Effect of sericin and possible interactions with dopaminergic and adrenergic receptors on the feed consumption and feeding behavior in chicken

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Abstract

This research aimed to study the effect of central infusion of sericin and possible interferences with dopaminergic and adrenergic receptors on the feed consumption of neonatal chickens. This study included 11 experiments with 4 groups in each group with 11 replications. In the first experiment, group 1 received ICV administration of the saline, and groups 2-4 received ICV injection of the sericin (0.125, 0.25, and 0.5 nmol), respectively. In the second experiment, saline, sericin (0.5 nmol), D1 receptor antagonist (SCH23390, 5 nmol), and Sericin + SCH23390 were administered. In experiments 3-11, AMI-193 (5 nmol), NGB2904 (6.4 nmol), L-741,742 (6 nmol), 6-OHDA (2.5 nmol), parazosin (10 nmol), yohimbine (13 nmol), metoprolol (24 nmol), ICI 118,551 (5 nmol), and SR 59230R (20 nmol) were injected instead of SCH23390. Then feed consumption was monitored up to 2 hours after the administration. Also, behavioral changes including the number of steps, jumps, feeding, drinking, and exploratory pecks were recorded for 30 minutes. According to findings, central infusion of sericin (0.25, and 0.5 nmol) declined meal consumption ($P<0.05$). Co-administration of the SCH23390 plus sericin meaningfully attenuated hypophagic effect of the sericin ($P<0.05$). Co-infusion of the ICI 118,551 plus sericin lessened sericin-induced hypophagia ($P<0.05$). Sericin significantly reduced the number of steps, jumps, exploratory, and feed peckings ($P<0.05$). These findings suggested sericin has hypophagic role in chicken and its effect mediates via D_1 dopaminergic and β_2 adrenergic receptors.

Keywords: Sericin, Dopaminergic, Adrenergic, Food intake, Chicken

1. Introduction

The cocoon layer of silkworm known as *Bombyx mori* and silk is consisted mainly of fibroin and 70% of silk protein is sericin [1]. In the textile industry, most of the sericin is removed from the cocoon without any application [2]. Recently, it is known that sericin has antioxidant action and inhibitory action of tyrosinase [1]. Fibroin improves models of memory impairment and as a cholinergic modulator maintains normal acetylcholine levels in the brain [1]. Sericin has anti-aging activity by oxidative stress, anti-inflammation, and apoptosis properties [3]. Injectable silk sericin enhances neuro-differentiation in severe ischemic stroke damage [4].

There is increasing interest in the effects of sericin on appetite regulation and energy expenditure. It has been reported that sericin can prevent high-fat diet-induced hyperlipidemia and overweight in mice [3]. Sericin administration decreased serum glucose and growth hormone levels and their expression in the brain while increasing insulin-like growth factor-1 (IGF-1) and growth hormone (GH) receptor expression in the diabetic rats' hippocampus [5]. Sericin intake enhances L-serine and L-tyrosine concentrations and noradrenergic turnover in the brain of mice [6]. It is reported ICV injection of the silk fibroin hydrogels (4% w/v) had no overt microglial/macrophage response and no harmful effects or mortality in the stroke model of rats [7].

Food intake can be controlled by impulses from the gastrointestinal tract and the brain [8,9]. Nervous systems that are effective in the production of these mediators in the nervous system can have remarkable effects on feeding behaviors [10-12]. Although some aspects of the regulation mechanism are similar between animals, there are some dissimilar mechanisms in the central feeding regulation among birds and mammals [13-15]. Adrenergic receptors are distributed throughout the CNS, particularly in areas like the hypothalamus and brainstem that control feeding behavior [10]. These receptors are categorized into alpha (α) and beta (β) types, with subtypes α1, α2, β1, β2, and β3 (Ciccarelli et al., 2013). Alpha adrenergic receptors, particularly α2, have been shown to stimulate meal consumption. For example, infusion of clonidine (an α2 receptor agonist) or NE into the paraventricular nucleus (PVN) stimulates feeding behavior in rats and domestic fowl [11]. This effect is blocked by yohimbine (an antagonist of α2 receptor) but not prazosin (an antagonist of α1 receptor) [12]. Dopamine (DA) is a main catecholamine neurotransmitter in the brain and plays a crucial role in appetite regulation. Dopaminergic (DAergic) neurons have been identified in different nuclei of the brain including the substantia nigra, ventral tegmental area and hypothalamus. To date, least 5 distinct sub-types of DA receptors have been identified

(D1-D5). Anatomical evidence suggesting both D1 and D2 receptors are involved in feeding regulation centres in the brain [12]. The projections of the DA have been identified from the ventral tegmental area (VTA) and into the ARC and NAcc. Feed intake was decreased via D1 and D2 receptors in rats. In addition, DAinduced hypophagia is mediated by D1 receptors in chicken, while other receptors (D2-D4) appear to have no role in appetite regulation [9].

Despite the great effort among scientists to understand the functional role of silk proteins in regulating food intake, studies on the physiological effects of silk proteins in relation to appetite regulation have been few. As, there are differences in central meal consumption regulation among birds and mammals, understanding the mechanisms of food intake is important, this research aimed to determine the effect of central sericin and possible interferences with dopaminergic and adrenergic receptors on the feed intake of chickens.

2. Material and Methods

2.1. Animals

484 meat-type (Ross- 308) one-day-old chicks obtained from a domestic hatchery were used (Mahan Co. Iran). The birds were placed in group cages for the first two days, and then kept in solitary confinement until the fifth day. Chicks had unlimited access to fresh drinks and a basic diet. The study protocole was approved by ethic commetee of the veterinary faculty, Islamic Azad University, Sciecne and Research Branch, Tehran, Iran.

2.2. Injection Procedure

ICV infusion was done at the age of five days. The heads of chickens were held by an acrylic device. A hole was made in the stencil and placed on the skull in the area of the right ventricle [16]. The needle of the Hamilton syringe was inserted 4 mm into the skull. Administrations were performed in a volume of 10 µL with no stress [17]. The accuracy of the infusion on the brain was determined by decapitation at the end of the study and confirmed by observing the blue color (Evans Blue) in the injected area [18].

