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EFFECT OF LEVELS OF DIETARY PROTEIN AND ADDITION OF PROBIOTIC (SACCHAROMYCES CEREVISIAE AND BACILLUS SUBTILIS) ON SERUM CONCENTRATIONS OF INSULIN, TSH AND GROWTH HORMONES IN AWASSI LAMBS

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Abstract : **To maintain the body on its metabolic activities, including catabolic and anabolic processes, it needs tools. Insulin, *thyroid–stimulating hormone* (TSH), growth hormone (GH) and other hormones are considered the tools that contribute to regulate metabolism in living organism. The studies that interest in that scope and applied on ruminants are too scarce, therefore the current research has studied the effect of levels of dietary protein and probiotic additives (live Saccharomyces cerevisiae more than 3.0×10^{13} CFU and bacillus subtilis more than 4.0×10^{9} CFU) on serum insulin, TSH and growth hormones concentrations in Awassi lambs. In this study 16 lambs were divided to into four groups. In the first group lambs were fed concentrate (12% crude protein, CP) without probiotics, in the second group lambs were fed the same diet plus 1 kg/ton of probiotic, in the third group lambs were fed concentrate diet (14% CP) without probiotics, and in the fourth one lambs were fed the same diet plus 1 kg/ton probiotic. Wheat straw was offered to all lambs adlibitum. The main results have shown that increasing CP level from 12 to 14% decreased ($P<0.01$) serum insulin concentration from 10.52 to 7.29, without affecting serum concentrations of both TSH and GH. Addition of probiotic had no effect on serum concentrations of all hormones. Regarding the interaction effect between level of dietary CP and addition of probiotic, results revealed that higher serum insulin concentration ($P<0.05$) was associated with blood samples collected from lambs fed 12% CP without probiotic. Higher ($P<0.01$) serum TSH concentrations detected in blood samples collected from lambs fed 12% CP without probiotic and 14% CP with addition of probiotic. In conclusion, only insulin levels were significantly affected by increasing CP levels, but interaction with addition of probiotic affected both insulin and TSH levels.**

Keywords: Catabolism, anabolism, Ruminants,supplements

I. INTRODUCTION

Probiotic are groups of microorganisms including yeasts or bacteria. It has multiple benefits; it helps growth of beneficial bacteria in the gut and increases the body immunity [1]. As well as it has good effects on hormones secretion, but [2] found that using high levels of it decreases thyroid stimulating hormone.

The pancreas has two types of tissues, one of these is an endocrine and other is an exocrine. The exocrine gland is called acini which secrets pancreatic digestive enzymes to bring about digestion and absorption the food. The other is endocrine gland, it's called the islets of Langerhans which secrete insulin, glucagon and somatostatin hormones involved in metabolism. The islets are made up of alpha (α) cells that secrete glucagon, beta (β) cells that secrete insulin (60% of all cells of the islets) and delta (δ) cells that secrete somatostatin [3]. Rough endoplasmic reticulum of β cells synthesizes insulin [4]. As glucose concentration is high, beta cells secrete insulin into bloodstream to support glycolysis to reduce glucose levels by removing the glucose from bloodstream to body cells [5]. Insulin binds target cell receptors to cause signal transduction which in turn stimulates the cell to link glucose transport proteins. This process leads to lower blood glucose level which inhibits release insulin through a negative feedback mechanism [6,7]. Also, insulin promotes protein synthesis by increasing integration of amino acids to maintain balanced body proteins [8, 9].

Dopamine stimulates hypothalamus to release thyrotropin releasing hormone (TRH), which signals anterior pituitary tropic cells to release TSH. Thyroid stimulating hormone stimulates the thyroid gland to release thyroxin hormones (T3, T4) into the bloodstream tissues use. Thyroid hormones biosynthesis is dependent upon the dietary iodine that is absorbed by the intestine after reduction to iodide.

Cells need theses hormones because possessing of myriad of physiological effects. It is needed for burning the nutrients (fuel), differentiation and development, oxygen consumption (The main target tissues of action of thyroid hormones with respect to the stimulation of oxygen consumption are skeletal muscle, cardiac muscle, liver, gastrointestinal tissues and kidney) and lipid, carbohydrate and protein metabolism [10].

