blood sampling, liver, and brain processing:

At four and six weeks post treatment, five quails from each treatment were randomly chosen and slaughtered. Blood samples were collected in heparinized tubes, centrifuged at 4000 rpm for 15 min. then plasma samples were decanted and stored frozen at -20°C until biochemical analysis were done. Liver and brain tissues of these quails were immediately removed as processed for subsequent determinations.

Determination of esterases activity: Activities of cholinesterase (ChE) in plasma and liver extracts were determined according the method described by Wills, (1972). Brain tissues were homogenized and extracted with acetone according to Fleming and Clark (1965). Since, acetyly cholinesterase enzyme (EC, 3.1.1.7) activity in brain extracts was measured by using the method of Elliman, *et al.* (1961).

Fat content of liver: Fatty acids composition of livers from treated quails was determined in one gram of homogenized liver tissue. Fat was then extracted with a mixture of chloroform and methanol (2:1) according to the method of Folch, *et al.* (1957). The fat content was determined gravimetrically after evaporating the solvent under a flow of nitrogen. Fatty acids were determined as methyl esters prepared according to the method of Metcalfe, *et al.*, (1966). The proportions of various fatty acids were determined by gas – liquid chromatography using two U- Columns 1.83 in long and 3 mm inside diameter filled with 6% of DEGS (diethylene glycol succinate).

Lipogenic activity in liver tissues (*in vitro*): Studies on lipogenesis were carried out with liver slices, 100 – 150 mg, which were incubated in 25 –ml Erlenmeyer flusks containing 3 ml of Ca- free Krebs – Ringer bicarbonate buffer, PH 7.4 according to Unbreit, *et al.*, (1964).

Statistical analysis: Statistical analysis was computerized by using statistical program SPSS (1997). All data were subjected to statistically analyses of variance and means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performances: - Table 2 showed the performance of quails fed on the two tested levels of CART. The achieved results that both studied doses didn't significantly affect body weight, but in general, the doses had little negative effects on live body weight, feed consumption, and on body weight gain and feed conversion. [-7.9 and -12.9% than control]. This may be attributed to the treatment decreasing effect of these drugs decrease transiently on live body weight during the first few weeks of treatment and thereafter then may increase body weight. These results are in close agreement with the finding reported by Ruetsch, *et al.* (2005). According to current concepts, appetite and feeding are regulated by a complex of neurotransmitters, neuromodulators, cytokines and hormones interacting with the hypothalamus including leptin and tumor necrosis factor system. (El-Wardany and El-Gendi, 2000 and Aufy, 2006. The pharmacologic mechanisms underlying body weight gain are presently poorly understood, but they may be as the results of that different activities at some receptor systems, which may induce an improved protein turnover and modulate lipid metabolism, however genetic predisposition of birds may a great deal in these responses.

Table (2): Effect of CART on the body weight, feed consumption and feed conversion of quails.

parameters	Age (Wks)	Group 1	Group 2	Group 3 (Control)
Body weight (g)	2	56.13±2.24	55.30±3.15	59.0±3.40
	4	155.47±10.57	149.23±7.58	143.95±5.52
	6	243.40±20.16	258.36±21.50	232.4±15.65
Average weight gain (g)	2-4	99.34±8.21	93.93±6.13	84.95±5.43
	4-6	87.92±9.41	109.13±13.50	88.46±6.12
	2-6	187.27±12.20	203.06±10.25	173.41±8.46
Total feed consumption (g)	2-6	678.01±31.60	699.37±35.14	681.82±26.3
Feed conversion		3.62±0.63	3.44±0.44	3.93±0.52

N.B. No significant differences were detected.

