

## Impact of sorbitol and l-carnitine on stimulating thyroid hormone, triiodothyronine and adenosine triphosphate level in broilers

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### Abstract

In order to provide more significant physiological benefits for the poultry sector, our research sought to assess the effectiveness of the two metabolic products, Sorbitol and L-carnitine, for energy production. In April and May, after three days of adaptation, 60 broiler chicks were partitioned into three groups (20/group). The regular food was given to the control group, while sorbitol 100 g/kg diet and L-carnitine 80 mg/kg diet were given to the second and third groups, respectively. After data analysis, the following conclusions were drawn: sorbitol and L-carnitine considerably increased TSH and T3 levels, but L-carnitine had superior benefits for ATP generation over sorbitol. On the other hand, sorbitol and L-carnitine significantly raise glucose levels while lowering triglyceride and cholesterol levels, except for L-carnitine, which does not affect cholesterol levels. The two products also positively affect growth efficiency by significantly increasing ultimate and earned body weight with only chest muscle from other organs weighted in the experiments. However, any treatments did not affect blood parameters estimated in this experiment. Histological variables of the intestine of broilers provide reliable indications of feed additive absorption. As a result, my data showed a positively influenced villi heightening by two treatments, while L-carnitine is more efficient in terms of villus width, villus surface area, and goblet cell number. In conclusion, L-carnitine was more efficient than Sorbitol in energy production, either of effects on thyroid production, ATP generation, growth-enhancing, and boosting absorption from the intestine.

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### Introduction

In chicken farming, feed energy from fat, carbohydrates, and protein is a crucial input that makes up around 75% of the expenditures associated with feed efficiency. Therefore, efficient energy and precise feeding are essential for chicken production to achieve sustainable development, especially during periods of growth or stress (1). The assumption is that the chicks will consume until their energy needs are met to trigger the required thermogenesis, which depends on the nervous mechanisms in poultry to produce thyroid hormones and other thermic hormones (2). The liver, as the metabolic center for the ATP-producing during glycolysis, offers energy for chicks, primarily through extreme physiological

variations as a result of hatching or/and sudden cellular proliferation, especially throughout late embryonic because the lipids of the yolk are taken in and deposited in the liver tissue as cholesteryl esters as well as ATP, playing a role as nucleotide precursors and for the reusing of carbon backbones (3). Sorbitol is a primary photosynthetic compound 6-carbon sugar alcohol observed in several fruits, as well as certain veggies and mushrooms, related to the sugar alcohol class (non-glucose monosaccharide) and is made from glucose 6-phosphate aside from being essential for osmoregulation in the cytosol under various abiotic stressors (4). It is also known as D-glucitol, following given orally, which is gradually absorbed in the gut through facilitated diffusion and has a significant impact on the

intestinal flora, mainly boosting the *Lactobacillus* colony, which triggers a minute increase in glucose and insulin level (5). L-carnitine belongs to B – hydroxy -Y-N -trimethyl aminobutyrate, and it is isolated from chicken embryos, characterized by solubility in water and found in animals, vegetation, and micro-organisms that deliver it both endogenously and exogenously. Additionally, it has a forced responsibility in the metabolism of fatty chains by directive them into the mitochondrial respiratory chain via a specific enzyme, acyltransferase (6,7). Adabi and his colleagues discovered that L-carnitine has indispensable offers, comprising growth preferment, immune response, and improving spermatogenesis, in addition to enhancing the animal's nutritional status through improved performance and even in a variety of stressful situations, as well as human being (8,9). Murali and his collaborators 2015 looked into the antioxidant possessions of carnitine because L-carnitine enhances the scale and action of the enzymatic form of antioxidants like glutathione and superoxide, as well as because it steadies phospholipids in cellular membranes and adjusts the mechanism of ion channels by turn down the number of oxidants (10). Furthermore, L-carnitine-enriched meals would increase the oxidization of lengthy fatty acids to create ATP, saving energy and maybe accelerating fat's protein-sparing effects (11). L-carnitine is an essential micronutrient for chickens' lipid metabolism and fuel creation. Besides that, it may improve broiler health and nutritional status, owing to its precursors, lysine and methionine (12).

