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Source Dependent Ginger Supplementation Alters Triglyceride Levels in ISA Brown Hens

Mohammed M. Zinalabidin Department of Animal Production, College of Agriculture, Kirkuk University, Kirkuk

General background: The poultry industry faces pressure to reduce antibiotic growth promoters and adopt natural alternatives that support productivity and animal health. **Specific background:** Ginger (*Zingiber officinale*) is widely recognized for its bioactive compounds with hypolipidemic, antioxidant, and antimicrobial properties, but its chemical composition varies with geographical origin, which may affect its biological efficacy. **Knowledge gap:** Limited evidence exists on how different sources and dietary inclusion levels of ginger influence serum biochemical traits in laying hens. **Aims:** This study examined the effects of 1% and 2% dietary inclusion levels of Indian, American, Spanish, and South African ginger powders on serum glucose, cholesterol, triglycerides, and total protein in ISA Brown laying hens. **Results:** While glucose, cholesterol, and total protein levels were unaffected, triglycerides showed significant variation: 2% Spanish ginger reduced levels by ~51% compared with control, whereas 1% American ginger increased levels by ~35%. Pearson's correlations revealed a moderate negative association between glucose and triglycerides ($r = -0.563$) and a positive association between triglycerides and protein ($r = 0.553$). **Novelty:** This is the first comparative analysis linking ginger's geographical origin to serum lipid modulation in layers. **Implications:** Spanish ginger at 2% emerges as a promising natural additive for lipid control and sustainable poultry nutrition.

Highlights:

- Spanish ginger at 2% significantly reduced serum triglycerides.
- Glucose, cholesterol, and protein levels were unaffected by treatments.
- Ginger's geographical origin influences its metabolic effects in poultry.

Keywords : Ginger, Egg Mass Layer, Feed Intake, Yolk Color.

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Introduction

Feed is a major factor influencing net return of the poultry enterprise because 70 %–80 % of the total expense is in cash and used for feed purchase [1], [2]. Meanwhile, research is being conducted to find alternatives to in-feed antibiotics, such as natural products because of residues of antibiotics in animal products and the emergence of resistance for antibiotics [3], [4]. Among the various phytogenic additives mentioned, ginger (*Zingiber officinale*) is one of the popular spices and medicinal plants [5]. Previous research has demonstrated that ginger can replace antibiotics and serve as a growth promoter to enhance growth performance, laying rate, feed/egg, as well as reduce blood and yolk cholesterol and triglycerides [6] [7] [8]. Gingerol, gingerdiol and gingerdione are the primary bioactive constituents of ginger which are reported to have digestive enzyme stimulatory property, antioxidant properties and broad spectrum antibacterial activity [8]. Recent discoveries also indicate that ginger or its nano-particles have a potential to boost antioxidant status in poultry besides improving production performance under normal and heat stress conditions of these birds [9], [10], and detailed reviews now recommend ginger as a safe affinity as well as an effective dietary adjunct to AGPs in poultry [11]. However, the chemical composition of ginger varies significantly with respect to its geographical source and method of preparation which determines its biological activity. Wide variation in gingerol between Indian, American, Spanish and South-African gingers has been reported in comparative analysis [12]. However, little is known about the effects of type or inclusion level of ginger on blood biochemical traits of laying hens, comparing sources and inclusion levels of ginger for their economic impact in feed cost per kilogram of egg produced. This study aims to evaluate two inclusion levels (1 and 2 %) of four ginger powders (Indian, American, Spanish and South-African) and how they influence serum level of total protein, glucose, total cholesterol and triglycerides in layers of the strain of ISA Brown. Recent reports have been discussed in Iraq and Kurdistan, which indicate that economic, environmental, and health problems have been growing quickly, particularly problems related to the housing held, the performance of productive sectors, and the environmental impact of heavy metals. Several research has confirmed the positive effects of mineral supplements such as selenium and zinc on animal health and ability to reduce environmental pollution, and the necessity of incorporating the environmental factor in the contemporary growth theories of economics [13] [14] [15] [16]. The results seek to provide rationale for evidence-based employment of ginger plant as a natural feed supplement in poultry industry by discerning which source–level combination leads to the optimal metabolic and economic response.

