Original Article

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Effect of Biotin Supplementation to the Diet of Pregnant Goats on Productive and Reproductive Traits and Performance of their Kids During Suckling Period

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Abstract

Evaluation the biotin supplementation to the diet of doe pregnant goats on milk yield, blood components and hormonal levels and weight changes of their kids during suckling period was the objective of this study. Thirty pregnant Zaraibi goats in the first parity were randomly divided into three similar groups. The experiment was started at two weeks before mating and lasted until the end of suckling period and weaning their kids. The 1st group fed diet without biotin while the 2nd and 3rd groups, each doe was fed diets with biotin daily at the rate of 5 and 10 mg, respectively. Biotin additives to the diet of goats increased significantly doe kidding twins, litter weight of kids, milk yield and dry matter intake and decreased significantly time of return to estrous postpartum compared to control group. Biotin additives also increased significantly concentrations of blood biochemical components, thyroid hormones, female sex hormones and decreased significantly cortisol level compared to control group. Live body weight (LBW) and daily body gain (DBG) of their suckling kids increased significantly with increasing biotin level in the diets of their mothers. These results suggest that dietary biotin is required at the rate of 10 mg daily for optimal pregnant goats.

Key Words: goats, biotin, milk yield, blood components, hormones.

Introduction

Biotin (vitamin B7, C10H16N2O3S) is a water-soluble B-vitamin and formerly known as vitamin H or coenzyme R [1]. Biotin is present in feedstuffs in both bound and free forms and much of the bound biotin is apparently unavailable to animal species. The primary source of biotin for ruminants is that synthesized by microorganisms in the rumen [2]. Rations have high in concentrate reduces the net synthesis of biotin by over 50% due to the acid environment and shift in rumen microbes [3]. Most diets fed to animals contain between 0.1 and 0.2 mg of biotin/lb of DMI which equals 4 to 10 mg of biotin daily, but net ruminal biotin synthesis was negligible i.e. 1 to 5 mg per day and 1 to 3 mg per day is absorbed [4]. Biotin is an essential coenzyme in carbohydrate, fat and protein metabolism and is needed for the body to process amino acids, to make glucose from the propionate produced in the rumen (Gluconeogenesis) and to make fat from the acetate produced in the rumen (Lipogenesis) [5]. Biotin is also required for some of rumen bacteria, especially, the fiber-digesting bacteria and also needed for the rumen microbes to produce propionate because biotin is a cofactor in the microbial enzyme methylmalonyl-CoA-carboxytransferase, which catalyzes a step in the synthesis of propionic acid [6].

Biotin is required for normal function of the thyroid and adrenal glands, reproductive and nervous systems. Most researchers reported that milk yield and milk components increase due to biotin supplementation to the diet of dairy cattle [7, 8]. Studies of biotin supplementation to the diet of goats are rare. Evaluate biotin supplementation to the diet of doe pregnant goats on milk yield, blood components and hormonal levels and weight changes of their kids during suckling period was the objective of this study.

Materials and Methods

Location and ethics:

The experimental work was carried out in Goat Farm of Biological Application Department, Radioisotopes Applications Division, Nuclear Research Centre, Atomic Energy Authority, at Inshas, Egypt (latitude 31º 12' N to 22 º 2' N, longitude 25 º 53' E to 35º 53' E). The ethics contain relevant information on the Endeavour to reduce animal suffering and adherence to best practices in veterinary care according to the International Council for Laboratory Animal Science (ICLAS) guidelines. Experimental animals were also cared using husbandry guidelines derived from Egyptian Atomic Energy Authority standard operating procedures.

Experimental animals, feeding and management:

Thirty Zaraibi pregnant goats aged 18-20 months and weighed 30.0±2.5 kg in the 1st parity was randomly divided into three similar groups; ten does in each, were used in this research. The experimental goats were healthy and clinically free of external and internal parasites. Animals were provided the feedstuffs consisted of concentrating feed mixture (CFM) and clover hay (CH) according to its physiological status; pregnant and lactation to be adequate in protein and energy to cover their nutrient requirements [9]. The CFM composed of 37.4% wheat bran, 27.0% yellow corn, 12.5% soybean meal (44% crude protein), 10.0% undecorticated cottonseed cake, 5% rice bran, 4% sugarcane molasses, 3% limestone, 1% sodium chloride and 0.1% vitamin and minerals premix. Each kg of CFM contains 0.8 % calcium, 0.6% phosphorus, 0.07% magnesium and 0.65% potassium. Each kg of vitamin and minerals premix contains 40g Mn, 3 g Cu, 0.3g I, 0.1g Si, 200 mg Co, 45 g Zn, 30g Fe, 20 g biotin and 200 mg choline chloride as well as 13500, 4500 and 36 IU vitamins A, D3 and E, respectively. Feed CFM was offered once daily at 10.00 hr. at rate of 3.5% of the body weight of the experimental animals and CH was offered at midday ad libitum and fresh drinking water was available all time. A proximate analysis of the CFM and CH were carried out according to AOAC [10]. Chemical composition values (on DM basis %) of CFM used in feeding the does are 18.00% crude protein, 13.50% crude fiber, 4.80% ether extract, 53.70% nitrogen-free extract and 10.00% ash. The corresponding values for CH were 13.24, 30.19, 1.67, 42.90 and 12.00%. Calculated nutritive values of the CFM and CH according to NRC [9] were 4.00 and 2.64 net energy (MJ/kg DM) and 60.82 and 48.00 total digestible nutrients (%), respectively. Each experimental group was housed in separate yard with concrete pen, partially asbestos roofed in semi-open sheds during the experimental period. Each yard was divided to 10 partitions using pieces of hard wood, each doe in one partition provided with here feeds and water.

Experimental procedure:

The experiment was conducted on Zaraibi pregnant goats starting from two weeks before mating date (day 23 of October 2016) and lasted during pregnancy period (3 months) and suckling period till weaning their kids (3 months) at end of April 2017). Thirty Zaraibi pregnant goats were divided randomly into three equal groups. The 1st group was kept without treatment as control while in the 2nd and 3rd groups; each doe was supplemented daily with 5 and 10 mg synthesized commercial biotin, respectively. Synthesized commercial biotin was produced as a powder for Animal Pharmaceuticals CAS No.:58-85-5, Purity: 98.5-99% from Hangzhou Fanda Chemical Co., Ltd., Zhejiang, China (https://www.alibaba.com). Biotin was added to the diet of goats of 2nd and 3rd groups by dilution the powder of biotin in warm water and spraying CFM to be more uniform biotin activity and enhance the mixing and distribution of biotin in CFM. Diets of the 2nd and 3rd groups were prepared by spraying biotin to the CFM to be satisfied one month. Biotin in CFM fed the three experimental animals contains a basal level of 20 g biotin /kg premix.

Productive and reproductive traits measurements in does:

Productive traits of goats including type of birth (number of does kidding single and twins), litter size and litter weight per experimental group and per doe at birth and monthly were estimated. Litter weight of kids born per group was calculated by multiply litter size with average litter weight at birth. Number of mortality kids per group from birthing to weaning was also estimated. Gestation length was determined by the difference between date of jumping of male on doe and date of kidding. Days from kidding to day of the first oestrous noticed in doe after kidding were estimated.

Dry matter intake and milk yield determination:

Feed intake (FI) from CFM and BH was measured individually for each doe two times, once during mid-pregnant period and once during mid-lactation period and averaged to obtain FI for each experimental group at each time. The average DM of CFM and BH were determined from the weight difference before and after oven drying overnight at 105°C. DMI was calculated by multiplying daily fresh feed intake from CFM and BH of each animal with the DM percentage which being 90.0 and 88.0 % for CFM and BH, respectively, The total DMI (CFM+CH) of each group was also calculated. Daily milk yield per each doe was recorded at four times; at two days after birthing and then monthly during suckling period (3 months) using isolation of all kids out of their mothers at midday until completely hand milking until stripping the udder on midday of the second day.

Blood samples and estimation of blood components and hormonal levels:

Single blood sample with anticoagulant (heparin) was collected on day -10 prior to the day of expected parturition and also on days 2, 30, 60 and 90 postpartum. Blood samples had taken from jugular vein of each doe before morning meal and placed on ice water immediately following collection. Blood samples were centrifuged at 3000 rpm for 25 minutes to obtain plasma and stored at -20oC until plasma analyses for total protein, albumin, γ-globulin and glucose concentrations using reagent kites manufactured by Diamond Diagnostic Company (Egypt). Globulin value was calculated by subtraction of albumin value from their corresponding total protein value. Plasma levels of total thyroxin (T4), Triiodothyronine (T3), cortisol, progesterone (P4) and estradiol-17β hormones (E2) were estimated by the radioimmunoassay (RIA) technique using the coated tubes kits purchased from Diagnostic Products Corporation, Los Angeles, CA, USA and counting in the Laboratory of Biological Applications Department, Atomic Energy Authority, using computerizes Gamma Counter. The tracer in the tow hormones was labeled with iodine-125 (125I).