This study intended to assess the consequences of two food additives, Sorbitol and L-carnitine, on energy utilization under typical physiological settings.

## **Materials and methods**

### **Ethical approve**

This study was approved by the institutional animal care and committee of veterinary medicine college / University of Mosul with certified No. UM.VET.2022.019.

### **Broilers management**

This work was done in March-April 2022 at the experimental farmhouse of the Veterinary College of Mosul University. The Al-Nibras hatchery has purchased 60-day broiler chicks (ROSS308), which will require three days to acclimate, then arbitrarily divided the chicks into three groups (20 chicks per group). During the 45-day rearing period, the birds were kept in separate land pens with an ambient temperature range of 32-33°C on the first day, then dropped 1°C later on the fourth day of age, then repeated to dropping 1ml every three days of bird age (13,14). Drinking water was provided *ad libitum* with the standard forage element given, agreeing to National Research Council (NRC) recommendation (15).

### **The layout of an experimentation**

The three groups encompass; the control group that received the ordinary diet, while the second and third groups received additions of Sorbitol at doses of 100 g/kg diet (16) and L-carnitine at 80 mg/kg diet (17), respectively. (Sigma, Poland's BIOPOINT).

### **Blood tests**

At 45 days, blood samples were drawn and parted into 2 portions, the first portion to collect serum after centrifugation 15 minutes / 3000 rpm and the other to obtain constituents of whole blood. Serum samples were stored at -26°C until the estimates of stimulating thyroid hormone (TSH) and triiodothyronine (T3) (AFIAS, Korea) (18). Also, the biochemical tests include glucose (RANDOX), triglycerides, and cholesterol via traditional kits (BIOLABO) (19). At the same time, the anticoagulant-treated tube was used to calculate red and white blood cells using the special solution Natt and Herrick (20), as well as Hb levels in g/dl using the traditional Sahil method (21).

### **Estimation of the ATP**

Using the HPLC technique, the ATP Content Assay Kit was used to estimate energy production (ATP). To begin, prepare standards at various concentrations (0.5µmol/ml, 0.1µmol/ml, 0.05µmol/ml, 0.01µmol/ml, 0.005µmol/ml). Then filter through 0.45µm of the organic filter membrane and 0.22µm of the aqueous filter membrane. The same membrane filters the mobile phase A (pure acetonitrile) and mobile phase B (configured) at 2:98. The second step is to turn on the switch buttons of HPLC, adjust the temperature to 27 °C, flow rate to 0.8 ml/minute, the wavelength to 254 nm, and volume of injection to 10 µl of ATP standard to obtain the standard curve within 10 minutes, the retention time of ATP being between 2.9 and 3.1 minutes. The third step is tissue (liver) extraction using the Kit procedure (Solarbio), followed by injection of 10 µl of the prepared sample into HPLC to determine the peak area and corresponding retention time. Then the concentration of the sample was obtained to analyze statically (22,23).

### **Growth efficiency and relative organs weight**

The preliminary body weight (g) was determined after three days of acclimation, but the ultimate body weight (g) was determined on the day of slaughter. The following equation computes the weight earning (g): ultimate body weight (g) - preliminary body weight (g) (24) At that time, the broilers were sacrificed, and the visceral organs, such as the heart, liver, gizzard, pancreas, and chest muscle, were weighed following body weight by applying the mathematical equation: weight of organ/weight of body × 100 (25). When weighed, only the liver was wrapped in foil paper and kept at -26°C until it was utilized for ATP extraction.