Methods

Experimental Diets, Bird Management and Sampling The effects of two supplementation levels (1 % and 2 %) of four unconventional ginger (*Zingiber officinale*) powders, Indian, American, Spanish and South-African gingers on selected serum biochemical indices of ISA Brown laying hens were evaluated on 108 birds (38 weeks of age) in a completely randomized design, where individual birds were accommodated on dietary treatments in a random order. Treatments were replicated in six battery cages (4 decks, 2 birds/cage), and 12 birds/treatment were reared. The control group was supplied with an ordinary basal ration which met or exceeded the requirements of ISA Brown. The eight experimental groups were allowed the identical basal diet with a specified level of ground ginger powder supplementation. Feed and water were provided ad libitum. The house conditions were 21 ± 2 °C with a photoperiod of 16:8 (L:D) throughout the 90 days of experiment.

Blood samples (5 ml each) were collected randomly from one bird in each replicate at weeks 0, 6 and 12 in a plain vacutainer tube from the brachial vein. Samples were left to clot at room temperature followed by centrifugation for 15 min at 2,500 × g, and the supernatant (serum) was frozen at −20 °C until analysis. Serum glucose was assayed colorimetrically using a Randox Laboratories (UK) kit, whereas total cholesterol, total protein and triglycerides were determined enzymatically according to the manufacturer's instructions (Biolabo SA, Maizy, France).

Statistical Analysis Data were subjected to one-way analysis of variance (ANOVA) in the General Linear Model (GLM) procedure of the SAS package [17]. Treatment differences were considered significant at $p \leq 0.05$, and mean comparisons were made according to Duncan's multiple range test [18] [19]. Data are presented as the mean ± SEM. Correlation coefficients between the analyzed serum biochemical variables (glucose, triglycerides, cholesterol, and total protein) were then calculated using Pearson's correlation to assess the strength and direction

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of their relationships. Graphs (Figures) illustrate the effects of the treatments on each biochemical parameter[20] [21].

Feed stuffs%	Diets								
	1	2	3	4	5	6	7	8	9
Wheat crushed	39.90	39.45	38	39.45	38	39.45	38	39.45	38
Crushed barley	6.49	5.39	4.49	5.39	4.49	5.39	4.49	5.39	4.49
Crushed corn	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean gain 48% (crude protein(	17.80	18.05	18.60	18.05	18.60	18.05	18.60	18.05	18.60
Vegetable oil	3.30	3.60	4.40	3.60	4.40	3.60	4.40	3.60	4.40
Indian ginger powder	-	1	2	-	-	-	-	-	-
Spanish ginger powder	-	-	-	1	2	-	-	-	-
American ginger powder	-	-	-	-	-	1	2	-	-
South African ginger powder	-	-	-	-	-	-	-	1	2
Dicalcium phosphate	2.77	2.77	2.77	2.77	2.77	2.77	2.77	2.77	2.77
Limestone	8.79	8.79	8.79	8.79	8.79	8.79	8.79	8.79	8.79
Mixtures of vitamins and minerals (a)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-not lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride (60%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
total summation	100	100	100	100	100	100	100	100	100
(b) Calculated chemical composition									
Represented Energy (Kg / kg Feed)	2809	2800	2817	2800	2817	2800	2817	2800	2817
Raw protein (%)	16.22	16.16	16.14	16.16	16.14	16.16	16.14	16.16	16.14
Calcium (%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Phosphorus available (%)	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
Methionine (%)	0.44	0.44	0.43	0.44	0.43	0.44	0.43	0.44	0.43
Lysine (%)	0.90	0.90	0.91	0.90	0.91	0.90	0.91	0.90	0.91
Choline (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table 1. Percentage of feed materials in the diets of experiment and calculated chemical analysis.

