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Involvement of corticosterone in food intake, food passage time and *in vivo* uptake of nutrients in the chicken (*Gallus domesticus*)

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Abstract 1. To evaluate the effect of corticosterone on nutrient transport, 10-week-old male chickens were grouped in 4 categories and treated as follows: sham-operated, adrenalectomised, corticosterone (4 mg/kg injected subcutaneously for 5 d) in both sham-operated and adrenalectomised. The food intake, food passage time and uptake of calcium, phosphorus and glucose were determined by standard procedures. 2. Corticosterone administration to both sham-operated and adrenalectomised groups stimulated significantly higher food intake, delayed food passage time and increased uptake of calcium, phosphorus and glucose, as compared to sham-operated control and adrenalectomised groups. 3. Corticosterone administration increased absorption of these nutrients significantly more in the adrenalectomised group than in the sham-operated controls. 4. Corticosterone also significantly elevated the plasma concentrations of these nutrients. The responses to the hormone were significantly greater in adrenalectomised birds. 5. It is concluded that corticosterone increases food intake and retention and the absorption of calcium, phosphorus and glucose in the alimentary tract.

INTRODUCTION

Two interrelated physiological systems are involved in stress responses. The 1st is the sympathomedullary system, evoking short-term responses associated with Cannon's 'flight or fight', mechanism; the 2nd is the hypothalamic-pituitary-adrenal axis, a long term response associated with Selye's adaptive stage of the general adaptation syndrome (Zulkifli and Siegel, 1995). It is of interest to evaluate the components of these 2 pathways which are associated with production traits. The role of corticosterone in the chicken has been considered in relation to feeding, because stress induces corticosterone release (El Halawani *et al.*, 1973; Eden and Siegel, 1975) and a decrease in food intake resulting from heat stress is responsible for severe growth depression (Dale and Fuller, 1980). Corticosterone has been shown to increase food consumption (Bartov *et al.*, 1980; Siegel and van Kampen, 1984; Covasa and Forbes, 1995) to reduce energy absorptive efficiency and to favour energy retention (Siegel and van Kampen, 1984). These observations do not quantify the effect of corticosterone on energy uptake. One assumption was that the reduction in absorptive efficiency may have been caused by intestinal distension as a result of the increase in water intake (Lepkovsky *et al.*, 1960). To our knowledge, information on the effects of corticosterone and adrenalectomy on food passage rate and direct absorption of individual nutrients are lacking in birds. The present

study was designed to investigate the effect of corticosterone on food intake, food passage rate and uptake of calcium, phosphorus and glucose in chickens.

MATERIALS AND METHODS

Initially, 50 10-week-old healthy White Leghorn chicks were allocated to this experiment although only 24 randomly selected birds were finally utilised. A group of 6 male birds were subjected to bilateral adrenalectomy under anaesthesia (thiopentone sodium). It was ensured that no adrenal tissue remained intact. At the end of the experiment, this was confirmed by thorough inspection of the original site of the adrenal. Another similar adrenalectomised group of birds was treated with corticosterone (4 mg/kg body weight, subcutaneously, for 5 consecutive d using propylene glycol as vehicle). 3rd group was kept as a sham-operated control, exposed to the same surgical/handling steps except for the removal of the adrenal gland. Half of the sham-operated birds ($n=6$) were treated with corticosterone (4 mg/kg, subcutaneously, for 5 consecutive d) and another half were treated with vehicle only, keeping other procedures in common. Thus, there were 4 groups (of $n=6$), namely: corticosterone in adrenalectomised or in sham-operated and vehicle alone in adrenalectomised or sham-operated.