Carcass traits: Findings in Table 3 show the relative weights of some organs for quails fed on two doses of CART. No significant differences between quails that fed on the control and CART diets, but the organs of the treated quails showed dependency for decreased weights than those of the control. These results are logically normal because the changes in the body weights of quails that fed on the CART were slightly decreased as compared to the control. It may be also due to the ability of CART treatment to affected on the relative weights of these organs. In this respect Sullivan, *et al.*, (1978) reported that some drugs of the mixture may be able to affect the digestive tract and delay the gastric emptying time and consequently quails didn't eat like control, (Hurwitz, *et al.*, 1982). Also, Clidinium may regulate the rate of entry of feed into the small intestine through its effect on

motility, (Mark et al. 1978), which may explain the changes in the relative weights of the vital organs as well as the general result of the productive performance traits.

Table (3): Relative weights % of carcass and some organs of growing quails.

Items	Group 1	Group 2	control Group 3
Live weight (g)	245.4±12.6	250.3±13.93	241±10.15
Carcass %	70.13±0.84	71.24±0.56	71.09±0.62
Heart %	0.96±0.012	0.96±0.016	0.93±0.012
Gizzard %	1.49±0.041	1.58±0.026	1.98±0.041
Liver %	2.25±0.11	2.39±0.13	2.7±0.12
Kidney %	0.71±0.01	0.64±0.006	0.77±0.01
Stomach %	0.33±0.01	0.34±0.01	0.39±0.02
Spleen %	0.04±0.002	0.03±0.001	.05±0.002

N.B. No significant differences were detected.

Plasma, liver and brain, acetylcholinesterase (ChE) activity

A- Plasma cholinesterase activity: Table 4 shows the effect of CART on the plasma ChE activity. No significant effects on plasma ChE during the two periods of feeding CART was found.

B- Liver ChE activity: From Table 4, it could shown that CART increased liver ChE activity especially in quails that fed 6.6 mg (47.7%) than the other groups (control and 3.3 mg). Also, age was more effective on the liver ChE activity.

C- Brain AchE activity: Brain AchE activity increased significantly by age and treatments (22%) especially in quails that fed on the lower dose (3.3 mg) than those of the control and those fed on the higher dose (6.6 mg) of CART.

Table 4: Effect of CART supplementation on Liver, brain and plasma cholinesterase (ChE) activity

Treatment	Brain AchE activity m mol /min / g			Liver ChE			Plasma ChE m mol /min / ml		
	Age		Overall	Age		Overall	Age		Overall
	4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks	
Group 1	18.6 ±0.06	26.5±0.0 ±0.03	22.55 b ±0.004	29.15 ±5.35	51.3 ±9.57	40.23a ±13.84	0.09 ±0.001	0.115 ±0.001	0.103 ±0.001
Group 2	22.4 ±0.04	33.2 ±0.04	27.8 c ±0.007	35.93 ±5.75	61.63 ±10.33	48.78b ±15.7	0.08± 0.002	0.07 ±0.001	0.73 ±0.02
Group 3 (Control)	15.2 ±0.02	21.7 ±0.05	18.45 a ±0.003	26.33 ±6.15	41.73 ±7.8	34.03a ±10.49	0.103 ±0.001	0.09 ±0.001	0.1 ±0.001
Overall	18.73* ±0.03	27.13* ±0.04		30.47* ±6.69	51.55* ±11.95		0.09 ±0.002	0.09 ±0.001	

a,b,c: Means in the same column with the same letters are not significantly different. (P<0.05).

* Significant (P<0.05)

Table 5: Effect of CART supplementation on lipogenic activity in liver and liver fat content.

Treatment	Lipogenic activity in liver m mol / min / g		Overall	Liver fat content % of fresh weight		Overall
	Age			Age		
	4 weeks	6 weeks		4 weeks	6 weeks	
Group 1	42.25 ±6.95	63.28 ±12.4	52.76 ± 9.03	2.7 ±0.03	3.7 ±0.08	3.2 ±0.08
Group 2	44.52 ± 8.62	79.43 ± 15.31	61.98 ±13.23	2.51 ±0.07	4.2 ±0.1	3.36 ±0.12
Group 3 (Control)	40.72 ±15.48	89.43 ±14.32	65.08 ±18.88	2.48 ±0.06	4.05 ±0.11	3.26 ±0.12
Overall	42.5* ±10.08	77.38* ±12.7		2.57* ±0.05	3.98* ±0.09	

* Significant ($P < 0.05$)

D- Lipogenic activity in liver: Table 4 showed the effect of CART on lipogenic activity in liver tissues. It could be easily observed that the age had an obvious and significant effect on the lipogenic activity especially after six weeks of age (Part II) (9.33%). On the other hand, the bird fed on the lower dose (3.3mg), [3.8%] recorded the least lipogenic activity as compared to groups (6.6 mg and control).