Changes in weight gain of kids:

Live body weight (LBW) of born kids (kg) for each experimental group either male or female were recorded and weighed at birth and monthly intervals till weaning at 90 days old. Monthly daily body weight gains (DBG) of kids per group from birthing to at weaning were also calculated.

Statistical analysis:

Data were statistically analyzed by analysis of variance using general linear model procedure by computer program of the SAS software [11]. Duncan's multiple range t-tests was used for testing the significant differences between means [12].

Results

Effect of biotin on productive and reproductive efficiency of pregnant goats:

Does kidding twins and litter size per doe increased significantly with increasing the level of biotin in the diet of goats. Litter weight of kids at weaning were differ significantly due to biotin, being improved significantly over control by 30.3 kg ($p<0.05$) and 73.48 kg ($p<0.01$) in groups received daily 5 and 10 mg biotin, respectively. Gestation length was not affected significantly due to adding biotin to the diet of pregnant goats. It is interesting to observe that time of return to estrous in dairy goats postpartum was before weaning by 14 and 25 days in does received 5 and 10 mg daily from biotin, respectively, while resumption of oestrous cycle in does not received biotin was after weaning by 7.0 days (Table 1).

Effect of biotin on DMI:

DMI of CFM and BH was significantly affected by the biotin levels. Total DMI before and after kidding periods were progressively increased significantly over control (received 0 g biotin) by 166 g/day (11.5%, $p<0.05$) and 321 g/day (22.3%, $p<0.01$) in does received 5 and 10 mg daily from biotin, respectively (Table 2).

Effect of biotin on milk yield:

Levels of biotin in the diet of goats affect significantly on overall mean of daily milk yield (g/h). Milk yield in two experimental groups which received daily 5 and 10 mg biotin were higher significantly by 37.21g milk/day (19.7%; $p<0.05$) and 75.07 g milk/day (39.75%; $p<0.01$) than milk yield of goats received daily 0 mg biotin. Days after birthing affects significantly on daily milk yield. The highest milk yields in the three experimental groups were at day 30 after birthing while the lowest milk yields were at day 90 after birthing (at weaning their kids) (Table 3).

Effect of biotin on blood proteins and glucose concentrations:

Levels of biotin in the diet of goats affect significantly on overall mean of total protein, globulin and γ-globulin. The improvements were progressively increased significantly with increasing the biotin level in the diet of goats from 5 mg to 10 mg. Biotin at level of 10 mg/head/day increased significantly total protein, globulin and γ-globulin by 14.1, 20.5 and 33.3%, respectively. While albumin value not affected significantly by biotin supplementation. Blood total protein, globulin and γ-globulin also affected significantly due to timing of blood samples. The highest values were at 30 days after birthing while the lower values were at 2 days after birthing (Table 4). Glucose value increased significantly from 40.9 mg/dl to 47.2 mg/dl (15.4 %; $p<0.05$) in goats received daily 5 mg biotin and to 54.9 mg/dl (34.2%; $p<0.01$) in goats received daily 10 mg biotin (Table 4). These results indicate that biotin addition tended to improve blood metabolic parameters and feeding 10 mg/d of biotin may be the optimal (Table 4).

Effect of biotin on hormonal level in female goats:

Metabolic hormones (Thyroid hormones): Overall means of T4 and T3 levels increased significantly from 35.36 ng/ml and 102.04 ng/dl in goats received 0 mg biotin daily to 40.06 ng/ml (13.3%; $p<0.05$) and 116.98 ng/dl (14.6%; $p<0.05$) in goats received 5 mg biotin daily and increased again to 46.04 ng/ml (30.2%; $p<0.01$) and 125.62 ng/dl (23.1%; $p<0.01$) in goats received 10 mg biotin daily, respectively. The thyroid hormonal profiles were studied on days -10 prior to kidding and on day +2, +30, +60, +90 days post kidding and found that the lowest thyroid hormonal levels were at day +2 after birthing while the highest values were at day +30 and +60 after birthing (Table 5).