The birds of all 4 groups were fasted overnight. Food passage rate, using chromic oxide as a marker, was determined on the 4th day of treatment (Esteve

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and Almird, 1994). On d5, from about 30 min after the last injection, the uptake study was carried out *in situ*, under anaesthesia. The first 8 cm segment of small intestine from the distal end of the duodenum was isolated by ligating at both ends and injected with a 5 g/kg aqueous solution of calcium carbonate. The next 8 cm segment was used for phosphorus uptake (injecting a 20 g/kg aqueous solution of sodium orthophosphate) and the 3rd 8 cm segment was used for glucose absorption, as described by Majumdar and Panda (1989). When handling the alimentary tract outside the body for ligation, infusion and blood collection purpose, a moist warm sterilised cloth (about 41°C) was used, with frequent replacement, in order to maintain conditions similar to those *in situ*. After infusion of the solutions indicated above, the intestine was inserted into the abdominal cavity. The intestine was exteriorised at 15, 30 and 45 min for blood sampling. Blood samples from the appropriate branches of the mesenteric veins were collected at 0 (just before injection), 15, 30 and 45 min post-injection, using a 26 gauge needle. Calcium, phosphorus and glucose concentrations were estimated in plasma using the standard methods of Nelson and Somogyi (1957) for glucose, the micro-colorimetric method of Taussky and Shore (1953) for phosphorus estimation and a Qualigens diagnostic kit Taussky and Shore (1953) for calcium estimation.

Statistical analysis

Differences between treatments for food intake and food passage rate were tested by one-way analysis of variance (Snedecor and Cochran, 1967). The treatment and period effects on uptake of calcium, phosphorus and glucose were analysed as a 2-way factorial design, following repeated-measures analysis of variance procedures described by Gill (1978). The means were compared by multiple-range test.

RESULTS

Food intake and food passage rate

The detailed results on food intake and food passage time are presented in Table 1. Food intake was significantly ($P<0.05$) greater in corticosterone-treated groups than in the vehicle-treated groups, irrespective of adrenalectomy. The time taken for

food passage through the alimentary tract was significantly greater in corticosterone-treated groups than in sham-operated controls. The corticosterone response was significantly ($P<0.05$) higher in the adrenalectomised group than in sham-operated controls. However, there was no significant difference between adrenalectomised and sham-operated control groups.

Absorption study

The detailed results on absorption of glucose, calcium and phosphorus are given in Tables 2, 3 and 4 and the increase in uptake of these nutrients after 15, 30 or 45 min of infusion are shown in Figures 1 to 3. The values of variance using a repeated measure model are given in Table 5. The treatment, period and treatment \times period effects were significant ($P<0.01$) for all calcium, phosphorus and glucose studies except for the treatment \times period interaction for calcium, which was significant at the 5% level (Table 5).

Glucose

The results related to glucose uptake are shown in Table 2 and Figure 1. Corticosterone in adrenalectomised birds produced significantly ($P<0.05$) higher mean glucose values at all times (zero, 15, 30 and 45 min) as compared to corticosterone in the sham-operated group. However, after deducting the zero value, the trend in glucose uptake at 15, 30 and 45 min. was reversed (Figure 1). Also, in both of the corticosterone-treated groups, the glucose values were significantly ($P<0.05$) greater than in the sham-operated control and adrenalectomised groups. However, there was no significant difference between the adrenalectomised and sham-operated groups.

Calcium

The detailed results on calcium uptake are given in Table 3 and Figure 2. Corticosterone treatment in adrenalectomised birds produced significantly ($P<0.05$) greater calcium uptake than the corticosterone-treated sham-operated group at the same time. The net uptake after deducting zero values remained greater in the corticosterone-adrenalectomised group. The adrenalectomised group showed a greater difference than the sham-operated group (Figure 2) However, there was no significant difference between adrenalectomised and sham-operated groups (Table 3).

Table 1. Mean values and standard error of food intake and food passage rate as affected by corticosterone and adrenalectomy in the chicken

Treatments	Average daily food intake (g)	Food passage time (min)*
Sham-operated	85.0 \pm 5.70 ^a	68.8 \pm 3.75 ^a
Adrenalectomised	85.3 \pm 3.87 ^a	70.0 \pm 3.89 ^a
Corticosterone in sham-operated	107.5 \pm 5.86 ^b	86.3 \pm 2.39 ^b
Corticosterone in adrenalectomised	110.0 \pm 8.16 ^b	95.0 \pm 2.04 ^c

* Time difference between start of intake of chromic oxide fortified food and 1st appearance in faeces. Values within a column with different superscripts differ significantly ($P<0.05$).