### Preparation of duodenum histological sections

The intestinal morphometric criteria take account of Villus height (VH), Crypt depth (CD), VH: CD length/depth ratio, Villus width (VW), and Villus Surface Area of the (VSA) according to the equation:  $2 \pi (VW/2) (VH)$  (26,27), and the number of Goblet cells (Field=0.06 mm<sup>2</sup>). Also, the photomicrograph shows mucosa, submucosa, and muscular layer thickness. A 4 cm piece of the duodenum was taken and fixed in buffered formalin at 10%. After that, it was lodged in paraffin, and microtome slides were obtained, then dyed with Hematoxylin and Eosin (25). With the help of the USB (2.0) colorful digital photo camera, all variables were tested by Scope Image 9.0-China. Using a 0.01 mm stages micrometer (Japan), the type of lens Olympus-CX31 used to calibrate.

### Statistic evaluation

Analysis of variance ANOVA one-way was applied to investigate the resultant documents. The Duncan Multiple Range Test was used to determine the significance of average variation at probability  $P < 0.05$  (28).

### Results

As interpreted in table 1, TSH and T3 levels are significantly higher ( $P < 0.05$ ) in broilers handled with Sorbitol and L-carnitine than in the non-treated group. In contrast, as measured by ATP generation, energy production is significantly ( $P < 0.05$ ), rising in the L-carnitine and Sorbitol treated collections, respectively more than in the control group.

Table 2 logged data from biochemical tests showed that additive groups had significantly ( $P < 0.05$ ) elevated glucose and lower triglyceride levels than controls. In contrast, when estimated in serum, the cholesterol level graduated from the highest level in L-carnitine to the lowest level in Sorbitol compared with control groups.

As a result, there is no any significant change ( $P > 0.05$ ) among groups in the preliminary body weight, but when Sorbitol and L-carnitine are added, the ultimate body weight and weight earning are statically ( $P < 0.05$ ) greater than they were with the control group (Table 3).

The relative organ weight to whole body weight in broilers fed Sorbitol, and L-carnitine demonstrations no significant ( $P > 0.05$ ) variance in organ weight, except for chest muscle getting higher in additive groups contrasted to controls, as well as hematological parameters were not statistically ( $P > 0.05$ ) different in all experimental groups (Tables 4 and 5).

Table 1: The influence of Sorbitol and L-carnitine on TSH, T3 hormones, and ATP levels in broilers

Groups	TSH ( $\mu$ lu /ml)	T3 (nmol/l)	ATP ( $\mu$ mol/ml)
Control	0.32±0.01b	0.88±0.18b	0.83±0.21c
Sorbitol	0.35±0.01a	1.33±0.49a	2.25±0.32b
L-carnitine	0.35±0.01a	1.61±0.15a	3.66±0.10a

The difference between groups is marked in the column with small letters when it is significant at  $P < 0.05$ .

Table 2: The influence of Sorbitol and L-carnitine on glucose, triglyceride, and cholesterol in broilers

Groups	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control	238.0±9.82b	142.60±14.90A	113.60±3.72a
Sorbitol	360.8±4.04a	55.60±1.69B	95.80±6.82b
L-carnitine	342.0±13.92a	50.60±1.56B	120.60±1.07a

The difference between groups is marked in the column with small letters when it is significant at  $P < 0.05$ .

Table 3: The influence of Sorbitol and L-carnitine on growth efficiency in broilers

Groups	Preliminary body weight (g)	Ultimate body weight (g)	Weight earning (g)
Control	77.80±2.69a	2326.40±56.49b	2240.60±58.51b
Sorbitol	76.60±4.10a	2575.20±76.80a	2498.60±77.36a
L-carnitine	81.00±1.22a	2591.40±40.39a	2510.40±40.15a

The difference between groups is marked in the column with small letters when it is significant at  $P < 0.05$ .

