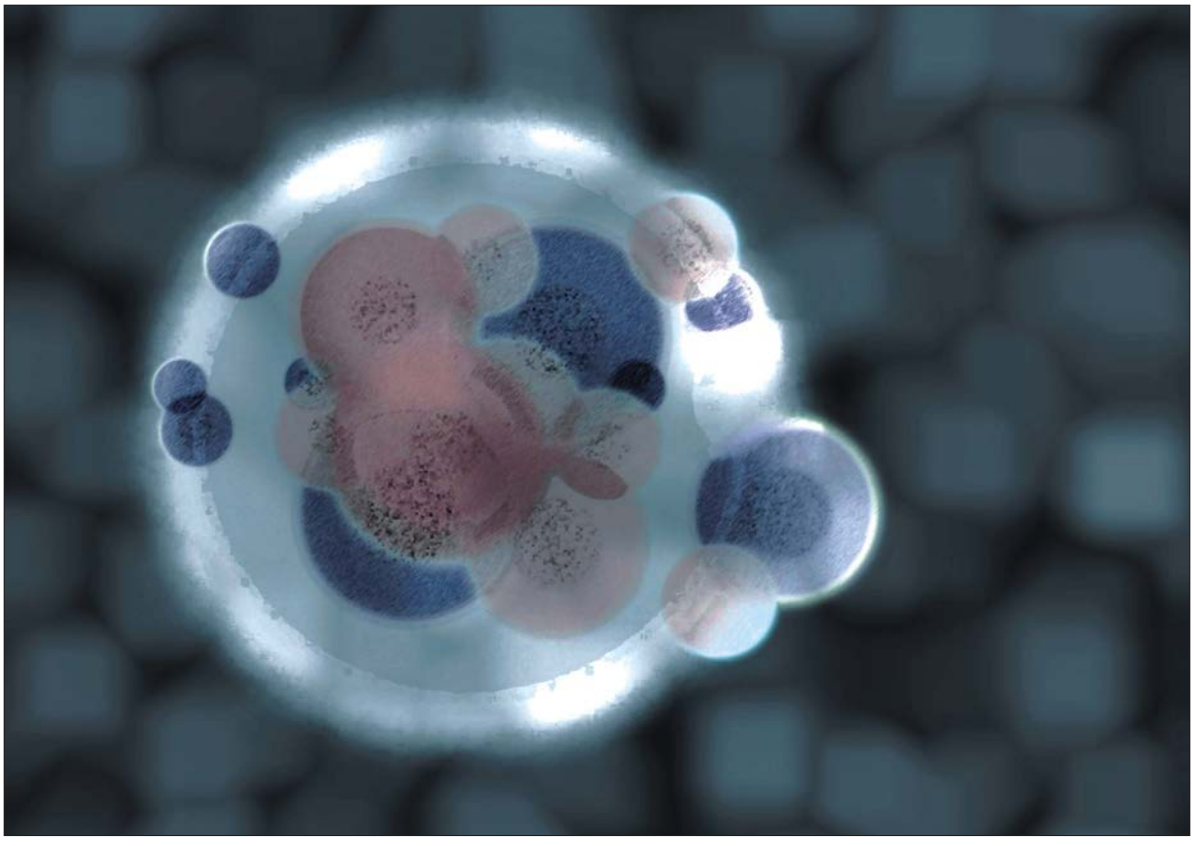

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Effect of Synbiotic Supplementation on Growth Performance, Blood Metabolites, Insulin and Testosterone and Wool Traits of Growing Lambs