Table 2. Effect of adrenalectomy and corticosterone on glucose absorption (mg/100 ml of plasma) in alimentary tract of the chicken

Treatment	Glucose concentration			
	Zero	5 min	30 min	45 min
Sham-operated	125.55±2.04 ^{Aa}	137.77±2.22 ^{Ab}	153.33±4.22 ^{Ac}	131.10±3.29 ^{Ab}
Adrenalectomised	139.99±4.87 ^{Aa}	148.88±3.29 ^{Ab}	162.22±2.33 ^{Ac}	139.99±4.55 ^{Aa}
Corticosterone in sham-operated	192.22±2.05 ^{Ba}	261.11±13.44 ^{Bb}	279.99±17.38 ^{Bc}	249.99±18.19 ^{Bb}
Corticosterone in adrenalectomised	268.88±2.22 ^{Ca}	325.55±9.80 ^{Cb}	353.33±4.55 ^{Cc}	307.77±13.82 ^{Cb}

^{A,B,C} superscripts denote column-wise significant ($P<0.05$) differences.

^{a,b,c} superscripts denotes row-wise significant ($P<0.05$) differences.

Table 3. Effect of adrenalectomy and corticosterone on calcium uptake (mg/100 ml of plasma) in alimentary tract of the chicken

Treatment	Calcium concentration			
	Zero	15 min	30 min	45 min
Sham-operated	8.26±0.51 ^{Aa}	8.78±0.45 ^{Ab}	9.11±0.37 ^{Ac}	10.23±0.42 ^{Ad}
Adrenalectomised	7.88±0.51 ^{Aa}	8.99±0.46 ^{Ab}	9.89±0.36 ^{Ac}	10.47±0.41 ^{Ad}
Corticosterone in sham-operated	9.58±0.23 ^{Ba}	10.59±0.34 ^{Bb}	11.97±0.32 ^{Bc}	11.40±0.28 ^{Bc}
Corticosterone in adrenalectomised	11.00±0.57 ^{Ca}	12.46±0.54 ^{Cb}	13.59±0.45 ^{Cc}	14.16±0.56 ^{Cd}

^{A,B,C} superscripts denote column-wise significant ($P<0.05$) differences.

^{a,b,c,d} superscripts denote row-wise significant ($P<0.05$) differences.

Table 4. Effect of adrenalectomy and corticosterone on phosphorus uptake (mg/100 ml of plasma) in alimentary tract of the chicken

Treatment	Phosphorus concentration			
	Zero	15 min	30 min	45 min
Sham-operated	4.17±0.11 ^{Aa}	4.69±0.08 ^{Ab}	4.77±0.15 ^{Ab}	4.24±0.13 ^{Aa}
Adrenalectomised	4.39±0.15 ^{Aa}	4.83±0.12 ^{Abc}	4.99±0.21 ^{Ab}	4.57±0.21 ^{Aca}
Corticosterone in sham-operated	5.42±0.10 ^{Ba}	5.97±0.11 ^{Bb}	6.45±0.15 ^{Bc}	6.04±0.09 ^{Bb}
Corticosterone in adrenalectomised	7.39±0.17 ^{Ca}	8.07±0.22 ^{Cb}	8.62±0.09 ^{Cc}	8.07±0.10 ^{Cb}

^{A,B,C} superscripts denotes column-wise significant ($P<0.05$) differences.

^{a,b,c} superscripts denotes row-wise significant ($P<0.05$) differences.

Phosphorus

The detailed results on phosphorus uptake are presented in Table 4 and Figure 3. Corticosterone treatments of the adrenalectomised group produced significantly ($P<0.05$) greater values at all times than did the corticosterone treatment of the sham-operated group. Both of the corticosterone-treated groups always showed significantly ($P<0.05$) greater phosphorus uptake than the sham-operated and adrenalectomised groups. However, no significant difference ($P<0.05$) was noted between sham-operated and adrenalectomised groups. Even after deducting the zero period values in each group, corticosterone administration increased the uptake phosphorus of P (Figure 3).