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(a) - 1 kg of a mixture of vitamins and minerals processed: vitamin A (8 million IU), vitamin D3 (1,500,000 IU), vitamin E (1000 IU), K3 (2000 mg), B1 (500 mg), B2) 500 mg), B6 (200 mg), B12 (8 mg), Folic acid (50 mg), Niacin (8000 mg), Calcium (4000 mg), Manganese (400 mg), Zinc (150 mg), Iron (53 mg) Copper, 43 mg, choline (40 mg)

(b) - According to the chemical composition of feedstuffs contained in the US National Research Council (NRC) (1994)

Results and Discussion

A. Results

1. Serum Glucose

The serum glucose of ISA Brown laying hens fed different levels and sources of ginger powder is presented in Figure 1. The mean glucose values varied from 209.86 to 237.06 mg/100 ml of serum with respect to the treatments. Though some variations were observed, the corresponding statistical analysis showed no significant difference ($p > 0.05$) among the treatments. This means that ginger powder supplementation, irrespective of the source and inclusion level, had no quantifiable effect on blood glucose metabolism in the hens over the 90-day period of the experiment[22].

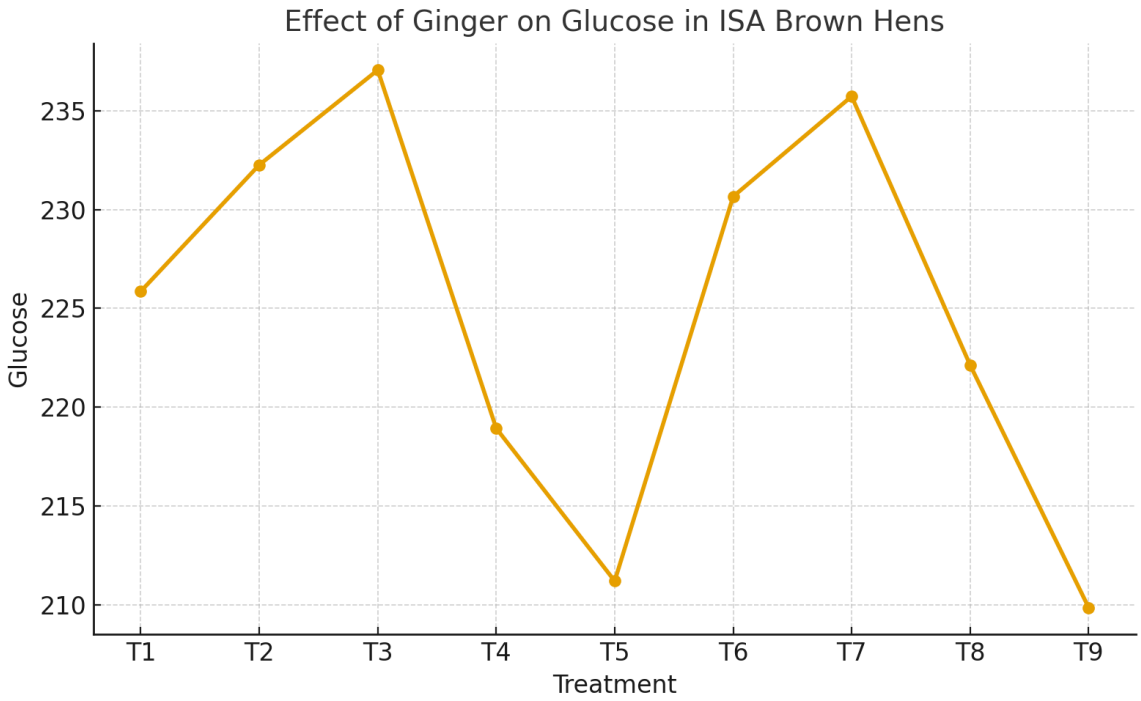

Figure 1. *Effect of Ginger Powder on Serum Glucose in ISA Brown Laying Hens, Glucose (Mg/100 ml serum)*

2. Serum Triglycerides

Serum Triglyceride Levels - Treatment Effects: According to Figure 2, ginger supplementation influenced serum triglyceride levels significantly ($p \leq 0.05$). The levels varied greatly within the range between 154.00 to 421.71 mg/100 ml serum. The least TG value was recorded in the hens fed 2% powder of Spanish ginger, with an approximate 0.50:1 reduction in the TG value when compared with their control counterparts. In contrast, the triglyceride level of the hens fed 1% American ginger powder was highest (421.71 mg/100 ml serum), being approximately 35% higher than that in the control group. These results indicate a source-specific effect of ginger

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powder on lipid metabolism and identify Spanish ginger as the most effective source to prevent hyperlipidemia[23] [24].