Table 4: The influence of Sorbitol and L-carnitine on relative organ weight in broilers

Groups	Heart (g/100g b.w.)	Liver (g/100g b.w.)	Gizzard (g/100g b.w.)	Pancreas (g/100g b.w.)	Chest muscle (g/100g b.w.)
Control	0.45±0.03a	2.01±0.01a	1.24±0.18a	0.19±0.07a	29.32±0.99b
Sorbitol	0.36±0.04a	2.14±0.19a	1.06±0.08a	0.16±0.01a	34.76±1.46a
L-carnitine	0.38±0.01a	1.90±0.05a	1.27±0.79a	0.15±0.01a	34.63±0.85a

The difference between groups is marked in the column with small letters when it is significant at  $P < 0.05$ .

Table 5: The influence of Sorbitol and L-carnitine on RBC, TLC, and Hb in broilers

Groups	RBC (10 <sup>6</sup> /mm <sup>3</sup> )	TLC (10 <sup>3</sup> /mm <sup>3</sup> )	Hb (g/dl)
Control	2.38±0.24a	35.05±2.85a	12.80±0.73a
Sorbitol	1.77±0.20a	37.57±4.07a	12.70±0.86a
L-carnitine	2.54±0.37a	39.05±2.00a	13.04±0.27a

The difference between groups is marked in the column with small letters when it is significant at P<0.05.

After a micromorphological analysis of the broiler duodenum, which exhibited significantly (P<0.05) an upsurge in villus height in two feed additive groups equaled to the control, but the crypt depth and the ratio VH: CD did not significantly (P>0.05)

vary between three groups while the villus width distinctive significantly (P<0.05) from the greatest in villus width of the broilers fed with L-carnitine compared to Sorbitol, but the two treated groups did not differ (P>0.05) from the control. The results indicate that feeding with L-carnitine causes a significant (P<0.05) increasing villus surface area compared with sorbitol and control groups. Moreover, the numeral of goblet cells for each field rises significantly (P<0.05) in the L-carnitine collection emulate to the control group, while adding Sorbitol to the chicken diet does not cause any variation (P>0.05) between the L-carnitine and control collections (Table 6). The two treated groups have thicker muscularis layers than the untreated group, according to histomorphometric assessments of the muscularis layer of the duodenum (Figures 1-9).

Table 6: The influence of Sorbitol and L-carnitine on histological criteria in broilers

Groups	Villus height [VH] (mm)	Crypt depth [CD] (mm)	VH: CD ratio	Villus width (mm)	Villus surface area (mm <sup>2</sup> )	Goblet cells (Field=0.06 mm <sup>2</sup> )
Control	1.51±0.06b	0.18±0.02a	9.16±1.59a	0.22±0.01ab	1.05±0.03b	41.80±5.58b
Sorbitol	1.98±0.05a	0.21±0.03a	9.81±1.78a	0.19±0.02b	1.21±0.13b	58.00±4.66ab
L-carnitine	1.91±0.05a	0.15±0.02a	12.73±1.12a	0.23±0.03a	1.39±0.18a	65.60±3.23a

The difference between groups is marked in the column with small letters when it is significant at P<0.05.



Figure 1: photomicrograph of broiler duodenum of the control group showing [1A] mucosa, [1B] submucosa, and [1C] muscularis with the histomorphometric measurements of the villi height, width, and crypt depth. H&E stain, 40X.

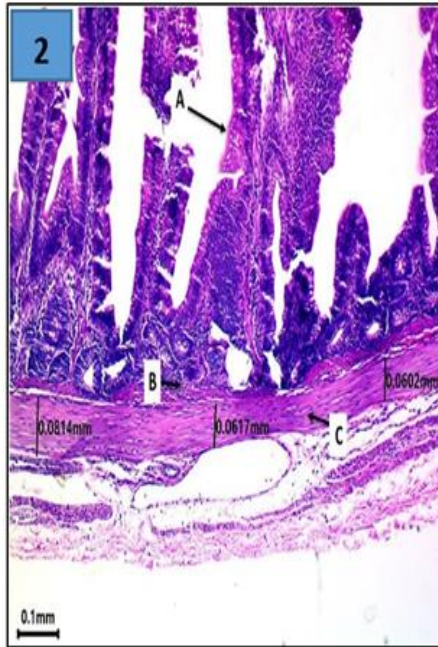


Figure 2: photomicrograph of broiler duodenum of the control group showing [2A] mucosa, [2B] submucosa, and [2C] muscularis with the histomorphometric measurements of the muscularis layer. H&E stain, 100X.