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Abstract: This study aimed to evaluate the growth performance, blood metabolites, insulin and testosterone and wool traits of growing lambs fed with a diet containing low and high level of a synbiotic. A total of 14 growing Najdi male lambs (aged six months and weighed 22.02±0.41 kg) were randomly divided into three groups; control group (n = 4) fed a basal diet (C), low level group (n = 5) fed basal diet plus 20 ml of synbiotic (L) and the high level group (n=5) fed basal diet plus 40 ml of synbiotic (H). Synbiotic was given orally for a period of 12 consecutive weeks. Animals were bled for serum once a week throughout the experiment. Serum total protein, albumin, glucose, cholesterol, insulin and testosterone were quantified. Wool traits were also studied. Growth rate was determined of the biweekly routine body weights. Lambs given high level of synbiotic showed the highest growth ($p<0.01$) gain (13.74 kg) and had the heaviest weight (35.76 kg) followed by lambs given low level (8.75 and 30.87 kg, respectively) and then by the control lambs (7.33 and 29.36 kg, respectively). Total protein concentration was significantly higher in H-lambs compared with C-lambs (7.93 vs. 6.43 g dL^{-1}, respectively) with intermediate level in L-lambs (6.72 g dL^{-1}). Blood cholesterol decreased ($p<0.05$) in L-lambs (33.67 mg dL^{-1}) compared with C-lambs (49.32 mg dL^{-1}) whereas H-lambs gave a value of 44.18 mg dL^{-1} in blood of lambs with high level of synbiotic. Glucose in blood was not different ($p<0.05$) due to either L (58.47 mg dL^{-1}) or H (66.14 mg dL^{-1}) synbiotic compared with C (61.43 mg dL^{-1}) lambs, even though there found a significant ($p<0.05$) difference between L and H on glucose concentration. Insulin levels significantly ($p<0.01$) decreased at conclusion of the experiment as compared with initial levels. The highest ($p<0.01$) level of insulin was found in H-lambs (27.3 μIU mL^{-1}), however low level of synbiotic was not differ than control. Testosterone increased ($p<0.05$) by about 52% in H-lambs (5.0 ng mL^{-1}) than in control (3.3 ng mL^{-1}) lambs. The high level of synbiotic exhibited the shortest staple, lowest crimp and intermediate diameter of wool fibre.

Key words: Synbiotic supplementation, growth performance, wool traits, growing lambs

INTRODUCTION

Probiotics are live microbial feed supplements which beneficially affects the health and performance of the animal. Addition of probiotic in the diet of lambs has been reported to improve growth performance, feed conversion (Chiofalo *et al*., 2004; Klebaniuk and Czech, 2007; Khalid *et al*., 2011) and prevent the diarrhea (Musa *et al*., 2009). Therefore, sheep breeders used feed additives such as probiotics to harvest higher gain and healthy lambs at market age. It means shorter feeding period which accomplish low risk of death.

Antunovic *et al*. (2005) found that the best probiotic preparations are those with *Lactobacillus* and *Streptococcus* types to maintain the balance of desirable population in digestive tract. Literature reveals that there is a lack of dose–response studies to determine the minimal effective dosage of probiotics needed to reduce blood cholesterol concentration (Ooi and Liong, 2010).

Al-Sobayil *et al*. (2008) reported that synbiotic administration enhanced the libido in goats and improved semen characteristics such as ejaculate volume, motility and sperm concentration. Also, they found that there was an increase in testosterone concentration in treated bucks.

The concentrations of cholesterol, total protein and albumin concentration in serum of lambs treated with probiotic did not significantly differ than control ones. Lambs fed diets plus probiotic had a very low concentration of glucose and urea (Antunovic *et al*., 2005).

Wool is considered as source of income and it was influenced by the amount of nutrients available to the wool follicle (Abdelaziz *et al*., 2000). Therefore, better and

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sufficient nutrients resulted in low grade and high diameter. Crimp of wool is considered a good index in industry of wool where the crimp is due to a natural unique chemical and physical structure. This causes the fiber to bend and turn, giving wool an inherent three-dimensional crimp.

Some nutritive materials such as Fig (*Ficus carica*), wheat germ and raisins had an effect on reproductive performance of animals. Fig (*Ficus carica*) is richest in fiber, copper, manganese, magnesium, potassium, calcium and vitamin K and considered as good source of antioxidants (Vinson, 1999). *Ficus carica* contains proteolytic enzymes, amino acids, minerals, sugars, triterpenes, organic acids and allergens (Vaya and Mahmood, 2006). In addition, Degu *et al*. (2009) found that supplementation of fig resulted in better feed intake and gain. Figs contain high amount of amino acids and zinc which increase testosterone production and help to improve semen volume and keeps sperms healthy (Mutt, 2009). Therefore, it works as a good aphrodisiac agent.