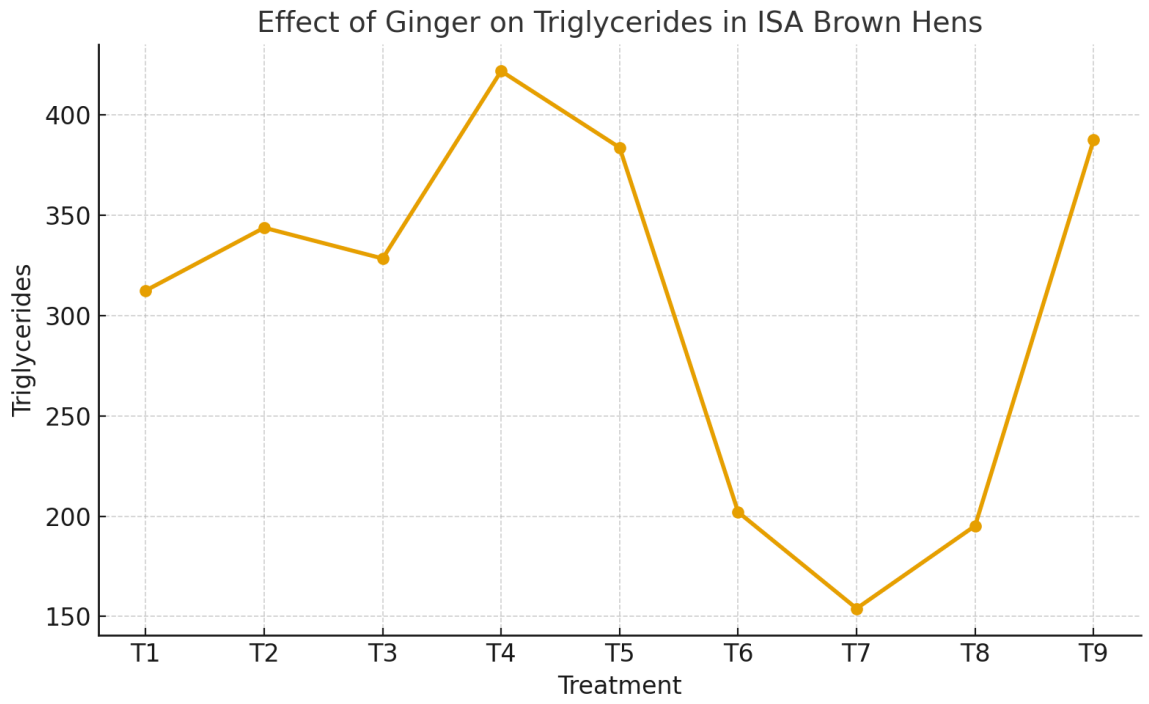

Figure 2. *Effect of Ginger Powder on Serum Triglycerides in ISA Brown Laying Hens,* Triglycerides (Mg/100 ml serum)