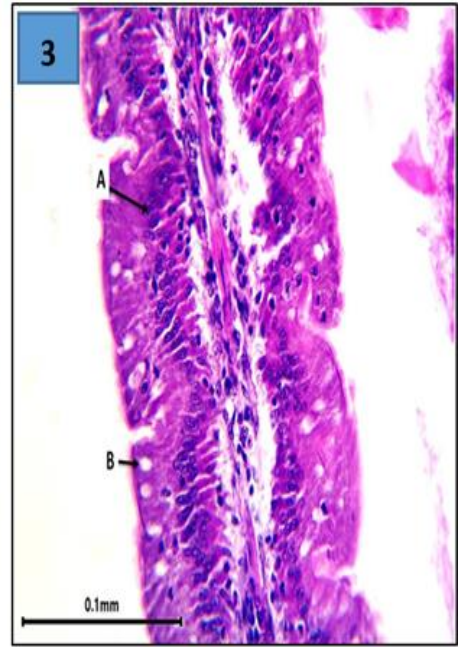


Figure 3: photomicrograph of broiler duodenum of the control group showing [3A] epithelial cells and [3B] goblet cells. H&E stain, 400X.



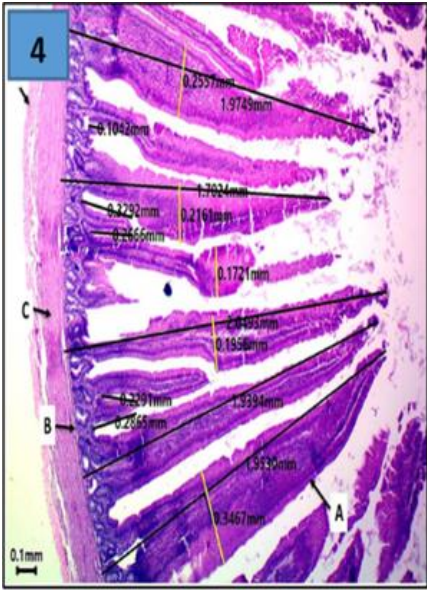


Figure 4: photomicrograph of broiler duodenum of the sorbitol group [4A] showing mucosa, [4B] submucosa, and [4C] muscularis with the histomorphometric measurements of the villi height, width, and crypt depth. H&E stain, 40X.

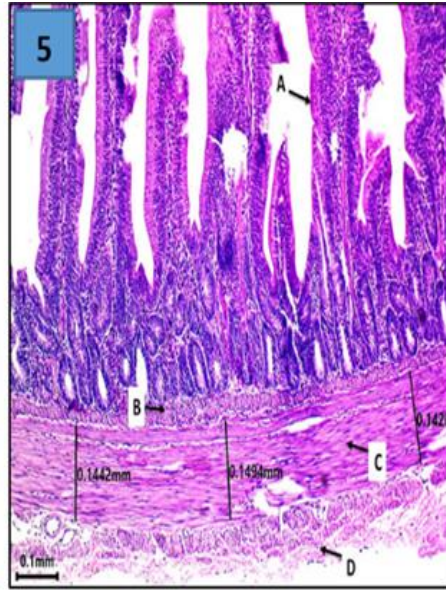


Figure 5: photomicrograph of broiler duodenum of the sorbitol group showing [5A] mucosa, [5B] submucosa, and [5C] muscularis with the histomorphometric measurements of the muscularis layer. H&E stain, 100X.