2.3. Grouping and Food Intake Measurement

This study included 11 experiments with 4 groups in each group with 11 replications. Before the study, birds were off feed for 3 hours (FD_3) and after the infusion, they were placed in their cages with free access to drink and meals. In the first experiment, group 1 received ICV administration of the saline, and

groups 2-4 received ICV injection of sericin (0.125, 0.25, and 0.5 nmol), respectively. In the second experiment, saline, sericin (0.5 nmol), D1 receptor antagonist (SCH23390, 5 nmol, tocris co.), and sericin + SCH23390 were administered. In the third experiment, chicken were injected with saline, sericin (0.5 nmol), D2 receptor antagonist (AMI-193, 5 nmol tocris co.), and sericin + AMI-193. In the forth experiment, ICV infusion was applied as saline, sericin (0.5 nmol), D3 receptor antagonist (NGB2904, 6.4 nmol, tocris co.), and co-injection of the sericin and NGB2904 (6.4 nmol). In experiment 5, the injection of the saline, sericin (0.5 nmol), D4 receptor antagonist (L-741,742, 6 nmol, tocris co.), and co-injection of the sericin and L-741,742 (6 nmol) were done. In experiment 6, ICV administration of hydroxylated analogue of dopamine (6-OHDA, 2.5 nmol, sigma co.), sericin (0.5 nmol) and sericin + 6-OHDA were done. In experiment 7, group 1 received ICV injection of the saline, group 2 received α1 receptor antagonist (parazosin, 10 nmol, sigma co.), group 3 was injected with sericin (0.5 nmol) and groups 4 received co-infusion of the parazosin and sericin. In experiment 8, group 1 received ICV injection of the saline, group 2 received α2 receptor antagonist (yohimbine, 13 nmol, sigma co.), group 3 injected with sericin (0.5 nmol) and groups 4 received co-infusion of the yohimbine and sericin. In experiment 9, the administration of the saline, sericin (0.5 nmol), β1 adrenergic receptor antagonist (metoprolol, 24 nmol, sigma co.), and sericin + metoprolol were done. In experiment 10, chicks received administration of the saline, sericin (0.5 nmol), β2 adrenergic receptor antagonist (ICI 118,551, 5 nmol, sigma co.), and in group 4 co-injection of the sericin and ICI 118,551 were applied. In experiment 11, group 1 received ICV injection of the saline, group 2 received β3 adrenergic receptor antagonist (SR 59230R, 20 nmol, sigma co.), group 3 was injected with sericin (0.5 nmol) and group 4 received co-infusion of the SR 59230R and sericin. Then the total feed consumption was calculated at 30, 60, and 120 minutes post-infusion [16,19].

2.4. Behavioral Evolutions

After the injection of sericin, each bird was placed in the monitoring boxes, and videotaped for half an hour. Behavioral evolutions were determined as the number of steps, jumps, exploratory, feeding, and drinking pecks (count-type behaviors) [20].

2.5. Statistical Analysis

Food intake and behavioral evolutions was determined based on % of body weight (%BW) and analyzed using the repeated measures analysis of variance (ANOVA) and displayed as mean ± SEM. To compare the means Tukey-Kramer test was used ($P<0.05$).

3. Results

As seen in the first experiment, central administration of sericin (0.125 nmol) had no effect on meal consumption but at levels of 0.25, and 0.5 nmol remarkably attenuated appetite in comparison to the placebo group ($P<0.05$) (figure 1).

Based on experiment 2, hyophagia was observed following the injection of sericin (0.5 nmol) in comparison to the placebo treatment ($P<0.05$). Infusion of the SCH23390 (5 nmol) had no effect on total meal consumption ($P>0.05$). Co-administration of the SCH23390 + sericin remarkably decreased hypophagia induced by sericin ($P<0.05$) (figure 2).

According to the third experiment, central injection of the AMI-193 (5 nmol) had no significant effect on appetite ($P>0.05$). Sericin (0.5 nmol) meaningfully decreased appetite compared to the placebo treatment ($P<0.05$). Co-injection AMI-193 + sericin did not cause significant changes in sericin-induced hypophagia ($P>0.05$) (figure 3).

As seen in the fourth test, central infusion of NGB2904 (6.4 nmol) had no effect on appetite ($P>0.05$). Administration of sericin (0.5 nmol) remarkably decreased feeding in comparison to control chickens ($P<0.05$). Combined injection of NGB2904 + sericin did not induce meaningful changes in the appetite reduction effect of sericin ($P>0.05$).

According to findings of the experiment 5, no change in feeding was observed by infusion of the L-741,742 (6 nmol) ($P>0.05$). Sericin (0.5 nmol) meaningfully decreased meal consumption than to control chicken ($P<0.05$). Sericin-induced hypophagia was not changed by co-administration of L-741,742 + sericin ($P>0.05$) (figure 5).

In the sixth experiment, infusion of the sericin (0.5 nmol) notably reduced appetite in chicken ($P<0.05$). Central injection of 6-OHDA (2.5 nmol) did not change feeding behavior ($P>0.05$). Co-administration of the 6-OHDA + sericin significantly diminished the effect of the sericin on feed consumption ($P<0.05$) (figure 6).

In the seventh experiment, meal intake was attenuated by injection of the sericin (0.5 nmol) ($P<0.05$). Central administration of parazosin (10 nmol) did not have a significant effect on appetite ($P>0.05$). No meaningful change was observed in the hypophagic effect of the sericin by Co-injection of the parazosin + sericin ($P>0.05$) (figure 7).

As seen in experiment 8, yohimbine (13 nmol) did not change meal consumption ($P>0.05$). Infusion of the sericin (0.5 nmol) decreased feeding ($P<0.05$). Co-administration of the yohimbine + sericin did not cause significant changes in sericin-induced hypophagia ($P>0.05$).