E- Liver fat content: From the same Table (4), it could be noted that age had a significant effect on liver fat content and this result is logic and expected, but the differences between treatments were not significant.

Benzi, *et al*, 1987 stated that the CART acting on the O₂ availability may interfere with the phospholipid and fatty acids metabolism and their action may differ according to the animal age.

Effect of CART on the fatty acids (FA) composition of liver lipids.

The findings presented in Table 5 showed the effect of CART on the fatty acids composition of liver lipids. It revealed that some of the unsaturated fatty acids increased while some others decreased in quails that fed on the CART than those of the control group. Other fatty acids were not significantly affected by these treatments (Arachidonic and Docosahexaenoic acids). From this table it could also be noted that the unsaturated fatty acids exceeding in group two than those of the control and group one during two studied periods. This may be due to the modification of saturated fatty acids to unsaturated fatty acids in the group that fed on 3.3mg of CART. However, this mechanism is not completely understood until now and may be as a result of the fact that the saturated FA elongated and desaturated to form monounsaturated and polyunsaturated. Also, the CART acting on microcirculation and metabolism may interfere with fatty acids metabolism and consequently their action could be changed according to the bird's age.

Table (6): Effect of CART on the fatty acids Profiles of liver lipids.

Groups	C14:01		Overall	C16:0		Overall	C16:01		Overall	C18:0		Overall	C18:01		Overall
	4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks	
G1	0.26 ±0.03	0.8 ±0.05	0.53 ±0.05	21.38 ±0.17	21.58 ±0.25	21.48 ±0.25	0.53 ±0.03	0.68 ±0.08	0.6 ±0.06	21.55 ±0.28	20.6 ±0.26	21.08 ±0.26	13.93 ±1.76	13.22 ±1.8	13.58 ±1.7
G2	0.33 ±0.04	0.3 ±0.06	0.31 ±0.05	22.25 ±0.19	22.63 ±0.2	22.44 ±0.18	0.4 ±0.06	0.2 ±0.04	0.3 ±0.05	20.88 ±0.09	22.13 ±0.13	21.5 ±0.12	15.15 ±1.64	14.33 ±1.98	14.74 ±1.74
G3 (control)	ND	0.2 ±0.04	0.01 ±0.002	22.5 ±0.18	23.35 ±0.25	22.93 ±0.21	0.2 ±0.04	0.28 ±0.06	0.24 ±0.4	19.4 ±0.24	23.1 ±0.22	21.25 ±0.29	14.7 ±2.03	13.8 ±2.31	14.25 ±2.07
Overall	0.195 ±0.03	0.43 ±0.05		22.04 ±0.17	22.52 ±0.23		0.38 ±0.04	0.38 ±0.06		20.61 ±0.22	21.94 ±0.22		14.59 ±1.73	13.78 ±1.9	

Table (6): Cont.

Groups	C18:02		Overall	C20:04		Overall	C22:06		Overall	Total saturated		Total unsaturated		Sat./unsat. ratio%	
	4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks		4 weeks	6 weeks	4 weeks	6 weeks	4 weeks	6 weeks
G1	15.98 ±1.11	18.02 ±1.08	17±3.15	7.58 ±0.09	7.98 ±0.12	7.78a ±0.1	108 ±0.9	8.28 ±0.91	9.14a ±1.27	42.93	42.18	48.28	48.99	89	86
G2	15.13 ±2.55	19.9 ±1.32	17.51 ±3.17	9.35 ±0.11	10.2 ±0.13	9.78b ±0.12	10.98 ±1.68	11.23 ±1.54	11.1b ±1.5	43.13	44.76	51.34	56.61	84	80
G3 (control)	15.73 ±1.67	18.5 ±1.25	17.11 ±2.02	9 ±0.12	9.05 ±0.07	9.03b ±0.091	10.95 ±1.68	10.33 ±1.93	10.64ab ±1.71	41.9	46.45	50.58	52.16	83	89
Overall	15.61 ±1.74	18.81 ±1.38		8.64 ±0.13	9.08 ±1.37		10.64 ±1.42	9.94 ±1.88		42.65	44.46	50.07	52.59	85.33	85