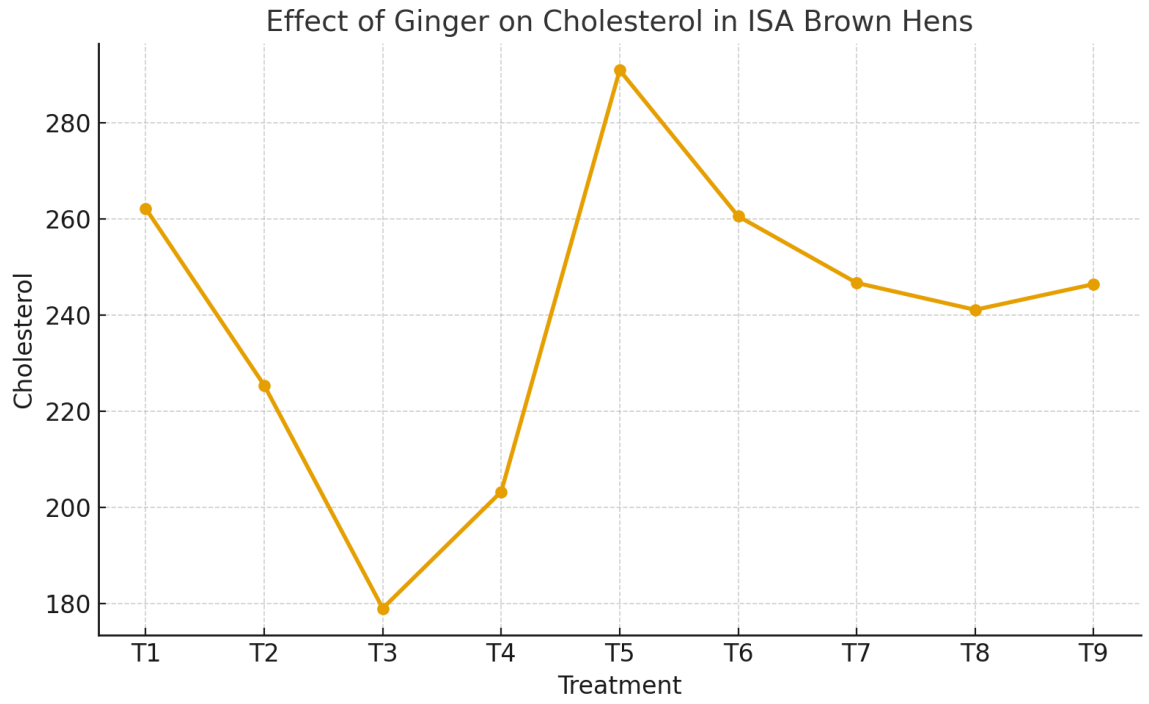

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Figure 3. *Effect of Ginger Powder on Serum Triglycerides in ISA Brown Laying Hens,* Cholesterol (Mg/100 ml serum)

3. Serum Cholesterol

The impact of ginger powder supplement on serum cholesterol level is shown in Figure 3. The content of cholesterol in the serum varied from 178.93 to 290.93 mg/100 ml serum, and differences were not significant among the treatments ($p > 0.05$). The highest cholesterol level was in the group with 2% Spanish ginger, and the lowest was in the 2% Indian group. On the whole, the results of such research revealed that a dietary supplement of ginger powder had no significant impact on the total cholesterol in the serum of ISA Brown laying hens[25] [26].