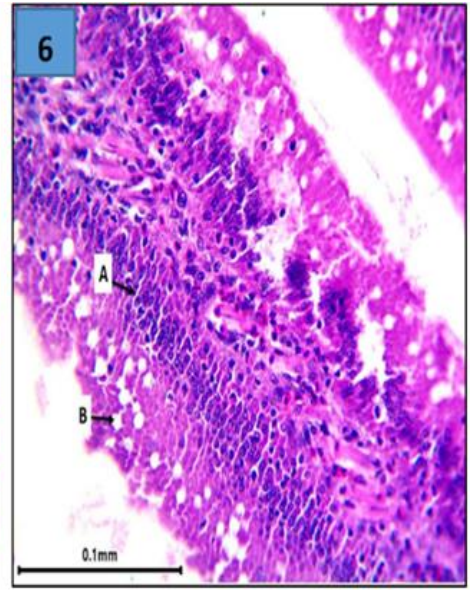


Figure 6: photomicrograph of broiler duodenum of the sorbitol group showing [6A] epithelial cells and [6B] goblet cells. H&E stain, 400X.

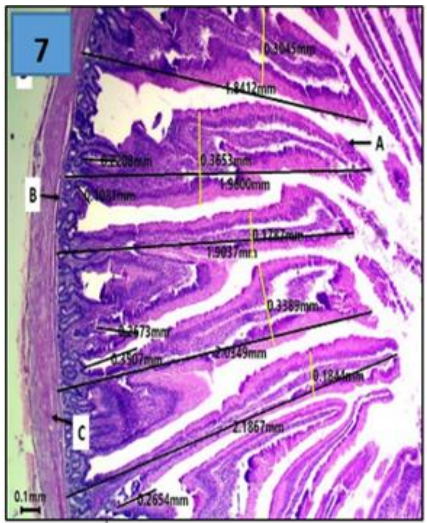


Figure 7: photomicrograph of broiler duodenum of the L-carnitine group [7A] showing mucosa, [7B] submucosa, and [7C] muscularis with the histomorphometric measurements of the villi height, width, and crypt depth. H&E stain, 40X.

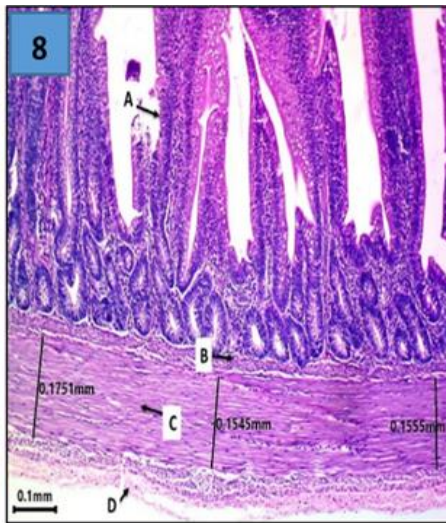


Figure 8: photomicrograph of broiler duodenum of the L-carnitine group showing [8A] mucosa, [8B] submucosa, and [8C] muscularis with the histomorphometric measurements of the muscularis layer. H&E stain, 100X.

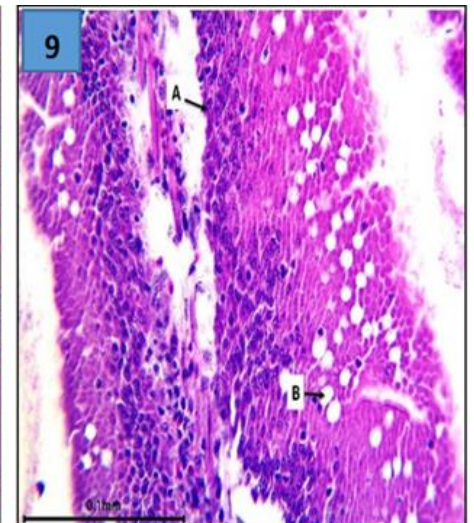


Figure 9: photomicrograph of broiler duodenum of the L-carnitine group showing [9A] epithelial cells and [9B] goblet cells. H&E stain, 400X.