a,b: Means in the same column with the same letters are not significantly different. $P \leq 0.05$.

ND = not detected

In Conclusion, CART supplementation in quail's diet could be recommended to decrease appetite and subsequently the feeding costs that represents not less than 60-70% of total costs. Thus the net revenue could be increases.

In general, more investigations have to be completed to elucidate the best doses of these drugs which can improve the feed digestion and absorption and the utilization.

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تعديل نشاط أنزيمات الكولين أستريز في البلازما والمخ والكبد بواسطة بعض المركبات الطبية وتأثيرها على صورة الأحماض الدهنية والأداء الانتاجي للسمان الياباني.

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*** قسم تغذية وانتاج الحيوان والدواجن - المركز القومى للبحوث.

**** قسم الإنتاج الحيوانى - كلية الزراعة - جامعة طنطا.

أجرى البحث على عدد ١٢٠ كتكوت عمر يوم من السمان الياباني وغذيت خلال الأسبوعين الأول والثاني من العمر على عليقة تغطي إحتياجات السمان الياباني النامي وبعد ذلك وزعت الطيور عشوائيا على ثلاث مجاميع تقريبا متساوية. وقد غذيت الطيور فى المجموعة الأولى والثانية على العليقة الأساسية مضافا إليها خليط من مادة CART بنسبة ٣,٣ و ٦,٦ مللجم/كجم عليقة على التوالي أما المجموعة الثالثة فغذيت على العليقة الأساسية بدون إضافات (كنترول) وعند الأسبوع الرابع والسادس تم عشوائيا أخذ خمس طيور من كل معاملة حيث ذبحت ومن ثم أخذت البلازما والكبد والمخ وتجميدها لحين اجراء التحاليل الخاصة بهم وأوضحنت النتائج الآتى :

- ١- لم تتأثر معنويا الطيور المغذاه على مادة الـ CART بالنسبة لوزن الجسم ومعدل استهلاك الغذاء ، الكفاءة الغذائية ، الأوزان النسبية للأعضاء ونشاط انزيم (ChE) Cholinesterase فى البلازما اثناء فترات التجربة وذلك بالمقارنة بالطيور الغير معاملة.
- ٢- تأثير نشاط Lipogenic فى الكبد معنويا خاصة فى الطيور التى غذيت على ٦,٦ مللجم من مادة الـ CART بالمقارنة بالمجاميع الأخرى .
- ٣- أدت التغذية على مادة الـ CART إلى نشاط انزيم ChE بالكبد خاصة فى الطيور التى غذيت على ٦,٦ مللجم مقارنة بالمعاملات الأخرى .
- ٤- تأثير معنويا محتوى الكبد من الدهون حيث لعب العمر دورا ايجابيا.
- ٥- زاد نشاط انزيم AChE زيادة معنوية فى المخ خاصة فى الطيور المغذاه على ٣,٣ مللجم من مادة الـ CART.
- ٦- أزداد تركيز مستوى الأحماض الدهنية غير المشبعة بالمقارنة بالأحماض الدهنية المشبعة فى الكبد وذلك فى الطيور المغذاه على ٣,٣ مللجم من مادة الـ CART بالمقارنة بالمجاميع الأخرى .

وقد خلصت الدراسة إلى أهمية استخدام خليط من مادة الـ CART فى تغذية السمان الياباني ، مع التوصية باستخدام وتجريب جرعات أخرى لما لهذا الخليط من تأثير على مركز الشبع ومن ثم تنظيم التغذية والنمو .