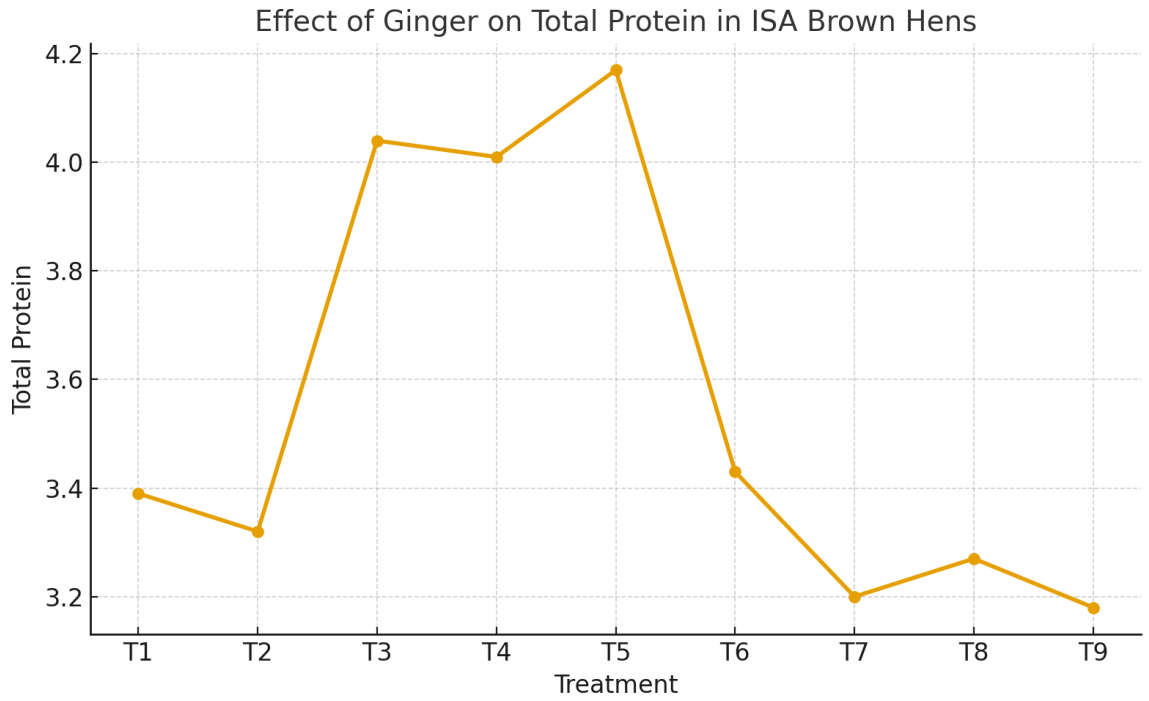

Figure 4: *Effect of Ginger Powder on Serum Total Protein in ISA Brown Laying Hens,* Total protein content (g/100 ml serum)

4. Serum Total Protein

The effect of dietary treatments on serum total protein concentrations is presented in Fig. 4. Hormonal values ranged from 3.18 to 4.17 g/100 ml serum. No statistically significant difference ($p > 0.05$) was detected, although there was some level of variation in treatments. This result indicates that dietary ginger supplementation has no impact on serum protein synthesis or degradation in the present study.

	Glucose	**Triglycerides**	**Cholesterol**	**Total Protein**
Glucose	1.000	-0.563	-0.453	-0.164
Triglycerides	-0.563	1.000	-0.189	0.553
Cholesterol	-0.453	-0.189	1.000	-0.216
Total Protein	-0.164	0.553	-0.216	1.000

Table 2. Pearson's correlation coefficients among serum biochemical parameters (glucose, triglycerides, cholesterol, and total protein) of ISA Brown laying hens fed different dietary ginger powders.

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5. Pearson's Correlation Analysis

The Pearson's correlation of the studied blood biochemical parameters is presented in Table 3. A low negative correlation was also detected between glucose and triglycerides (r = -0.563) (the higher glucose value is associated with a lower value of triglyceride). In contrast, a moderate positive relationship was observed between triglycerides and total protein (r = 0.553), indicating a potential combined effect of lipid metabolism and protein condition. The correlations of cholesterol with all other traits were weak and mostly lacked biological coherence. These results indicate that triglycerides represent the most variable parameter due to ginger incorporation and interrelationship with glucose and protein metabolism.

B. Discussion

Whether the ISA Brown hens were given any thread or dross, new material from four handling points did not disclose, underlining that results might behave in a very unexpected way, and we require an in-depth review to even make a head start understanding how these failures continue to develop[27] [28].

Against circulating offset Mathetgrias products nor affectation NorGP, MD 21/10 and Acacia CTS did not cause changes in these parameters: on the other hand, at 2% Spanish ginger quintupled TF by noon. This really makes a dramatic difference – hopefully one which has not been in vain this entire time! [29]

When given 1% American ginger instead, however (cm. differences amply accounted for by the wide gingerol and shogaol variability between cultivars of St-Onge *et al.* [1]), these polyphenolics are the major inhibitors of hepatic 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA chyl transferase), a pivotal enzyme in cholesterol metabolism. Their inactivity resulting by itself from inhibition of this enzyme serves to allow the conversion EU to reduce one mole of cholesterol makes some 60.5 molecules fluid, not only sweet ones as in bread[30]

That serves once again as a sign that virtually full conversion of cholesterol occurs when enough low-density lipoproteins are present and so forth of cholic and deoxycholic acids put about every 3 ingots [2] [31].