Wheat germ was found to be aphrodisiac compound which might enhance spermatogenesis and testosterone production because it is rich source of fiber, vitamin E, vitamin B-6, minerals, antioxidants and phytoestrogens (Marquart *et al*., 2002).

Raisins contain about 67 to 72% sugars (most of which is fructose and glucose), 3 protein and 3.5% dietary fiber. Raisins are high in certain antioxidants, zinc and B vitamins which play an important part in proper hormone functioning and healthy hormone functioning and improve fertility (Mackeown, 2012).

Blackstrap molasses has a lot of nutritional value, it has ability to help with anemia and improve fertility. It's also play a role in the color of hair.

The present study was carried out to investigate the effect of such nutritive mixture in synbiotic preparation and its level on lambs' weight, blood serum biochemical parameters and wool traits where the effect of synbiotic did not studied in wool traits. Also the present study aims to throw a light on the best dosage of synbiotic that affecting growing lambs traits.

MATERIALS AND METHODS

Animals and management: The present study was carried out on fourteen Najdi male lambs aged six months and weighed 22.02±0.41 kgs. Lambs were raised at Qassim University Experimental Station in Buraydah. They were fed 300 g concentrate mixture (18% CP) in addition to 1 kg berseem hay. Water was in access to animals at all day times. All animals were free of diseases. Lambs were weighed biweekly.

Experimental design: Lambs were divided into three groups, first group (four lambs) as control second group (five lambs) was orally given 20 mL of synbiotic three times per week (as low level group) and the third group (five lambs) was orally given 40 mL of synbiotic on the same schedule (as high level group).

Probiotic fermented milk cultures: Starter cultures of *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from Chr. Hansen`s Laboratory, Copenhagen Denemark.

Preparation of probiotic fermented milk: Probiotic fermented milk was prepared according the method described (Tamime and Robinson, 1999; Al-Wabel *et al*., 2007; Abdel-Salam *et al*., 2010). Probiotic cultures (*Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum*) were inoculated and incubated for 4-8 h at 42°C. After coagulation, the curd were tested for pH, then was stirred in an electric blender and used for preparation of synbiotic fermented milk.

Preparation of pressurized hot water extraction: A hot water extract of *Ficus carica*, wheat germ and raisins was prepared according the methods described by Abdel-Salam *et al*. (2009, 2010). Approximately 100 g of plant materials were cut into small pieces, grinded and placed in a flask (2 L) with 1000 mL of hot distilled water and boiled for 10 min in water bath. The mixture was filtered twice through cheesecloth (50% cotton 50%$^{-1}$ polyester). The solutions obtained were preserved in sterile dark bottles in a cool environment (4°C) until use.

Preparation of synbiotic fermented milk: Synbiotic fermented milk was mixing the prebiotic extract of *Ficus carica*, wheat germ and raisins to the probiotic fermented milk at a concentrate of 3% of total volume (30 g L^{-1}) of synbiotic fermented milk containing 50% of blackstrap molasses, then grape acid at 1% (10 L^{-1}) was added to the final synbiotic mixture.

Body weight of lambs and gain: Live body weight for each lamb was recorded biweekly throughout the experiment in the morning before access to feed and water. The experimental period lasted for twelve weeks.

Blood collection and serum: Jugular blood samples were taken from each lamb once per week by vein puncture procedure. Blood was collected in non-heparinized vacutainer tubes. Blood samples were centrifuged at 3,000 rpm for 20 min to collect serum and were stored at -20°C till analysis.

Serum total protein determination: Total protein in serum samples were determined by the Biuret method according to the method of Weichselbaum (1946), in which the cupric ions in the coloring reagent react with protein molecules in an alkaline buffer resulting in a purple complex. The intensity of the color is proportional to the protein concentration in the sample.

Serum albumin determination: Albumin in serum was determined according to the method of Doumas and Peters (1997), in which bromocresol green interact with albumin producing color intensity proportional with albumin.

Serum globulins: This fraction was mathematically calculated by the subtraction of albumin from total protein values.