In test 9, administration of the metoprolol (24 nmol) did not change feeding ($P>0.05$). ICV infusion of the sericin (0.5 nmol) suppressed feed intake ($P<0.05$). Co-infusion of metoprolol + sericin had no meaningful effect on sericin-induced hypophagia ($P>0.05$) (figure 9).

Based on figure 10, sericin (0.5 nmol) significantly decreased appetite ($P<0.05$). Administration of the ICI 118,551 (5 nmol), had no remarkable effect on cumulative meal consumption ($P>0.05$). Co-injection of the ICI 118,551 + sericin remarkably attenuated hypophagic effect of the sericin ($P<0.05$) (figure 10). In experiment 11, infusion of the SR 59230R (20 nmol) did not change feeding behavior ($P>0.05$). Central administration of the sericin (0.5 nmol) suppressed meal intake ($P<0.05$). Adminstion of SR 59230R and sericin together did not affect decreasing appetite caused by sericin ($P>0.05$) (figure 11).

As shown table, sericin significantly reduced the number of steps, jumps, exploratory, and feeding pecks 15 minutes after the infusion ($P<0.05$), and no meaningful difference was seen after 15 minutes post administration ($P>0.05$).

Table. Count-type behaviors after ICV injection of control solution (saline) or Sericin

		Time post-injection (minutes)					
Behavior	**Groups**	**5**	**10**	**15**	**20**	**25**	**30**
	Control	122.50±10.12 [a]	141.23±4.17 [a]	265.31±21.13 [a]	321.54±18.11	377.45±34.22	465.64±46.17
	Sericin (0.125 nmol)	125.62±10.22 [a]	133.14±6.51 [a]	264.23±18.16 [a]	331.22±15.13	364.24±31.10	478.37±53.21
Steps	*Sericin (0.25 nmol)*	102.44±3.24 [b]	126.55±3.25 [b]	203.26±12.15 [b]	286.23±16.32	353.64±35.12	472.20±31.15
	Sericin (0.5 nmol)	91.44±211 [c]	104.34±3.11 [c]	169.12±8.33 [c]	287.54±17.76	334.10±30.21	458.54±34.22
	Control	1.55±0.21 [a]	2.11±0.11 [a]	3.65±0.14 [a]	5.32±0.11	6.65±0.31	6.21±1.03
	Sericin (0.125 nmol)	1.54±0.11 [a]	2.15±0.13 [a]	3.63±0.17 [a]	5.31±0.12	6.24±0.24	6.20±0.14
Jumps	*Sericin (0.25 nmol)*	0.67±0.10 [b]	1.22±0.11 [b]	2.41±0.15[b]	5.43±0.32	6.31±0.28	6.24±1.24
	Sericin (0.5 nmol)	0.21±0.10 [c]	0.94±0.10 [c]	1.85±0.16 [c]	5.46±0.20	6.32±0.26	6.26±1.63
	Control	45.21±8.24 [a]	86.12±12.31 [a]	109.15±16.69 [a]	134.58±22.48	152.20±17.11	176.42±15.11
	Sericin (0.125 nmol)	43.54±8.31 [a]	83.44±9.33 [a]	106.24±13.22 [a]	133.36±14.16	148.41±19.12	172.39±16.41
Exploratory pecks	*Sericin (0.25 nmol)*	26.11±5.78 [b]	62.64±6.46 [b]	84.34±9.21 [b]	122.24±19.58	142.64±21.30	154.24±17.27
	Sericin (0.5 nmol)	20.34±4.31 [c]	52.65±4.33 [c]	45.52±5.14 [c]	121.14±22.43	140.36±10.10	151.14±13.87
	Control	137.64±18.55 [a]	335.15±69.34 [a]	523.62±64.27 [a]	612.28±99.52	732.11±87.27	851.342±118.12
Feeding pecks	*Sericin (0.125 nmol)*	135.71±24.64 [a]	328.11±59.21 [a]	525.54±51.50 [a]	620.38±89.17	746.34±86.38	843.24±101.08
	Sericin (0.25 nmol)	104.24±31.23 [b]	226.34±34.31 [b]	431.14±43.28 [b]	511.42±85.13	682.54±57.28	742.22±99.25

	Sericin (0.5 nmol)	46.55±24.03 [c]	157.55±21.22 [c]	325.34±23.11 [c]	476.52±41.11	679.54±49.24	720.21±87.17
	Control	1.11±0.11	3.35±0.11	3.34±0.90	4.54±0.11	6.34±0.12	6.33±0.11
Drink pecks	*Sericin (0.125 nmol)*	1.05±0.12	3.34±0.10	4.16±0.97	4.34±0.18	6.54±0.20	6.78±0.16
	Sericin (0.25 nmol)	1.04±0.10	2.33±0.08	3.95±0.28	4.64±0.14	6.67±0.43	6.64±0.25
	Sericin (0.5 nmol)	1.03±0.11	3.54±0.07	3.94±0.29	4.76±0.10	6.34±0.21	6.56±0.21
Data are expressed as mean ± SEM. (n= 11 chicks per group). Different letters (a, b and c) indicate significant differences between treatments at each time ($P < 0.05$).							