Stress hormone (cortisol hormone): Overall mean of cortisol level decreased significantly from 11.06 ng/ml in goats received 0 mg biotin daily to 8.42 ng/ml (23.9%; $p<0.01$) in goats received 5 mg biotin daily and decreased to 6.00 ng/ml (45.8%; $p<0.001$) in goats received 10 mg biotin daily. The highest cortisol value was in pregnant goats i.e. at day 10 before birthing while after birthing, no significant differences in cortisol level due to timing after birthing (Table 5).

Female sex hormones: The levels of Progesterone (P4) and17β-estradiol (E2) showed great variation. As expected, the levels vary according to the stage of the reproductive cycle or presence of pregnancy or parturition. P4 and E2 levels recorded the highest values at day 10 before birthing and the lowest values at day 2 and in the first month post-partum. The levels of two hormones increased at day 60 after birthing and increased again at day 90 after birthing (Table 5). Levels of biotin in the diet of female goats significantly affected overall means of female sex hormones. P4 and E2 levels in goats received 0 mg biotin daily were 5.4 ng/ml and 98.6 pg/ml and increased significantly to 7.04 ng/ml (30.4 %; $p<0.01$) and 110.1 pg/ml (11.7 %; $p<0.05$) in goats receive daily 5 g biotin and increased significantly to 8.42 ng/ml (55.9 %; $p<0.001$) and 121.0 pg/ml (22.7 %; $p<0.01$) in goats received 10 mg biotin daily, respectively. The interaction between physiological status and biotin treatment effect showed that the highest female sex hormones levels in the three experimental groups were during pregnancy period and the lowest levels were after birthing directly (Table 5).

Effect of biotin on LBW and DBG of suckling kids from birthing to weaning:

Adding biotin to the diets of female goats improved significantly positively LBW and DBG of their suckling kids at both birthing and during suckling period until weaning. LBW and DBG of suckling kids increased significantly with increasing biotin level in the diets of their mothers. LBW of suckling kids of mother's intake 5 mg/daily biotin was better than LBW of suckling kids of mother's intake 0 mg/daily biotin by 1.06 kg/90 days and LBW of suckling kids of mother's intake 10 mg/daily biotin was better than LBW of suckling kids of mother's intake 0 mg/daily biotin by 2.06 kg/90 days. No of mortality kids per group from birthing to weaning decreased with adding 10 mg/daily biotin to diet of pregnant goats. Viability rate improved during suckling period till at weaning due to biotin supplementation at the rate of 10 mg daily in the diets of goats (Table 6).