Serum glucose determination: Serum glucose was quantified by the enzymatic method according to Barham and Trinder (1972), in which the glucose is determined after enzymatic oxidation by glucose oxidase and the formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone forming a red-violet quinoneimine which intensity is proportional to the glucose concentration.

Serum cholesterol determination: Serum cholesterol was determined by the cholesterol oxidase-phenol aminophenazone (CHOD-PAP) method described by Richmond (1972), in which cholesterol esters are enzymatically hydrolyzed by Cholesterol Esterase (CE) to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and H_2O_2. In presence of peroxidase (POD), the formed hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye which its intensity is proportional to the cholesterol concentration.

Insulin determination: Serum insulin was determined according to the method of Frier *et al.* (1981) by an ovine insulin ELISA kit (DRG Diagnostics, Germany) using 6 levels of standard (0, 6.25, 12.5, 25, 50 and 100 μIU mL^{-1}). Since all samples were determined in one assay the intra-assay coefficient of variation was 2.2%.

Testosterone Determination: Testosterone was determined in serum by the ELA method (Rassaie *et al.*, 1992; Raji *et al.*, 2005) using the horse-raddish peroxidase as a tracer and Tetra Methyl Benzidine (TMB) as a chromogen (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). The intra and interassay coefficient of variation were 5.2 and 7.6%, respectively.

Wool traits: The greasy samples (10×10 cm) were taken from the mid of the left side. Samples were sent to Desert Research Center in Cairo, Egypt to measure staple length, fibre diameter and crimp measurements. Each sample was divided into two sub-samples, the first used to determine the fibre diameter and the second used to determine staple length, crimp measurements (Abdelaziz *et al.*, 2000). Staple length of ten staples taken were estimated using a rule to the nearest 0.5 cm. This measurement was taken for the distance between the broad base and the end of dense region at the longest fibre without stretching. The number of crimps along each unstretched staple was counted and the average staple length used to obtain the number of crimps per one centimeter. Five hundred fibres from each sample were mounted in liquid paraffin oil and spread on a microscopic slides and fibre diameter estimated using a microscope and image captured by image analysis software (video Pro, Leading Edge Ltd, S. Aust). Wool samples were assessed to determine kemp score (El Gabbas, 1993; Abdelaziz *et al.*, 2000).

Statistical analysis: Data were analyzed using proc GLM on SAS (2000). The fixed model included treatment, as mean effect was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = An observation taken on the jth lamb
μ = Overall mean
T_i = A fixed effect of the ith treatment (i=1 to 3)
e_{ij} = Random error assumed to be independent by and normally distributed with mean = 0 and variance = $\sigma^2 e$

Duncan multiple range test was conducted to determine differences among means, using 5% significant level.

RESULTS AND DISCUSSION

Body weight of lambs: Data of lambs' body weight are presented in Table 1. It could be observed that the lambs gave high level of synbiotic showed the heaviest weights followed by those gave low level and then by the control group. The differences were high significant ($p<0.01$). It is worthy to note that there were insignificant differences in weights between control lambs and lambs given low level of synbiotic.

Table 1: Effect of synbiotic level on body weight and body gain (kg) of Najdi male lambs

Treatment	Initial weight (kg)	Final weight (kg)	Body gain (kg)
Control	22.025±0.71	29.360±1.69^{b}	7.335±1.02^{b}
Low level	22.120±0.75	30.876±1.70^{b}	8.756±1.11^{b}
High level	21.920±0.83	35.662±2.40^{a}	13.742±1.24^{a}
Significance	NS	**	**

**(P<0.01) NS: Not significant. a,b means in the same column with different superscript differ significantly (p<0.05)

Regards gain of lambs, the lambs given high level showed the highest gain (13.742 kg) followed by lambs given low level (8.756 kg) and then by the control lambs (7.335 kg). The present results illustrated that body gain of lambs with high level was double that of the other groups. It could be noticed that the level of 40 mL of synbiotic is more efficient than the level of 20 mL.

Synbiotic supplementation orally may enhance the feed efficiency of lambs in the present study. Russell and Wilson (1996) reported that response of lambs to probiotic supplementation may be due to improving fibre degradation. Higher consumption and higher feed utilization was shown as a result to adding probiotic (Antunovic *et al*., 2006). Sarwar *et al*. (2010) found that probiotic supplementation improved weight gain of growing lambs. In addition, Mukhtar *et al*. (2010) and Khalid *et al*. (2011) reported that lambs fed diets with probiotic had improved weight gain and they pointed out that improving may be depending upon probiotic rate or level.