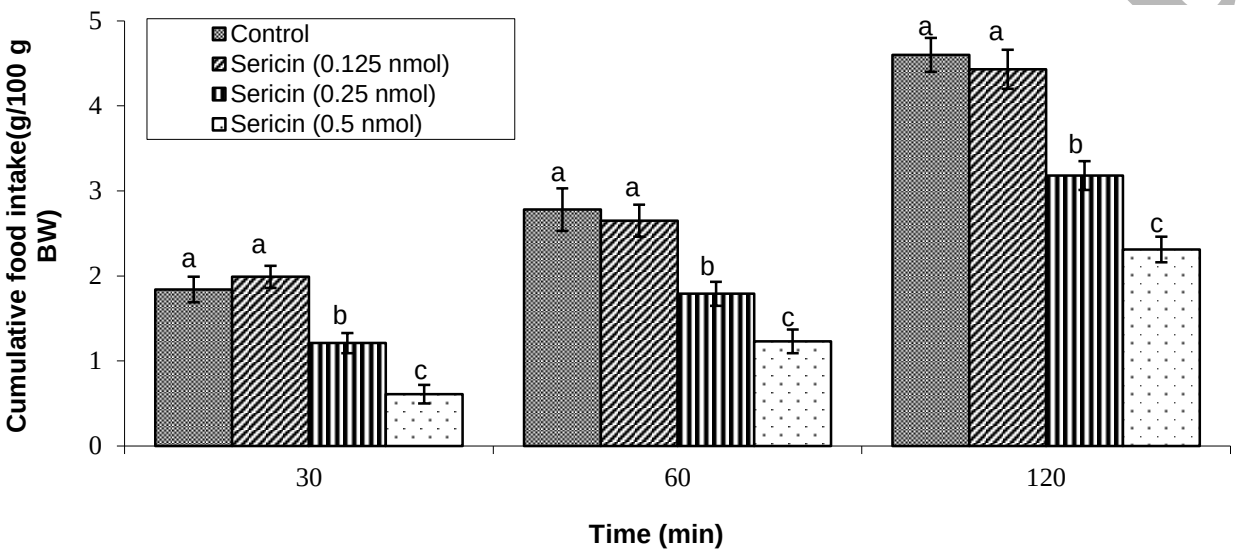


Fig 1. Effect of ICV injection of Sericin (0.125, 0.25, and 0.5 nmol) on cumulative food intake in neonatal chickens (n=44). Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments (P < 0.05).

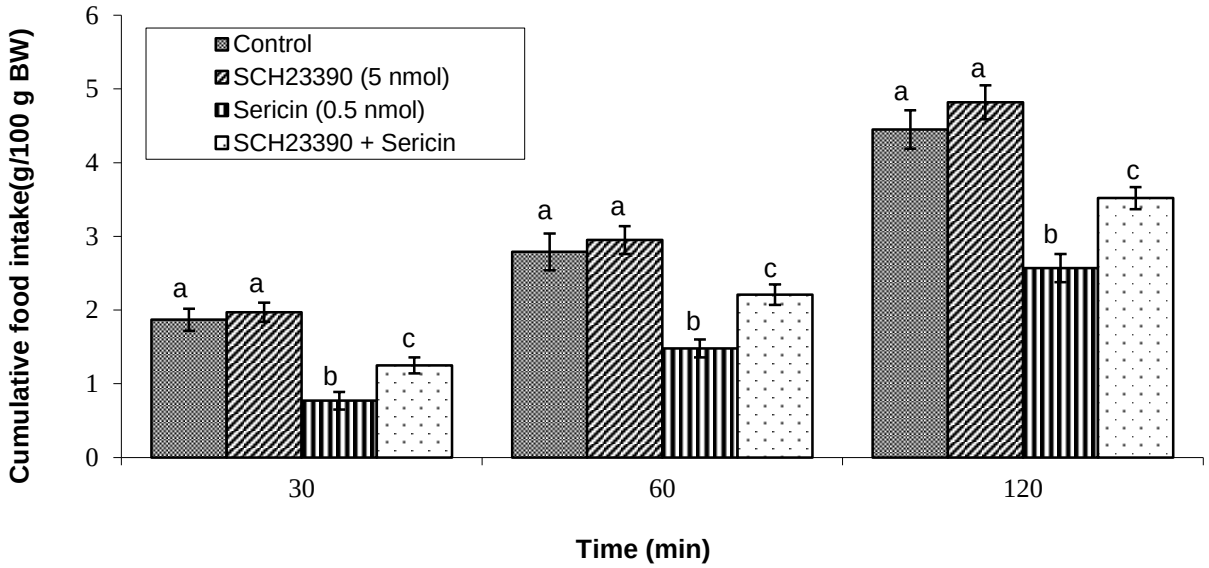


Fig 2. Effect of ICV injection of SCH23390 (5 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). SCH23390: D_1 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments ($P < 0.05$).

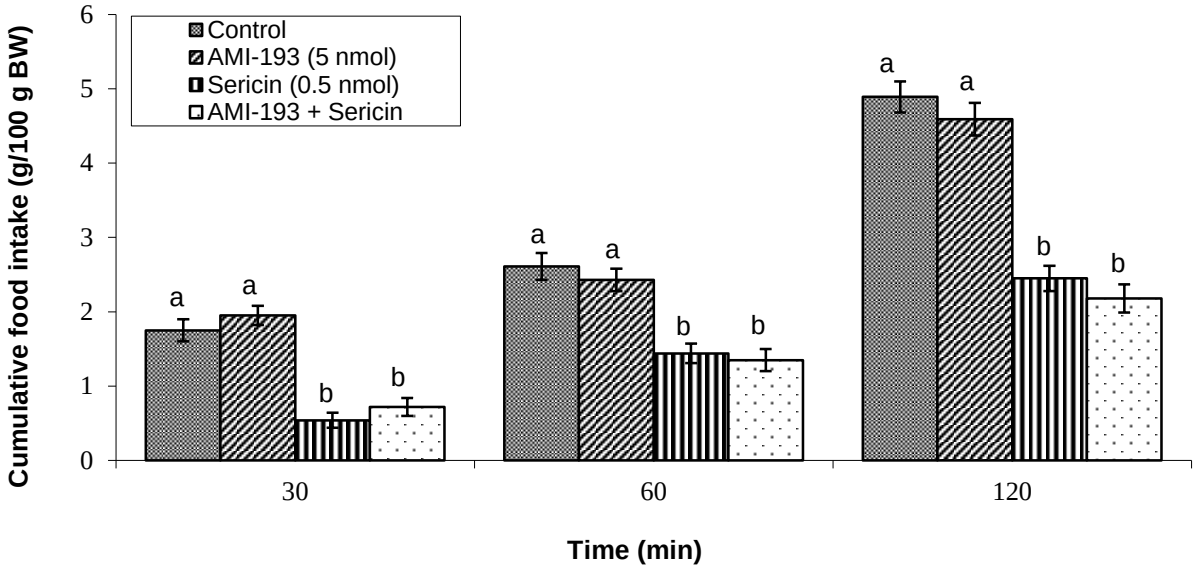


Fig 3. Effect of ICV injection of AMI-193 (5 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). AMI-193: D_2 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).