## **Discussion**

Own analysis revealed that Sorbitol elevated TSH, T3, ATP, and glucose levels while reducing triglyceride and cholesterol level caloric expenditure and thyroid hormone status were known to be interrelated. Hence, my results are similar to that studied by Iwen and your groups, which demonstrated that increased glucose levels are a precursor to sorbitol. caused excess production of the thyroid to encourage a hypermetabolic condition (production ATP) accompanied by high resting energy consumption, lessened cholesterol, markedly augmented lipolysis, and gluconeogenesis (29,30). On the other hand, L-carnitine produces more ATP than Sorbitol, and L-carnitine did not change the cholesterol value from normality. L-carnitine is well known as one of the most crucial amino acids for chickens because it aids in the beta-oxidation of lipids by the mitochondria to produce energy. As a result, Tufarelli and his colleagues in 2020 demonstrated the highly significant value of T3 and cholesterol levels as the control group when treated with L-carnitine and discussed the causes of thyroid hormones' considerable function is to regulate oxidation reactions; thus, any noticeable functional alteration is expressed in an altered metabolic rate (31). Likewise, Rehman and other researchers' work supported that L-carnitine-supplemented meals would promote oxidation to produce ATP and conserve energy (6). Also, the groups who received L-carnitine supplements had greater T3 and T4, which are thyroid hormones that improve energy expenditure (32). There is another explanation, L-carnitine has been proven to raise serum insulin-like growth factor I (IGF-I) levels, which encourages the growing of the bird with an anabolic impact on striated muscular tissue by boosting protein uptake and consequently increasing the propagation and distinction of stem cells of the muscle satellite cells (33).

In terms of growth efficiency, there were no discernible differences between the two treated groups when Sorbitol and L-carnitine were compared in terms of body weight gain for the current study, although both treatments increased ultimate body weight, weight earning, and form weight organs just the chest muscle when compared to the control group. My outcomes are consistent with some researchers who used commercial products with hepatoprotective ingredients, such as Sorbitol (2 mg) and L-carnitine (0.25 mg) as two of the substances that considerably boosted broiler growth by improving final body weight and weight gain, as well as the histologic structure of the liver (34). The mechanism of Sorbitol's beneficial impact on animals' growth is that, as a sugar alcohol, it can raise blood sugar, boost bile production, promote fat digestion via fermentation into short-chain fatty acids in the intestine, and optimize energy homeostasis. (35). Hossinezhad and his coworkers claimed that L-carnitine enhanced body weight and thigh and breast muscles and lowered the feed conversion ratio,

although the gizzard, heart, and liver weights were unaffected. As a result, adding 200 mg/kg of L-carnitine to broilers has a good effect on lowering cholesterol (HDL, LDL, VLDL) and triglycerides, and as a consequence, the fat percentage will be diminished (36). Recent findings contradict previous findings that L-carnitine changed for the better red cell stabilization and erythropoiesis (37).

Both Sorbitol and L-carnitine elevated villus length compared to the control. However, villus widths differed between the two treatment groups, with L-carnitine indicating greater breadth than Sorbitol, but the two groups did differ from the control. Furthermore, L-carnitine has a better positive effect than Sorbitol on the absorptive surface area of villi and the number of goblet cells. The gut is regarded as a crucial organ for absorbing and digesting as well as enzymes for digestion, microbiome, and gut absorbent surface area since the chick overcomes stages of growth after hatching that require parallel advances in nutrition use (38). As a result, we concur with Fang and Niu that a considerably longer villus height was discovered in the group treated with (0.6 g/kg) of L-carnitine, which possibly able to counteract the impact of a high diet rich in fat and resulted in enhanced fish growth (39). Knowing the metabolic fuel that increases the population of goblet cells and the length of microvilli to promote intestine absorptive capacity has enormous therapeutic promise. However, the mechanisms and dietary factors that support these activities are still unknown, as is how variations in enterocyte metabolism affect the efficiency of nutrient absorption in general and macronutrient absorption in particular (40). Carnitine is essential for the intrinsic metabolism of fats in the enterocyte, which coordinated approach energy levels by safeguarding and supporting intestinal microflora (the gut microbiota can regulate several biological functions, including dietary absorption, lipids, and glucose balance), improving absorption, and indirectly facilitating butyrate absorption, which offers 70% of the energy needed by colonocytes. Moreover, carnitine is crucial for preserving the colonic microbiota's high-fiber fermentation capacity, leading to the hypothesis that L-carnitine can enhance villi absorption and height and width (41).