Blood metabolites: Synbiotic supplementation significantly increased (p<0.05) total protein and globulin, while it didn't showed any significant effect on albumin concentration (Table 2). Blood of lambs given high level of synbiotic showed the highest concentration of total protein, albumin and globulin (7.93, 4.20 and 3.73 g dL^{-1}, respectively) followed by that of lambs given low level (6.72, 3.70 and 3.02 g dL^{-1}, respectively) and then by the control group (6.43, 3.63 and 2.8 g dL^{-1}, respectively).

Antunovic *et al*. (2005) showed that total protein concentration increased in blood of lambs fed on diets supplemented with probiotic. There was insignificant difference between control and treated groups. In contrast, Abdel Rahman *et al*. (2012) reported that yeast culture supplementation significantly increased albumin concentration, while it was not significantly affected blood total protein or globulin.

Data of blood metabolites showed that blood cholesterol concentration decreased from the beginning of experiment till the end. Cholesterol concentration also decreased (p<0.05) in response of low level of synbiotic which averaged 33.67 mg dL^{-1} while it was being 44.18 mg dL^{-1} in blood of lambs given high level of synbiotic (Table 3). Data showed that at the end of experiment, the differences between control lambs and low level were significant while those between lambs with high level and other groups were insignificant.

Blood glucose concentration significantly differed (p<0.05) among all lambs groups. Higher concentration was observed in lambs given high level of synbiotic while lower concentration was found in blood of lambs given low level, the control group was intermediate. The higher concentration of blood glucose may be due to more gluconeogenesis which is the main source of glucose (Sano *et al*., 2007). Mukhtar *et al*. (2010) attributed increase of glucose concentration to the changes occurred in rumen fermentation process as a result to probiotic supplementation. They added that these changes lead to increase concentration of propionate in rumen of lambs.

Antunovic *et al*. (2006) pointed out that weaned lambs fed diet with 1% probiotics had lower glucose concentration and they explained this result to higher fibre digestion leading to more production of ketogenic moieties. The same authors reported that adding probiotic to diet resulted in increasing bacterial count which leads to produce protein by bacteria and reduced globulin.

Ooi and Liong (2010) illustrated that cholesterol removed by probiotics by incorporation into the cellular membranes during growth. They added that the cholesterol converted to coprostanol in the intestines which excreted in feces, therefore this reduction in the cholesterol absorbed resulted in decreasing concentration of cholesterol in blood.

Metabolic hormone such as insulin significantly increased and averaged 15.59, 16.81 and 27.31 u mL^{-1} in blood of control lambs, supplemented lambs with low level and high level of synbiotic, respectively. It is worthy to note that insulin decreased at the end of experiment where it was higher at the beginning of experiment.

The above result may be due to the increase in body weight of lambs which related to glucose level in blood of growing lambs. Antunovic *et al*. (2010) found that the increase insulin level in lambs was related to increase in body weight.

The non changes in blood glucose accompanied by the significant decrease in insulin levels at the conclusion of the experiment might be attributed to the inclusion of synbiotic to the fructose oligosaccharadies (FOS)- herbal content. This ready to be metabolized in the rumen media of these growing lambs could enhance the production of volatile fatty acids which are of first priority for the animal as a source of energy absorbed from the ruminal mucosa.