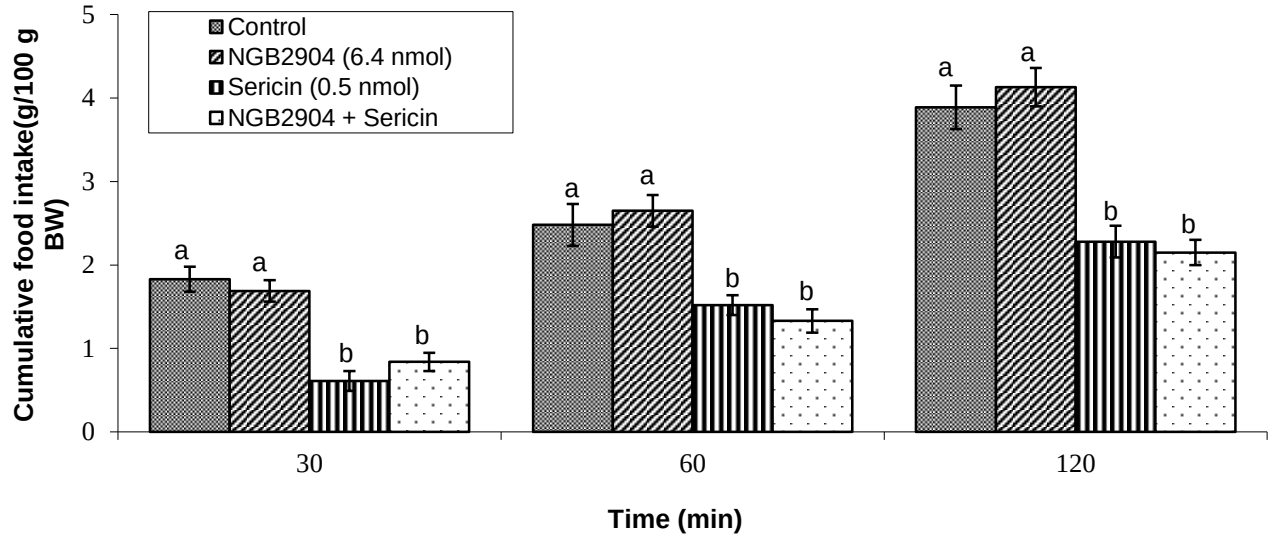


Fig 4. Effect of ICV injection of NGB2904 (6.4 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). NGB2904: D_3 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).

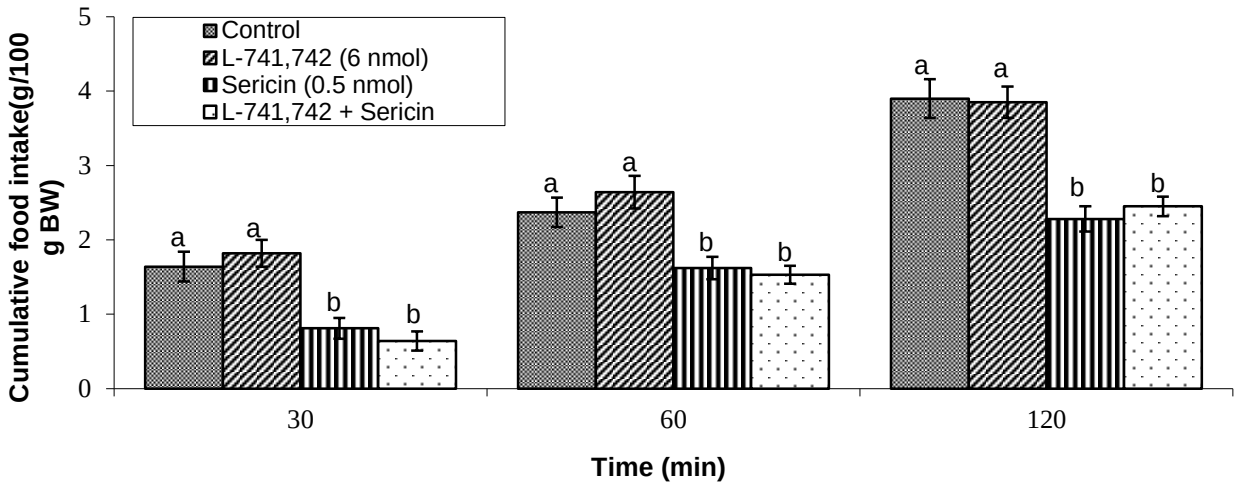


Fig 5. Effect of ICV injection of L-741,742 (6 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). L-741,742: D_4 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).