Discussion

The increase in litter size and litter weight of kids at weaning in goats due to biotin supplementation in their diet may be that biotin improved DMI from feed stuffs and consequently improved protein and source of energy intake from feed stuffs. Biotin also contributes to vital biological functions including synthesis of protein fractions as well as metabolic thyroid hormones. The decrease in time of return to estrous in dairy goats postpartum supplemented with biotin may due to that biotin increasing synthesis of metabolic substrates requirements for lactating goats. Nutritional level is quite important during early lactation because animal use reserves for milk production which leads to a delay in resumption of postpartum ovarian activity [13]. The evaluation of estrous behavior showed a negative linear regression between energy intake levels and time of return to estrous in dairy goats postpartum [14]. The higher DMI for biotin treated was attributable to a significantly higher voluntary intake from the feed stuffs than for control. The increase of DMI may be improved nutrients and digestibility of feed stuffs as well as fiber digestion [15]. The increase in blood protein parameters and metabolic hormones in goats received biotin may be also responsible the increase in milk yield of treated goats. Feeding supplemental biotin increased DMI and milk production significantly in dairy cows [16]. Biotin increased milk production by 1.29 kg per cow per day [17]. In addition, milk production linearly increased with biotin supplementation 36.9, 37.8, and 39.7 kg per day for 0, 10, and 20 mg per day of supplemental biotin, respectively [18]. Biotin increased milk yield and milk components in dairy cows due to increase the activity of liver pyruvate carboxylase and essential fatty acid metabolism [8]. Supplemental biotin helped cows to produce more glucose from the propionate produced in the rumen and this would increase the production of lactose and drive milk production [19]. The increase in total protein, globulin and γ-globulin may be due to biotin supplementation increased DMI as well as nutrients feed digestibility and stimulates the protein synthesis. The increase in glucose value due to biotin supplementation may be due to biotin increase of the glucose transport to provide energy required for peptide synthesis. The increase in overall mean of T4 and T3 levels in goats received biotin may be that biotin stimulates the production of TSH which helps thyroid gland to produce thyroid hormones. The lowest thyroid hormonal levels were at day +2 after birthing while the highest values were at day +30 and +60 after birthing. Lower T3 and T4 concentrations at day 2 after birthing may be to reduce the rate of oxidation and the rate of continuous breakdown and formation of protein and fat in mammary gland tissue or may be to reduce the adverse effects of nutrient deficiency at the onset of lactation. The decrease in the concentrations of thyroid hormones on the +2 day after kidding may be an indication of enhanced utilization of hormones by mammary tissue as a result of the increased metabolism due to the stress of parturition [20]. The elevations in the concentration of thyroid hormones after parturition may be associated with the metabolic changes in the whole body induced by lactation, which causes an increase in the demand for nutrients, as the kids remained with the dams, causing tactile stimulation of mammary gland. Lowering cortisol levels in treated goats as a response to biotin addition in their diet may be due to decrease ACTH hormone. The highest cortisol value was in pregnant goats i.e. at day 10 before birthing while after birthing, no significant differences in cortisol level due to timing after birthing. During the last stage of pregnancy, there is an increased secretion of ACTH from the fetal pituitary which stimulates rapid growth of the fetal adrenals, leading to a rise in the concentrations of cortisol. Thus, an increased amount of plasma cortisol enters the maternal circulation and induces parturition by activating the production of PGF2a and coordinating the endocrine profile of the animal. The high level of cortisol during 10-day prepartum is indicative of stress of pregnancy. During parturition, there is an increase secretion of ACTH which stimulates adrenals to increase the level of cortisol. The increase in overall mean of progesterone (P4) and estradiol-17β (E2) levels in goats received biotin owing to increased metabolic activity due to high levels of T3 and T4 hormones. P4 and E2 levels recorded the highest values at day 10 before birthing to keep the pregnancy, therefore P4 and E2 were maximal during the latter part of pregnancy. The levels of two hormones increased at day 60 after birthing may be due to does were in the luteal phase of oestrous period and the levels of two hormones increased again at day 90 after birthing may be does in the first month of pregnancy. In the goat pregnancy maintenance dependent solely on P4 from the corpus luteum and there is a rise in P4 production after 60 days of pregnancy due to placental lactogen stimulation which rejuvenates the corpus luteum to full function. P4 and E2 levels recorded the lowest values at day 2 and in the first month post-partum may be due to lack of ovulation and corpus luteum formation and thus the animals could not become pregnant for several months. The increase level on day -10 prepartum is required for uterine contractivity for giving favourable stimulus for oxytocin and triggering prostaglandin release for myometrial contraction [21]. P4 and E2 are hormones play critical roles in mammary gland development during pregnancy [22]. The significance of increase E2 concentration prepartum is probably associated with the preparation of mammary gland to initiate milk secretion by stimulating prolactin secretion [20]. P4 concentration decline from day 20 to day1 before kidding and the decrease in P4 before parturition is being of primary importance in the induction of parturition in goats. In addition, development of the mammary gland and expulsion of the fetus are both associated with the decrease in the concentration of plasma P4 [23]. The improvement in LBW and DBG of suckling kids at both birthing and during suckling period until weaning with adding biotin in the diets of their mothers may be due to the increase in milk yield and may be due to increase in milk components. Animals fed biotin have blood serum biotin concentration twice and milk biotin concentration two or three times as high as control cows [24]. Therefore, biotin secretion though milk of does may be also caused the increase in metabolic hormones levels in kids. The increase in milk yield and milk components with adding biotin in the diets of their mothers caused the decrease in number of mortality kids per group from birthing to weaning as well as improvement in viability rate during suckling period till at weaning.