DISCUSSION

Corticosterone stimulated greater food intake in both adrenalectomised and sham-operated groups. In part, the results are consistent with those of Bartov *et al.* (1980), Siegel and vag Kampen (1984) and Covasa and Forbes (1995). To our knowledge, there is no published information to confirm or contradict the effect of adrenalectomy. Corticosterone slowed food passage rate in the alimentary tract of sham-operated birds and this response was further ($P<0.05$)

reduced in adrenalectomised birds (Table 1). However, no significant difference prevailed between adrenalectomised and sham-operated groups. These results showed that the response to corticosterone in this respect increased with adrenalectomy. This may result from some factor present in the adrenal which may directly or indirectly counter-act the effect of corticosterone on food passage rate. Indirect support for the effects of corticosterone administration come from observations that heat stress induces corticosterone release (El Halawani *et al.*, 1973; Eden and Siegel, 1975) and also slows down the food passage rate (Wilson *et al.*, 1980). It is assumed that corticosterone reduces the food intake effect of stressors as this hormone was found to stimulate food intake and prolong food retention in alimentary tract and, in turn, may improve nutrient digestion and absorption by increasing time of contact with digestive enzymes and absorptive walls, and may also increase the digestibility of dietary fibre by allowing more time for microbial fermentation (Washburn, 1991).

The treatment \times period interactions for calcium, phosphorus and glucose uptake were significant, showing that the absorption pattern varied with time. The higher glucose value at time zero resulting from corticosterone treatment of

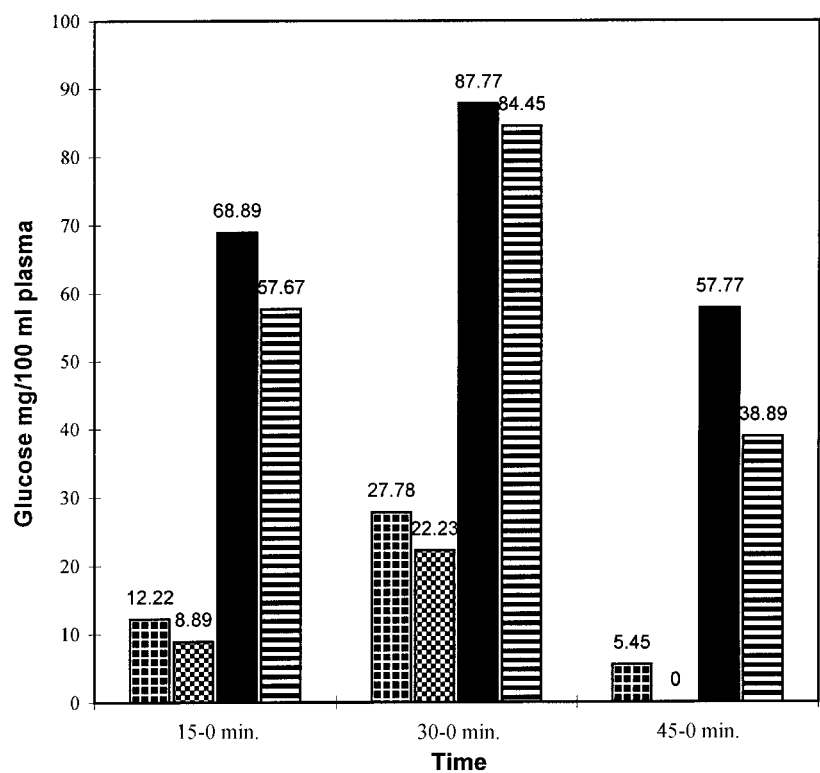


Figure 1. Increase in glucose concentration in intestine at 15, 30 and 45 min post-infusion over zero values. ■ Sham-operated, ▨ Adrenalectomised, ■ Corticosterone sham-operated and ▤ Corticosterone adrenalectomised.