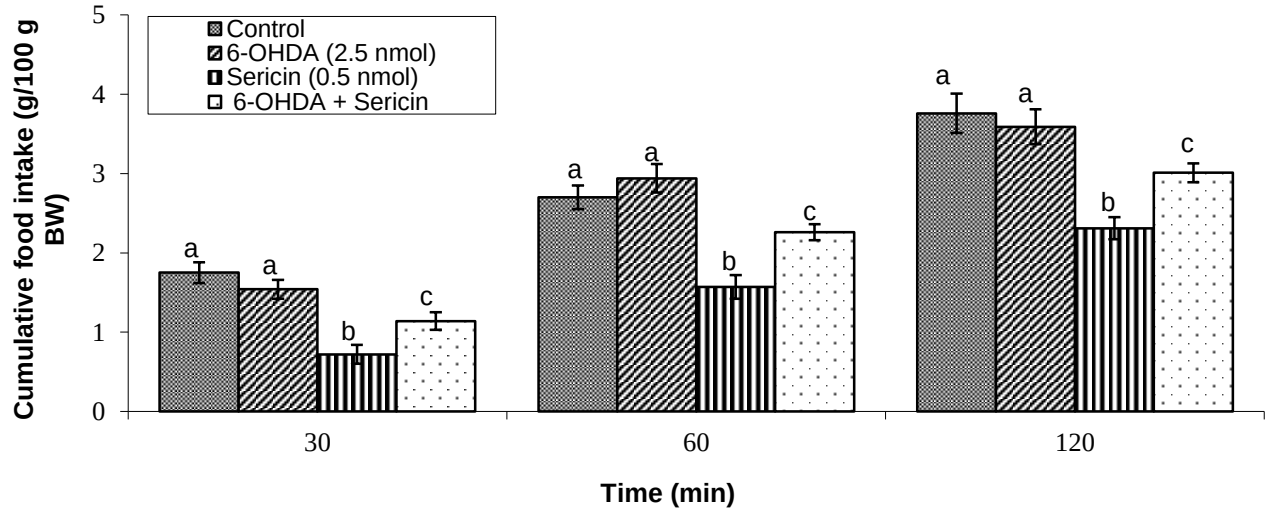


Fig 6. Effect of ICV injection of 6-OHDA (2.5 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). 6-OHDA: 6-hydroxydopamine. Data are expressed as mean ± SEM. Different letters (a, b, and c) indicate significant differences between treatments ($P < 0.05$).

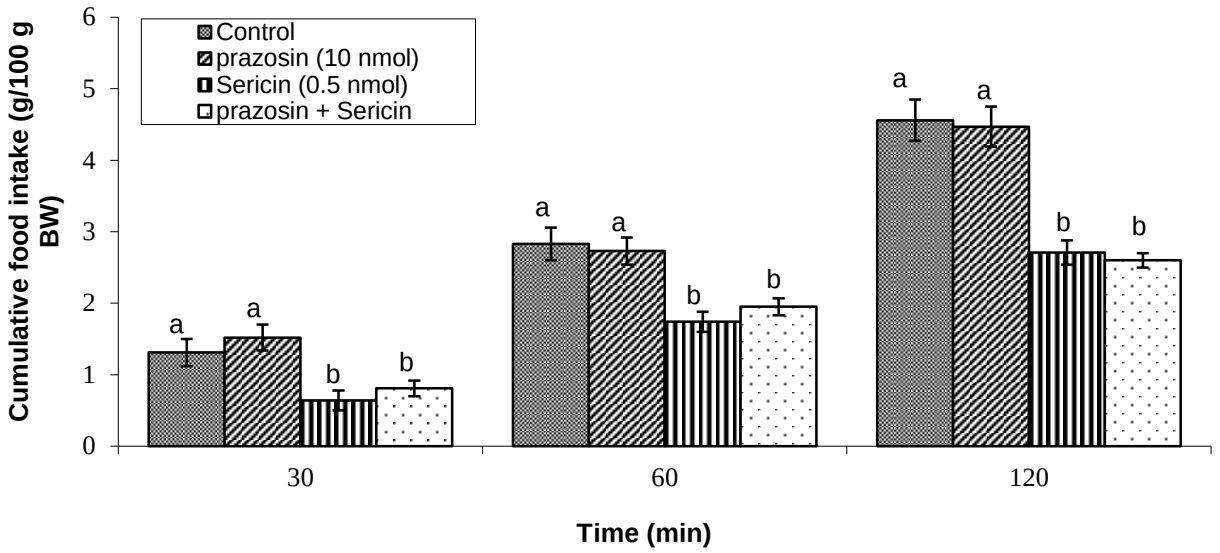


Fig 7. Effect of ICV injection of parazosin (10 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). parazosin: α_1 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).

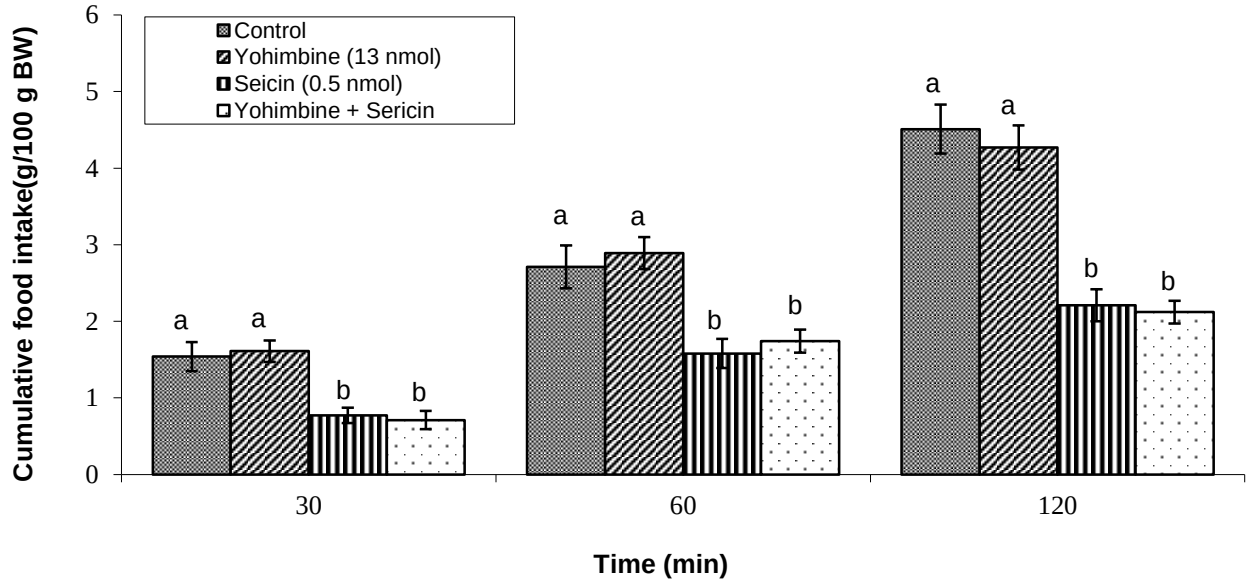
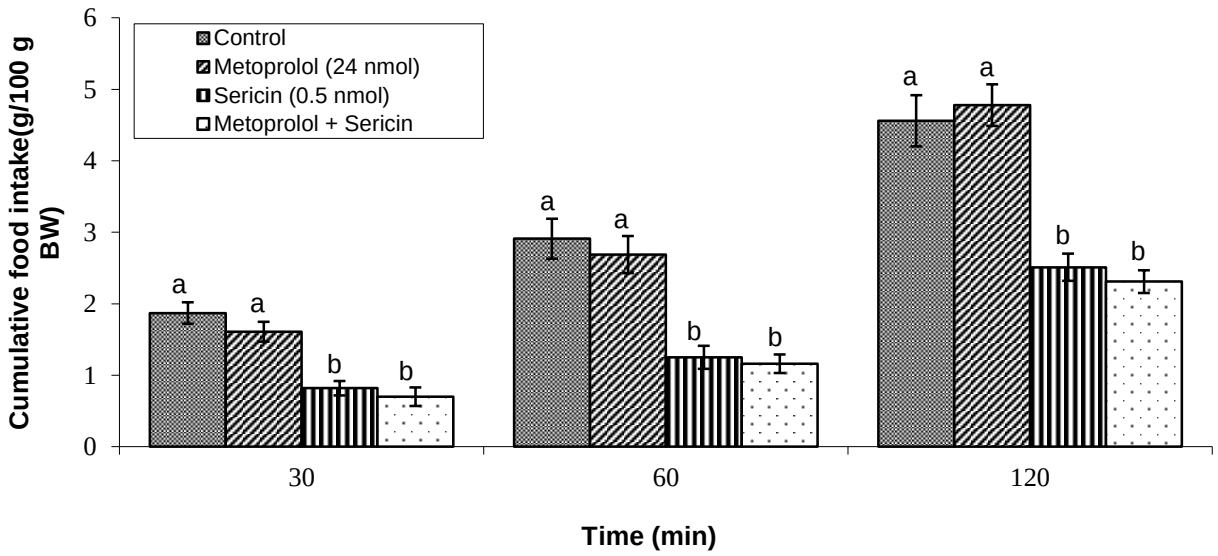