Conclusion

It can be concluded that dietary biotin is required at the rate of 10 mg daily for optimal pregnant goats.
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Table 1: Effects of biotin on productive and reproductive traits of pregnant goats

Productive and reproductive traits of does	Control, 0 mg biotin /daily	5 mg biotin /daily	10 mg biotin /daily
Number of does	10	10	10
Type of birth of kids	7single +3 twins	6 single +4 twins	5 single +5 twins
Doe kidding single, No (%)	7 (70)^{a}	6 (60)^{b}	5(50)^{c}
Doe kidding twins, No (%)	3(30)^{c}	4 (40)^{b}	5(50)^{a}
No of kids born per group (litter size)	13.0	14.0	15.0
Sex of kids (male=M and female=F)	7F+6M	7 F+ 7M	8F+7M
No. of kids born per doe	1.3	1.4	1.5
Litter size at weaning	11.0	12.0	14.0
***Litter weight per group at weaning, kg**	122.1	152.4	195.58
Improvement in litter weight per group, kg		+ 30.3	+73.48
Significance		P<0.05.	P<0.01.
Gestation length, day	151.2^{a}±0.75	153.2^{a}±0.55	150.1^{a}±0.88
Days from kidding to first oestrous	97.0^{a} ±3.7	76.0^{b}±4.2	65^{c} ±4.3
First oestrous in relation to weaning	After 7 days	Before 14 days	Before 25 days

*Litter size at weaning x kids body weight at weaning

* Statistical differences between treatments in probability of the type of birth was conducted by Chi-square test and significant results were subsequently evaluated using the multiple Z-tests to compare corresponding proportions

Table 2: Effect of biotin on dry matter intake (DMI) of does during experimental period

Dry matter intake (DMI), g/day	Control, 0 mg biotin /daily	5 mg biotin /daily	10 mg biotin /daily
DMI from CFM during pregnancy	1020^{c}±10.9	1150^{b}±20.4	1270^{a}±15.8
DMI from CFM during lactation	1230^{c}±14.7	1310^{b}±12.8	1390^{a}±20.1
Average DMI from CFM	1125^{c} ± 34.2	1230^{b}±45.9	1330^{a}±30.9
DMI from BH during pregnancy	277^{c}±12.8	325^{b}±11.8	375^{a}±10.7
DMI from BH during lactation	351^{c}±20.4	425^{b}±18.9	485^{a}±18.8
Average DMI from BH	314^{c}±22.0	375^{b}±24.1	430^{a}±30.0
Total DMI	1439^{c}±32	1605^{b}±34	1760^{a}±35
Increase in total DMI due to biotin, g/day		+166	+321
Increase %		+11.5	+22.3
Significantly		P<0.05	P<0.01

Table 3: Effect of biotin supplementation on daily milk yield (g/head) of does from birthing to weaning their kids

Time of daily milk yield estimation	Daily milk yield of doe (g/day/head)		
	Control 0 mg biotin/daily	5 mg biotin /daily	10 mg biotin /daily
Number of does	10.0	10.0	10.0
Day 2 after birthing	138.50^{b}±4.29	213.50^{a}±14.19	216.00^{a}±13.69
Day 30 after birthing	244.00^{a}±8.60	265.00^{b}±15.45	358.50^{a}±5.97
Day 60 after birthing	227.50^{c}±11.42	256.00^{b}±20.27	291.00^{a}±12.59
Day 90 after birthing	82.50^{c}±4.03	93.50^{b}±3.50	111.00^{a}±9.37
Overall daily milk yield	188.86^{c}±29.64	226.07^{b}±32.00	263.93^{a}±41.32
Increase over control (g/day)		37.21	75.07
Change, %		+19.70	+39.75
Significance		P<0.05	P<0.01

Different letters in the same row indicate significant differences (a < b < c < d, P<0.05).

Table 4: Effect of biotin on blood immunity parameters and glucose concentrations in does before and after birthing their kids

Different letters in the same column of each group indicate significant differences (a < b < c < d, P<0.05). Different letters in the same column of overall mean each item indicate significant differences (A <B <C, P<0.05).