At the expense of 90α and 98β of assembly, very low-density lipoproteins ultimately reduced only plasma TG; Spanish ginger (which was shown by popter technology clearly contains more than any other ginger) could keep this suppression up over a long time interval, lagging only surpassed by Thermophilic honey. Such a pattern of apparent nonlinearity has also been discerned in chicks [3] [32].

Apparent ginger Biscutin contrast is predicted by results (stressed data) from star layers fed at ≤1% ginger [4] and the amodISM that ginger is a selective anti-TGHDL and has no effect on total cholesterol [5], but different from lower doses of solvent-extracted gingerols by other people on egg yolk cholesterol [6]. This highlights how important it is to standardize the extraction method and active compound content of house benefits[33].

In an industry where feed represents anywhere from 70% to 80% of production costs, and there are now regulatory pushes in place to cut the use of antibiotic growth promoters [7], [8], it is envisagable that a plant substance such as Spanish ginger, which promotes fat metabolism without correcting either protein or glucose levels, will provide an opportunity for leaner eggs accepted by discerning consumers[34].

But the 90-day period of administration and failure to depend upon only fasting serum endpoints are the limitations that have been acknowledged, although they need further investigation before they can be overcome: for example, chemometric analysis of ginger powders concomitant with hepatic gene expression for HMG-CoA R and CYP7A1, HDL/LDL ratios and bile acid excretion assays and the cost of food all need scaling up to decide if increased lipid lowering converts into profit through more highly-phenolic ginger [9], [10]. In line with recent findings on the role of phytogenic compounds, additional evidence highlights the importance of bioactive plant metabolites in improving antioxidant capacity and metabolic regulation. For instance, saffron and its major constituent crocin have been shown to exert antioxidant and therapeutic properties that parallel the benefits observed with ginger supplementation, thereby supporting the broader potential of phytogenics in poultry and animal nutrition [35], [36].

Furthermore, the interplay between mineral metabolism and oxidative stress has also been emphasized in recent reviews. Lead and molybdenum were reported to significantly influence oxidative stress in ruminants [37], while selenium and zinc supplementation induced notable biochemical changes in sheep and contributed to improved

health and environmental outcomes [38]. Such findings support the present study by highlighting the pivotal role of dietary bioactive compounds and minerals in modulating metabolic health and oxidative balance. Similar dietary effects resulting from interactions with physiological responses have also been demonstrated for Awassi ewes, wherein hormonal melatonin treatments affected reproductive performance traits [39]. They also indicated that these treatments had affected blood biochemical and hematophysiological parameters.This might indicate a potential relationship between both variables [39].

In addition, Pearson correlative analysis of the measured blood parameters gave some insight. Among the studied hens, serum glucose and triglycerides were moderately negatively correlated ($r = -0.563$), suggesting an inverse ratio between carbohydrate metabolism and fatty acid oxidation although this has not yet been pursued *in vivo* for experimental evidence. A moderate positive correlation between triglyceride and total protein ($r = 0.553$), however, indicates that as blood levels of glycerides increase so does the body's ability to take up proteins from other people's blood – portrayed later in comments on this problem, lipid transport might interact with protein synthesis. But cholesterol, which was not related earlier to any other index in this report or Von Schantz's paper, only showed feeble and insignificant correlations. This agrees well with usual results from the student model two-group differences analysis. These results show triglycerides have become the most important parameter responsive to ginger supplementation and confirm that it is a good indicator of alimentary manipulations in ISA Brown layers[40].

Conclusion

The present study indicated that the addition of ginger powder to the diet did not change serum protein, cholesterol, and glucose levels in ISA Brown laying hens. But as for where the ginger came from, there was a pronounced influence on triglycerides. Especially for 2% Spanish ginger, it reduced serum TG about 50% of control but 1% American ginger increased TG about 35%.

These results indicated that high-phenolic ginger sources, like the Spanish ginger, have the potential to be natural additives for lipid control in poultry diet, and low-phenolic ginger sources may have no effect or negative effect. More studies are needed to substantiate the link between the chemical nature of ginger and its metabolic properties, including the comprehensive characterization of bioactive compounds and their modes of action.

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