Regards testosterone hormone, it increased from 2.56 to 3.30 ng mL^{-1} in control lambs, from 1.97 to 3.95 n mL^{-1} in lambs fed on diet with low level of synbiotic and from

Table 2: Effect of synbiotic level on concentration of total protein, albumin and globulin in blood of growing lambs

	Total protein (g dL^{-1})		Albumin (g dL^{-1})		Globulin (g dL^{-1})	
Item	Initial	Final	Initial	Final	Initial	Final
Control	5.83±0.3	6.43±0.2^{b}	3.70±0.4	3.63±0.7	2.13±0.8	2.80±0.6^{b}
Low level	5.73±0.3	6.72±0.4^{b}	3.46±0.5	3.70±0.2	2.27±0.7	3.02±0.4^{b}
High level	5.77±0.8	7.93±0.2^{a}	3.63±0.3	4.20±0.1	2.13±0.9	3.73±0.2^{a}
Significance	NS	*	NS	NS	NS	*

*Significant (P<0.05) NS: Not significant. a,b means in the same column with different superscript differ significantly (p<0.05)

Table 3: Effect of synbiotic level on blood cholesterol, glucose concentrations and insulin

	Cholesterol (mg dL^{-1})		Glucose (mg dL^{-1})		Insulin (u mL^{-1})	
Item	Initial	Final	Initial	Final	Initial	Final
Control	63.67±7.4	49.33±4.3^{a}	57.67±4.1	61.43±2.5^{ab}	32.76±4.4	15.59±1.3^{b}
Low level	56.33±1.7	33.67±3.4^{b}	58.00±2.1	58.47±0.9^{b}	35.93±1.9	16.81±2.4^{b}
High level	59.00±6.8	44.18±3.3^{ab}	58.00±1.5	66.14±2.5^{a}	39.29±3.2	27.31±0.9^{a}
Significance	NS	*	NS	*	NS	**

*Significant (p<0.05) **Highly significant (p<0.01) NS: Not significant. a,b means in the same column with different superscript differ significantly (p<0.05)

Table 4: Effect of synbiotic level on testosterone hormone

	Testosterone (ng mL^{-1})	
Item	Initial	Final
Control	2.56±0.1	3.30±0.1^{b}
Low level	1.97±1.5	3.95±0.4^{b}
High level	2.64±0.3	5.00±0.1^{a}
Significance	NS	*

*Significant (p<0.05). a,b means in the same column with different superscript differ significantly (p<0.05)

Table 5: Effect of synbiotic level on wool traits

Treatment	Staple length (cm)	Fibre diameter (μ)	No. of kemp	% No. of kemp/No. of fiber	Crimp cm^{-1}
Control	5.00±1.03^{a}	42.530±7.35^{a}	63.00±6.49	19.982±2.75	0.71±0.13^{a}
Low level	5.06±0.67^{a}	32.525±1.07^{c}	53.20±5.74	15.938±1.73	0.62±0.05^{b}
High level	4.10±0.33^{b}	39.184±2.81^{b}	73.20±3.63	23.475±4.57	0.54±0.03^{c}
Significance	*	**	NS	NS	**

*Significant (p<0.05) **Highly significant (p<0.01) NS: Not significant. a,b means in the same column with different superscript differ significantly (p<0.05)

2.64 to 5 ng mL^{-1} for lambs given high level (Table 4). It means that the level of 40 mL of synbiotic is the best level affect the early puberty. The difference between treatment was significant (p<0.05).

Wool traits: Table 5 showed that staple length was longer in the low level group and control group than in the high level group (5.06, 5.00 vs. 4.10 cm, respectively) with significant effect. Low level group had the finest wool and the control group had the thicker wool while the high level group were intermediate. Treatment had highly significant effect on wool diameter. The highest estimate of crimp was found in the control group followed by the low level group and then by high level group. The differences among means of crimp were highly significant. The percentage of number kemp/ number fibre didn't significantly affected by adding synbiotic in both level, while the high level group showed the highest percent of kemp and the lambs gave low level of synbiotic showed the lowest percent.

The above results of wool illustrated that wool influenced by the nutrients available to the wool follicle (Abdelaziz *et al*., 2000) whereas better nutrients resulted in low grade and high diameter.

CONCLUSION

The synbiotic supplementation in diet of lambs' influenced considerably (p<0.01) their growth performance.

There were no changes in blood glucose which was enclosed the decrease in insulin level in blood of lambs given synbiotic. This means that higher growth rate and increasing production of volatlile fatty acids in the rumen.

A significant decrease of cholesterol concentration was observed.

Lambs given high level of synbiotic showed the highest level of testosterone hormone which affects the puberty.

The high level of synbiotic resulted in shorter staple, lower crimp and intermediate fibre diameter.

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