## **Conclusions**

It concludes that sorbitol and L-carnitine food fortification motivate TSH and T3, but L-carnitine intake is more efficient for ATP production than Sorbitol. As a result, the two pure products change metabolism by raising glucose and lowering triglycerides, while only the Sorbitol-treated group decreases cholesterol levels. As a result, the two metabolic products cause villi lengthening, L-carnitine more in villus width, villus surface area, and goblet cell population of the broilers duodenum histological segment. These results reflect an advancement in ultimate, earned body weight yet



only chest muscle relative weight, not other organ weight, while blood constituents are unaffected.

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### Conflict of interest

According to the researcher, there are no any conflicts of interest associated to the publishing of this paper.

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## تأثير السوربيتول ول - كارنتين على مستوى الهرمون المحفز للدرقية، ثلاثي يوديد الثايرونين والادينوسين ثلاثي الفوسفات في دجاج اللحم

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### الخلاصة

لغرض الاستفادة الكبرى لقطاع الدواجن، سعى بحثنا إلى تقييم فعالية مُنتجَي التمثيل الغذائي، السوربيتول ول - كارنتين لإنتاج الطاقة. خلال شهر نيسان وإيار وبعد ثلاثة أيام من التأقلم قسم ٦٠ فرخ من دجاج اللحم إلى ثلاث مجموعات (٢٠ / مجموعة). تم إعطاء الغذاء العادي للمجموعة الضابطة، بينما تم إعطاء السوربيتول (١٠٠ غم / كغم من العلف) ول - كارنتين (٨٠ مغم / كغم من العلف) للمعاملتين الثانية والثالثة على التوالي. أظهرت النتائج أن كل من السوربيتول ول-كارنتين حفزا على زيادة مستويات كل من الهرمون المحفز للدرقية وثلاثي يوديد الثايرونين بشكل كبير، ولكن كان لاستخدام ل-كارنتين تأثيراً معنوياً أكبر لتوليد الادينوسين ثلاثي الفوسفات بالمقارنة مع السوربيتول. ومن ناحية أخرى، رفع السوربيتول ول - كارنتين مستويات الكلوكرز بشكل كبير مع خفض مستويات الدهون الثلاثية والكوليسترول، باستثناء ل - كارنتين ، الذي لم يكن له تأثير على مستويات الكوليسترول. المنتجان اللذان استخدمنا في الدراسة لهما أيضاً تأثيرات إيجابية على كفاءة النمو من خلال زيادة الوزن النهائي والمكتسب بشكل كبير مع عضلات الصدر فقط من الأعضاء الأخرى التي تم وزنها في التجارب، لكن معايير الدم التي تم تقديرها في هذه التجربة لم تتأثر بأي من العلاجات. وتوفر المتغيرات النسجية لأمعاء دجاج التسمين مؤشرات موثوقة لامتصاص هذه الإضافات العلفية. أظهرت البيانات ارتفاعاً إيجابياً ملحوظاً في الزغابات المتأثرة بالعلاجين، في حين أظهر ل-كارنتين كفاءة أكثر من حيث عرض الزغابات، ومساحة سطح الزغابات، واعداد الخلايا الكأسية. يستنتج من البحث أن ل - كارنتين أكثر كفاءة مقارنة من السوربيتول من حيث إنتاج الطاقة إما من خلال التأثيرات على إنتاج الغدة الدرقية، وتوليد الادينوسين ثلاثي الفوسفات فضلاً عن تعزيز النمو وزيادة الامتصاص من الأمعاء.