Aminergic neurons of hypothalamus influence GH secretion. Catecholamine stimulates the hypothalamus to release growth releasing hormone (GRH) which in turn influences somatotroph (anterior pituitary) to release GH. GH has many activities; it brings out increase in fatty acids by increasing lipolysis, it causes breaking down of vascular polysaccharides to glucose and it results in releasing of somatomedins (growth factors) from liver, kidney and muscle. Somatomedins are mitogenic for many types of somatic cells and has a great importance in growth. Interestingly, the human GH receptor is specific for human GH and doesn't bind GH of other species, whereas the GH receptor from lower forms is able to bind GH of more than one species [10].

II. Material and Methods

Experimental diets comprised of concentrate and roughage diets. Concentrate diets were prepared by mixing their ground ingredients (wheat barn, barley, yellow corn, and soybean meal) after performing their chemical analysis. These diets were prepared locally with addition of salt, vitamins and mineral mix and probiotic at specific levels of 0 and 1 kg/ton of feed as recommended by manufacturer. Two concentrate diets were prepared on basis of CP levels of 12 and 14% on dry matter (DM) basis. In each concentrate diets levels of ingredients were estimated to secure the standard ratio of rumen degradable nitrogen (RDN) to metabolizable energy (ME) of about 1.34g RDN/MJ of ME [11]. Roughage was ground wheat straw. Concentrates were offered to lambs at 2.5% of body weight, whereas, straw was offered on *adlbitum* basis. Quantities of concentrate diet offered to each lamb were adjusted weekly according to changes in their body weights. Table 1 show levels of ingredients in concentrate diets. Table 2 shows chemical compositions of these ingredients, concentrate diets, and wheat straw.

Table 1: Components of concentrate diet (%)

Concentrate diets	barley	WB	YC	SBM	Urea	NaCl	V-M
12%CP	58	30.75	8	0.63	0.62	1	1
14% CP	37	35	23	2.26	0.74	1	1

WB, Wheat bran; YC, Yellow corn; SBM, Soybean meal; V-M, Vitamin-minerals mix

Table 2: Chemical composition of concentrate ingredients, concentrate diets and wheat straw (%)

Diets and ingredients	DM (%)	% of DM					
		Ash	OM	CP	EE	CF	NFE
Barley	91.78	5.65	94.35	10.16	1.99	6.71	75.49
Wheat bran	91.75	5.48	94.52	14.27	3.77	13.96	62.52
Yellow corn	91.18	2.22	97.78	9.27	3.51	4.2	80.80
Soybean meal	91.93	7.87	92.03	45.48	1.83	5.37	39.35
Urea	-	-	-	287.5*	-	-	-
Concentrate 1	88.11	5.69	94.31	12.53	3.39	4	74.39
Concentrate 2	88.44	5.79	94.21	14.51	3.13	4.14	72.43
Wheat straw	88.07	7.09	92.91	3.22	1.86	37.69	51.14

DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; CF, Crude fiber; NFE, Nitrogen free extract; * 46× 6.25

Experimental animals

Sixteen Awassi sheep were used in this study. Animals were bought from local market with average body weight18.76±2.35kg and 4-6 months of age. Sheep were transferred to place of the study and viewed to veterinary tests to ensure its safety and presence of disease. Sheep were injected with Ivermectin against external parasites (3ml/sheep). Sheep were also drenched with Vinazole against intestine and liver worms (35 ml/100kg BW). Gentamicin Fort was used (5ml/sheep) to treat diarrhea single case. Sodium carbonate was used in limited cases to overcome nutritional problems associated with high consumption of concentrate in some. Sheep were weighed using digital balance and randomly allotted into 4 treatment with 4 sheep per each. Sheep were housed in individual pens provided with separated containers for concentrate diets, wheat straw and clean water. Sheep were gradually adapted to consume concentrate diets before the start of the study. Preliminary period was ranged 10-20 days. Concentrate diets were offered twice a day, morning meal at 8 AM and evening meal at 4 pm.

Sampling and measurements

Samples of blood were collected at the end of the experiment from each animal. Blood withdrawn from the jugular vein by needle with syringe (5 ml) and emptied it in plain tubes and centrifuged (5000 r/m. for 5 minute) to collect the serum. Serum was collected from the precipitated blood by micropipette and transferred into eppendorf tubes which were stores frozen at -20◦C until hormonal determinations. After 3 days of serum collection, the hormonal determinations have been performed using Snibe Maglumi 1000 apparatus and Snibe diagnostic kits [Maglumi (CLIA) Shenzhen new industries biomedical engineering CO., Ltd.].

Statistical analysis

The data obtained were statistically analyzed according to factorial experiments (2 × 2) in completely randomized design (CRD) to evaluate the effect of the main factors studied in the experiment and interaction between them. Statistics analysis was performed with SAS [12].