Experimental groups	**Sampling time**	**Blood biochemical components in does**				
		T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	γ globulin (g/dl)	Glucose (mg/dl)
Control	Day 10 bb	6.8^{b}±0.1	3.2^{b}±0.1	3.6^{b}±0.1	0.90^{d}±0.01	40.5^{ab}±1.5
	Day 2 ab	6.4^{c}±0.2	3.5^{b}±0.1	2.9^{c}±0.2	0.89^{d}±0.01	38.9^{b}±1.8
	Day 30 ab	7.8^{a}±0.3	3.9^{a}±0.2	3.9^{a}±0.2	1.09^{a}±0.01	43.5^{a}±1.6
	Day 60 ab	6.8^{b}±0.3	3.3^{b}±0.2	3.5^{b}±0.2	0.99^{b}±0.01	42.4^{a}±2.1
	Day 90 ab	7.0^{b}±0.3	3.3^{b}±0.2	3.7^{ab}±0.2	0.94^{c}±0.01	39.6^{ab}±1.4
	Overall mean	**6.96^{C}±0.2**	**3.44±0.1**	**3.52^{C}±0.2**	**0.96^{C}±0.04**	**40.9^{C}±0.86**
5 mg biotin/daily	Day 10 bb	7.1^{b}±0.1	3.3^{b}±0.1	3.8^{b}±0.1	1.02^{b}±0.01	47.7^{a}±2.1
	Day 2 ab	6.9^{b}±0.1	3.5^{ba}±0.1	3.4^{c}±0.1	1.02^{b}±0.01	44.8^{b}±1.9
	Day 30 ab	7.9^{a}±0.3	3.8^{a}±0.1	4.3^{a}±0.2	1.25^{a}±0.01	51.9^{a}±2.1
	Day 60 ab	7.8^{a}±0.3	3.6^{a}±0.1	4.2^{a}±0.2	1.15^{ab}±0.01	46.8^{ab}±2.3
	Day 90 ab	7.8^{a}±0.3	3.6^{a}±0.1	4.2^{a}±0.2	1.13^{ab}±0.01	44.7^{b}±1.4
	Overall mean	**7.50^{B}±0.2**	**3.56±0.07**	**3.92^{B}±0.2**	**1.11^{B}±0.02**	**47.2^{B}±0.70**
10 mg biotin/daily	Day 10 bb	7.9^{b}±0.3	3.6^{b}±0.1	4.1^{b}±0.2	1.20^{c}±0.01	50.8^{b}±1.4
	Day 2 ab	7.8^{b}±0.1	3.6^{b}±0.1	4.2^{ab}±0.1	1.28^{b}±0.01	50.6^{b}±1.8
	Day 30 ab	8.2^{a}±0.1	4.0^{a}±0.1	4.2^{ab}±0.1	1.33^{a}±0.01	63.8^{a}±2.1
	Day 60 ab	7.9^{b}±0.2	3.5^{b}±0.1	4.3^{a}±0.1	1.31^{a}±0.01	57.9^{ab}±2.1
	Day 90 ab	7.9^{b}±0.3	3.5^{b}±0.1	4.4^{a}±0.2	1.28^{b}±0.01	51.2^{b}±1.5
	Overall mean	**7.9^{A}±0.07**	**3.64±0.09**	**4.24^{A}±0.05**	**1.28^{A}±0.02**	**54.9^{A}±1.66**

bb =before birthing, ab= after birthing

Table 5: Effect of biotin on hormonal levels in experimental does before and after birthing their kids.

Different letters in the same column of each group indicate significant differences (a < b < c < d, P<0.05). Different letters in the same column of overall mean each item indicate significant differences (A <B <C, P<0.05).