Fig 8. Effect of ICV injection of yohimbine (13 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). Yohimbine: α_2 receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).



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222 **Fig 9.** Effect of ICV injection of metoprolol (24 nmol), Sericin (0.5 nmol) and their combination on
223 cumulative food intake in neonatal chickens (n=44). Metoprolol: β_1 adrenergic receptor antagonist. Data
224 are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between
225 treatments ($P < 0.05$).
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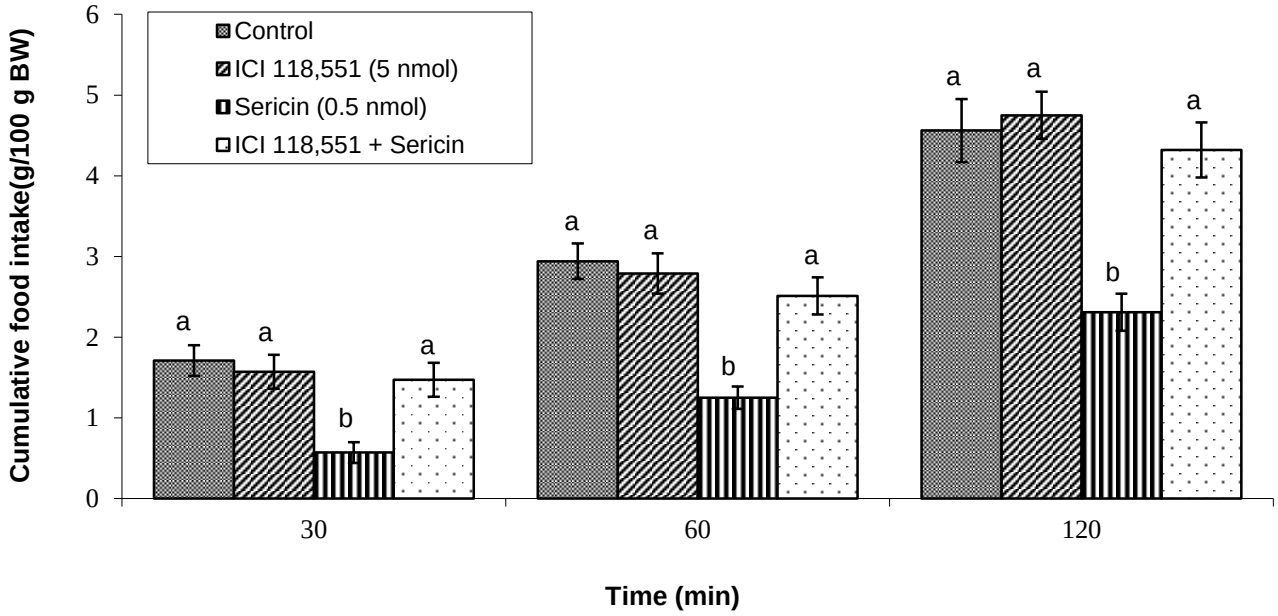