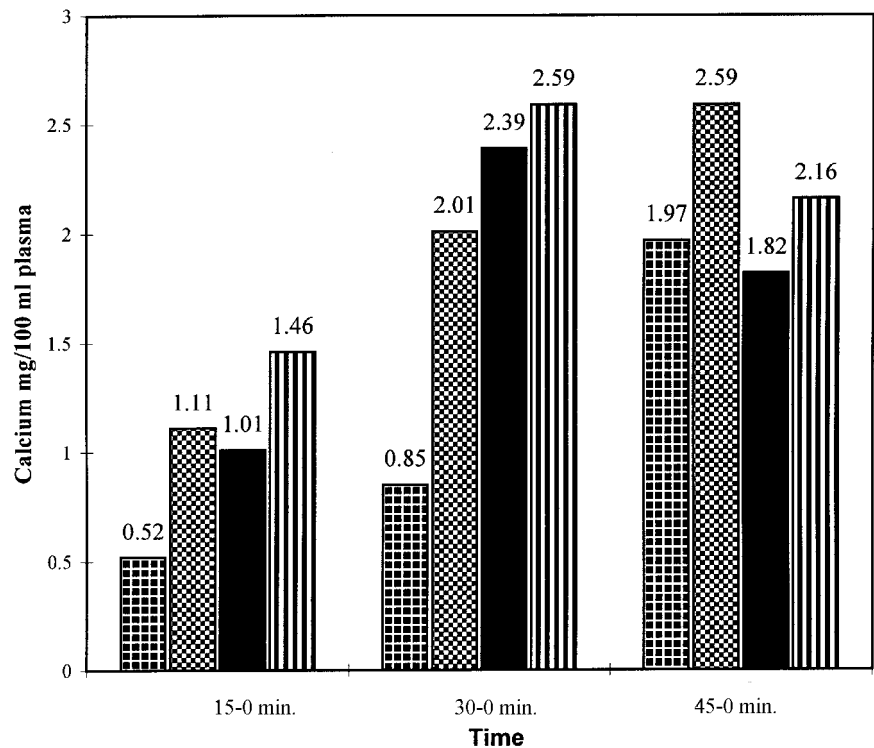


Figure 2. Increase in calcium concentration in intestine at 15, 30 and 45 min post-infusion over zero values. ■ Sham-operated, ▨ Adrenalectomised, ■ Corticosterone sham-operated and ▤ Corticosterone adrenalectomised.

adrenalectomised birds, compared with the sham-operated group may have been caused by a greater metabolic response to corticosterone in the former case. Dietary corticosterone has been reported to change the metabolism of carbohydrate, lipid and protein in a dose-related manner (Siegel and van Kampen, 1984). Corticosterone also increases

protein breakdown and fat deposition (Sharpe *et al.*, 1986), so a change in glucose concentration at time zero is to be expected. The increase in absorption of glucose under the influence of corticosterone could not be confirmed or contradicted from other published data. However, heat stress in the rat, hamster and also in the chicken increased the