III. Results

Effect of dietary protein levels and addition of probiotic on serum hormone concentrations in Awassi lambs was shown in table 3. Results revealed that increasing CP level from 12 to 14% significantly decreased (P<0.01) serum concentration of insulin from 10.52 to 7.29. Serum concentrations of TSH and GH were not affected by level of CP. Serum concentrations of TSH and GH were not affected by addition of probiotic though slight decrease were detected.

Table 3. Effect of dietary protein levels and addition of probiotic on serum hormone concentrations in Awassi lambs (mean ± SE)

Hormones	Level of CP (%)		Addition of probiotic, kg/tone	
	12	14	0	1
Insulin	10.52^{a} ± 0.54	7.29^{b} ± 0.50^{b}	9.38 ± 0.74	8.43 ± 0.83
TSH	0.217± 0.027	0.231 ± 0.020	0.231 ± 0.022	0.217 ± 0.025
GH	0.706± 0.061	0.663 ± 0.03	0.700 ± 0.055	0.670 ± 0.045

Means with different letter are significantly differed at (P<0.01)

Regarding effect of interaction between level of dietary CP and addition of probiotic (table 4), results showed that higher (P<0.05) serum concentration of insulin was detected in blood samples collected from lambs fed concentrate diet of 12% CP without addition of probiotic. Addition of probiotic decreased (P<0.05) serum concentrations of insulin at low level of CP. higher (P<0.05) serum concentration of TSH were detected in blood samples collected from lambs fed concentrate diet of 12% CP without addition of probiotic and those fed concentrate diet of 14% CP with addition of probiotic. Serum concentrations of TSH were reversely responded to addition of probiotic as interacted with level of CP.

Table 4- Effect of interaction between level of dietary protein and addition of probiotic on serum hormone concentrations in Awassi lambs (mean ± SE)

Level of CP	12%		14%	
Probiotic	0	1 kg/ton	0	1 kg/ton
Insulin	11.043^{a} ±0.397*	10.013^{b}±1.033^{b}	7.723^{bc} ± 0.751	6.88^{c} ± 0.719
TSH	0.282^{a}± 0.020**	0.152^{b} ± 0.015	0.180^{b} ± 0.012	0.282^{a} ± 0.002
GH	0.785± 0.093	0.627 ± 0.069	0.615 ± 0.033	0.712 ± 0.059

Means with different letter are significantly differed at *(P<0.05) or ** (P<0.01)

IV. DISCUSSION

Metabolism is controlled by Insulin, thyroid hormones, GH (somatotropin) and other hormones [13]. GH, Insulin, T3 and T4 play important role in inhibiting proteolysis [14]. The process in which amino acids like alanine and glutamine are converted to glucose in liver, brain, testes and in the erythrocytes is called gluconeogenesis [15]. Insulin mechanism on carbohydrates is by secreting pancreatic beta cells the insulin into blood stream to increase glycolysis and lower glucose by removing it of glucose to body cells [5]. An Insulin secretion is increased by increasing ingested proteins leads to enhancing amino acid uptake by cells [15].

Thyroid hormones have multiple physiological effects. It modulates oxygen consumption, basal metabolic rate and lipid, carbohydrate and protein metabolism [10]. Thyroid hormones and GH increase the gluconeogenesis and protein synthesis but decreases lipogenesis [16]. The current research has shown

inconsistent results with what stated in the literature. However, [17] found that hormones have a key role in normal body functions and neural activity may prevent these normal functions. Moreover, secretion of glucagon and insulin can be influenced not just by blood glucose, amino acids and adrenal hormones but by neural activity [18].

In addition, the thyroid hormones secretion is regulated by complex system that involved hypothalamus-pituitary-thyroid gland feedback control loop and influenced by neural activity [10]. The main components of the neural control are hypothalamic (corticotropin-releasing hormone CRH) – pituitary – adrenal (norepinephrine) axis where CRH plays an important role in changing GH, TRH and TSH secretion, affecting growth and thyroid functions [19].

V. CONCLUSION AND RECOMMENDATION

In conclusion, there are no effect of levels of dietary protein and addition of probiotic on growth hormone concentration, while the current study shows the swinging of insulin and TSH concentrations. Living being hormones aren't only affected by diet and ingredients levels but also affected by neural effects which include all the circumstances encompassing the animals such as temperature, humidity, ventilation and others. Therefore, study the effect of conditions surrounding the Iraqi animals on hormonal, enzymatic and other parameters changes is recommended.

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