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228 **Fig 10.** Effect of ICV injection of ICI 118,551 (5 nmol), Sericin (0.5 nmol) and their combination on
229 cumulative food intake in neonatal chickens (n=44). ICI 118,551: β_2 adrenergic receptor antagonist. Data
230 are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between
231 treatments ($P < 0.05$).
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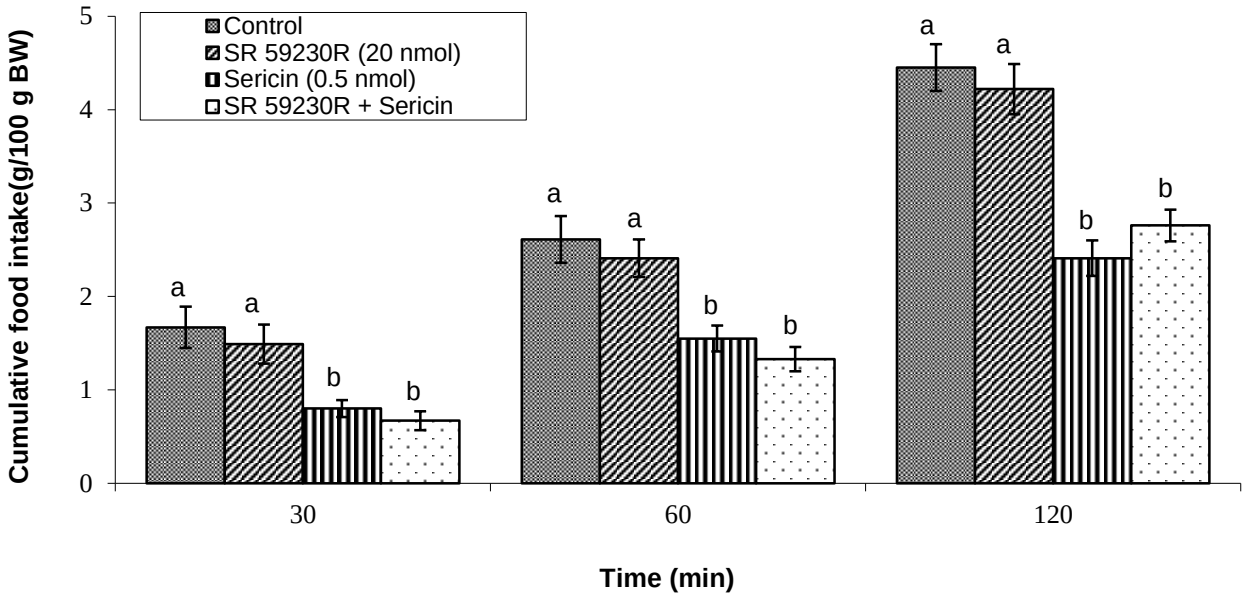

Fig 11. Effect of ICV injection of SR 59230R (20 nmol), Sericin (0.5 nmol) and their combination on cumulative food intake in neonatal chickens (n=44). SR 59230R: β_3 adrenergic receptor antagonist. Data are expressed as mean ± SEM. Different letters (a and b) indicate significant differences between treatments ($P < 0.05$).

4. Discussion

This study is actually the first report on the effect of central sericin and possible interferences with dopaminergic and adrenergic receptors on meal consumption in newborn chickens. According to the findings, ICV infusion of sericin (0.25, and 0.5 nmol) decreased appetite in FD3 chicken. Bombyx mori are being increasingly sought after and sericin is a natural, highly hydrophilic globular protein, with a molecular weight of less than 400kDa, and together with fibroin, forms the silk thread [21]. Supplementation of the sericin in high-fat diet intake improves glucose tolerance hypolipidemic effects, increases plasma adiponectin levels, and decreases leptin, resistin, and TNF-α concentrations [22]. ICV injection of adiponectin leads to hypophagia [23]. Based on previous research, there is no report of ICV injection of the sericin even in mammals and scarce information exists on its role in appetite regulation [24]. Appetite regulates by stimulatory or inhibitory mediators in the central part of the nervous system. ICV administration of the leptin suppressed appetite in both broilers and leghorns [25]. Perhaps hypophagic role of the sericin mediates by the influence of other neurotransmitters.

As observed, co-injection of the D_1 receptor antagonist + sericin decreased hypophagic effect of the sericin. Also, co-injection of the β_2 adrenergic receptor antagonist + sericin suppressed sericin-induced hypophagia.

The dopaminergic system has a key role in appetite regulation in both mammalian and avian, however, differences were seen between them. For instance, D1 and D2 agonists diminish food intake in rats while in chicken only D1 receptors are responsible for dopamine-induced hypophagia and other receptors may have no role [26]. Also, just β2 adrenergic receptors have a role in appetite in chicken and the anorexic effect of leptin is perhaps modulated by β2 adrenergic receptors in chicks [27]. β-adrenergic receptors in the brain simplify the growth of novel inhibitory avoidance memory and synaptic plasticity. Sericin prevents damage caused by oxidative stress in cholinergic and dopaminergic neurons [3]. Dopaminergic and adrenergic receptors act on the hypothalamic nuclei which neuropeptide Y (NPY) and agouti-related protein (AgRP) are associated with hyperphagia, whereas pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcripts (CART) cause hypophagia [28]. Sericin consumption may have a stimulating effect on noradrenergic nerve function [6]. Oral administration of sericin efficiently transports L-serine and L-tyrosine to the brain, motivating noradrenergic activity while decreasing dopamine metabolites in the brain [6]. Perhaps sericin-induced hypophagia mediates by direct regulating these neurons or by primary influence on D1 dopaminergic and β2 adrenergic receptors, then they act on neurons of the ARC. Sericin has a protective potential against diabetes-induced damage in sciatica-related nerve cells, which is shown by an increase in nerve growth factor and a decrease in NPY expression in the spinal cord [29].

Oral gavage of sericin (2.4 g/kg for 35 days) increased the insulin-PI3K/AKT signaling pathway in the liver and reduced hippocampal neuronal apoptosis [5]. However, there are no reports on the effect of central infusion of sericin on the regulation of meal intake for comparison. In conclusion, these findings suggest that sericin has a hypophagic effect on chicken and its effect is mediated through dopaminergic D1 and β2 adrenergic receptors. Based on the limitations of the currents study, we were not able to determine gene experession or IHC staining for obtained results. Also, as mentioned there were no similar studies to compare our findings in the poultry model. Further experiments are needed to understand the direct effects of sericin-induced hypophagia in chickens.

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None

Authors' Contribution

N.M: data collection

M.Z: supervisor, methodology

B.V: supervisor

S.H: advisour, methodology

Conflict of interests

The authors report no conflicts of interest.

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Data Availability

Data available by request

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