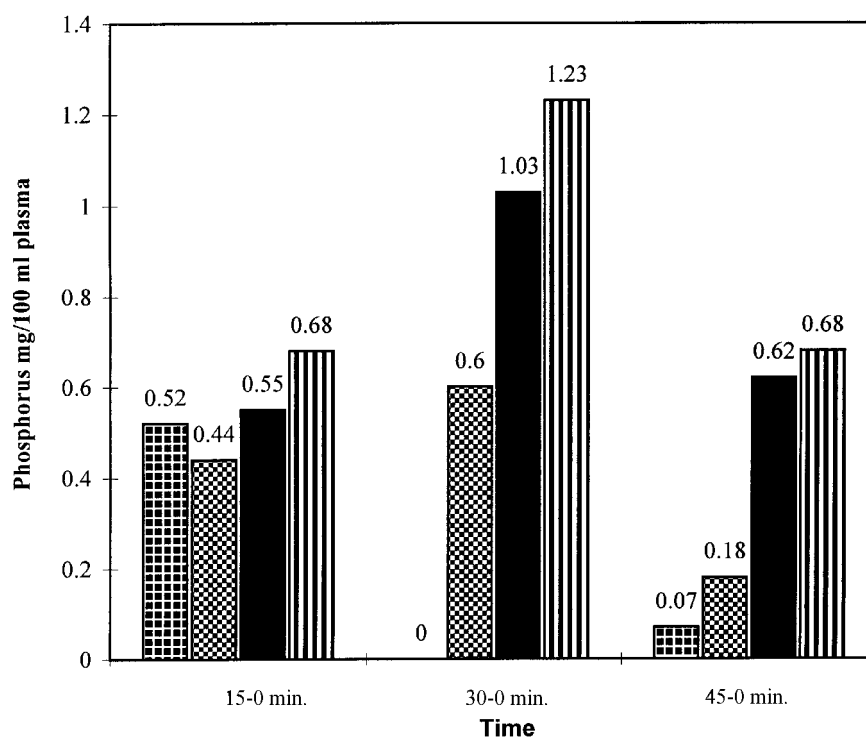


Figure 3. Increase in phosphorus concentration in intestine at 15, 30 and 45 min post-infusion over zero values. ■ Sham-operated, ▨ Adrenalectomised, ■ Corticosterone sham-operated and ▩ Corticosterone adrenalectomised.

transfer of glucose and galactose (Carpenter and Musacchia 1974, 1978; Mitchell and Carlisle, 1992) and also elevates the corticosterone concentration in turkeys and chickens (El Halawani *et al.*, 1973; Eden and Siegel, 1975). The present finding that the corticosterone facilitates the uptake of glucose is therefore indirectly supported. Corticosterone influenced the uptake of both calcium and phosphorus; concentrations at all stages of sampling were significantly ($P < 0.05$) higher in both of the corticosterone-treated groups.

No direct evidence supporting this observation has been found elsewhere but certainly the significance of corticosterone in relation to calcium and phosphorus has been suggested to extend the survival time of bird under heat stress (Yousef, 1985). Also, the corticosterone-treated adrenalectomised group had significantly higher calcium and phosphorus concentrations than that of the corticosterone-treated sham-operated control. This difference may be caused by the presence of endogenous corticosterone in the latter group or some factor influencing this parameter may be

present in the adrenal gland which in turn interacts with corticosterone and affects absorption rate. However, no difference was noted between adrenalectomised and sham-operated groups. After deducting the zero value from 15, 30 or 45 min post-infusion, the trend of net values among different treatments (Figures 2 and 3) also remained similar to the total concentrations of calcium and phosphorus in the respective groups (Tables 3 and 4).

Under heat stress, a major factor responsible for decreased production is the reduction in food intake (Dale and Fuller, 1980). Release of corticosterone under heat stress has also been reported (El Halawani *et al.*, 1973; Eden and Siegel, 1975). In the present study, corticosterone not only induced a decrease in food intake, but it also slowed the food passage rate, which may improve digestion, as discussed above, and may also increase the absorption of nutrients. The adverse effect of stress on production traits may, therefore, not be mediated through corticosterone at these rates of food intake, food passage rate or absorption. The response to corticosterone treatment was greater

Table 5. Analysis of variance using a repeated-measures model

Source of Variance	DF	Mean sum of squares		
		Ca	P	Glucose
Between treatment	3	72.462**	64.536**	163669.70**
Error-1	20	3.499	0.402	1431.30
Between Periods	3	25.109**	3.218**	14183.50**
Treatment × Period	9	0.688*	0.161**	1974.33**
Error-2	60	0.261	0.039	393.07

* $P < 0.05$, ** $P < 0.01$.

in the adrenalectomised group suggesting that the factor/hormone antagonising the favourable effect of corticosterone may be of adrenal origin. This antagonistic effect may result from the involvement of another pathway of stress (the sympatho-medullary axis).

It is concluded that corticosterone stimulates food intake, reduces food passage rate and increases glucose, calcium and phosphorus absorption rates in the chicken and thus there may be a factor or hormone in the adrenal which antagonises the corticosterone effects.

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