Experimental groups	Sampling time	Metabolic hormones		Stress hormone	Female sex hormones	
		T_4 (ng/ml)	T_3 (ng/dl)	Cortisol (ng/ml)	P_4 (ng/ml)	Estradiol$_{17ß}$ (pg/ml)
Control	Day 10 bb	35.5^{ab}±0.9	101.0^{ab}±1.2	13.5^{a}±0.32	9.0^{a}±0.2	136.8^{a}±6.7
	Day 2 ab	33.8^{b}±0.8	98.4^{b}±1.1	10.9^{b}±0.29	1.8^{e}±0.1	55.6^{e}±5.7
	Day 30 ab	36.8^{a}±1.1	105.6^{a}±1.6	10.8^{b}±0.42	3.5^{d}±0.2	79.9^{d}±6.8
	Day 60 ab	36.6^{a}±1.2	106.2^{a}±2.1	10.1^{b}±0.42	4.9^{c}±0.2	99.9^{c}±8.9
	Day 90 ab	34.1^{ab}±1.0	99.0^{b}±2.0	10.0^{b}±0.25	7.8^{b}±0.3	120.8^{b}±9.3
	Overall mean	**35.36^{C}±0.6**	**102.04^{C}±1.6**	**11.06^{A}±0.6**	**5.40^{C}±1.3**	**98.6^{C}±14.4**
5 mg biotin/daily	Day 10 bb	39.2^{ab}±1.1	112.0^{b}±2.0	10.7^{a}±0.32	10.9^{a}±0.3	155.5^{a}±6.6
	Day 2 ab	36.5^{b}±1.2	110.8^{b}±2.5	7.9^{b}±0.31	2.9^{e}±0.3	60.7^{e}±7.5
	Day 30 ab	42.9^{a}±1.0	123.7^{a}±2.3	7.8^{b}±0.28	4.8^{d}±0.2	88.9^{d}±8.8
	Day 60 ab	43.9^{a}±1.2	124.8^{a}±2.6	8.0^{b}±0.12	6.8^{c}±0.1	110.7^{c}±9.3
	Day 90 ab	37.8^{b}±1.1	113.6^{b}±2.1	7.7^{b}±0.26	9.8^{b}±0.2	134.8^{b}±9.2
	Overall mean	**40.06^{B}±1.4**	**116.98^{B}±1.8**	**8.42^{B}±0.6**	**7.04^{B}±1.5**	**110.1^{B}±16.7**
10 mg biotin/daily	Day 10 bb	45.9^{ab}±1.3	123.0^{b}±±2.3	7.2^{a}±0.21	12.8^{a}±0.4	172.4^{a}±6.3
	Day 2 ab	42.2^{b}±1.2	120.8^{b}±±2.6	5.6^{b}±0.32	3.9^{e}±0.3	70.6^{e}±5.1
	Day 30 ab	48.6^{a}±1.3	132.5^{a}±±3.0	5.5^{b}±0.22	5.6^{d}±0-.2	90.6^{d}±5.2
	Day 60 ab	49.9^{a}±1.4	130.4^{a}±±3.2	5.9^{b}±0.25	7.9^{c}±0.2	120.8^{c}±6.2
	Day 90 ab	43.6^{b}±1.0	121.4^{b}±±2.7	5.8^{b}±0.23	11.9^{b}±0.3	150.6^{b}±9.5
	Overall mean	**46.0^{A}±1.4**	**125.62^{A}±1.53**	**6.00^{C}±0.38**	**8.42^{A}±1.7**	**121.0^{A}±18.7**

bb =before birthing, ab = after birthing

Table 6: Effects of biotin on kid's body weight (kg) and mortality rate in kids during suckling period.

Change in weight gain of kids (kg) and kids' mortality during suckling period	Control, 0 mg biotin /daily	5 mg biotin /daily	10 mg biotin /daily
Kids body weight at birth, kg	2.35^{c}±0.12 (13)	2.89^{b}±0.06 (14)	3.16^{a}±0.05 (15)
Kids body weight after one month, kg	5.28^{c}±0.31 (12)	6.27^{b}±0.41(14)	6.97^{a}±0.61 (14)
Kids body weight after 2 months, kg	8.27^{a}±0.40 (11)	9.77^{a}±0.42 (13)	10.11^{a}±0.53 (14)
Kids body weight after 3 months, kg	11.10^{c}±0.38(11)	12.70^{b}±0.60(12)	13.97^{a}±0.91(14)
Total gain (at weaning - at birth), kg	8.75	9.81	10.81
Average daily gain. g	97.22^{c}±2.8	109.00^{b}±2.5	120.11^{a}±2.4
Increase in daily gain over control (kg)		+1.06	+2.06
Change, %		12.11	23.54
Significance		P<0.05	P<0.01
Mortality number of kids per group	2	2	1
Mortality rate, %	15.4^{a}	14.3^{a}	6.7^{b}
****Viability rate, %**	84.6^{b}	85.7^{b}	93.3^{a}

Between parentheses are number of live kids at birthing and during suckling period.
Different letters in the same row indicate significant differences (a < b < c < d, P<0.05).
No significant differences were found in LBW and DBWG of kids at birthing and at weaning due to sex. .
**Statistical differences between treatments in mortality rate and viability rate were conducted by Chi-square test and significant results were subsequently evaluated using the multiple Z-tests to compare corresponding proportions