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Influence of bee venom on productive performance and immune status in male goats

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ABSTRACT

The objective of the present work was to evaluate the effect of bee venom (BV) on productive performance and immune response of Zaraibi male goats. Fifteen males aged (52 weeks) with average body weight (BW) of 17.87 kg were divided into three groups (5 goats / each). The first treatment (T1) was control group fed on basal diet and injected with 1 m1 of distilled water /male. The second (T2) and third (T3) treatment groups were fed on control diet and injected with 1 ml of 250 or 500 mg/L of BV /male, respectively. The three treated groups were injected intramuscularly two times / week for 4 weeks. The collected data at 2 (W2) and 4 (W4) weeks post BV injection showed that (T2) and (T3) groups had higher body weight gain (BWG), performance index (PI), production efficiency factor (PEF), lower feed intake (FI) and better feed conversion ratio (FCR) than (T1) group. The (T2) group displays a prominent and higher levels in total protein (TP) and globulin (Glob) than (T1) and (T3) groups at W2 and W4 post injection. Total antioxidant capacity (TAC) and immunoglobulin G (IgG) concentration of (T2) group were significantly more than those of (T1) and (T3) groups at W2 . Lysozyme concentration was significantly higher in (T2) group at W4 than (T1) and (T3) groups. The study concluded that injection of Zaraibi male goats with BV especially 250 mg/L can ameliorate productive performance, and immune response which reverberates positively on economic efficiency.

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INTRODUCTION

There is a constant focus on productive performance of small ruminants and its relation with feeding status consistent with nutritional gap under Egyptian conditions. Hence, Advices of using herbal plants and honey bee products were given to form a healthy product without chemical residues that endanger for human health. **Rabie et al. (2018)** suggested that animal diets can be supplemented with BV derived material to improve all performances and provide health advantages. BV is known as apitoxin and can be regarded as one of the most efficient natural supplements because of its distinctive structure and plenty of advantageous enzymes and peptides **(Kim et al. 2019)** . Chemical analysis describes that BV has pharmacologically active mixture of peptides like (melittin, apamin, adolapin) ,enzymes like (phospholipase A2 , hyaluronidase), amino acids and volatile compounds (**Elkomy et al. 2021)**. It also has pheromones and minerals such as Ca and Mg (**Wehbe et al. 2019).** Supplementation of BV in drinking water as an alternative to antimicrobial growth promoter is reported by **Han et al. (2010)**. Also, intramuscularly injection with BV results in best body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) in chicken (**Ali and Mohanny 2014)** and in rabbits **(Elkomy et al. 2023)**. An improvement in the immune status of broilers administrated with BV is recorded by **Abeer et al. (2022). Da-hye Kim et al. (2019)** mentioned an increase in antioxidant capacity and fatty acid metabolism in poultry supplemented with BV. Also, (**Abd El-Aziz et al. 2024)** concluded that BV supplementation to rabbits in drinking water could enhance final weights, bolster antioxidant status and mitigate the presence of pathogenic bacteria.

Therefore, this experimental work was designed to evaluate the effect of intramuscularly injection of BV on the productive performance and some immune responses of Zaraibi male goats.

MATERIALS AND METHODS

All experimental animals were belonged to EL-Serw Research Station, Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt.

Collection of BV :

Samples of bee venom (BV) were collected from the Craniolian hybrid (*Apis mellifera*) bees during summer season of 2023. Electric gathering of BV was done according to **Benton et al. (1963)** and purification was performed according to **Metwally (2016).** BV was collected, dried at 27 °C for 30 minutes and stored frozen until injection time.

Animals and experimental design

Fifteen healthy Zaraibi male goats aged 52 weeks old with an initial average body weight 17.87± 0.38 kg were randomly assigned into three equivalent treatment groups (5 males / group). The 1st treatment (T1) group was acted as a control and received a basal diet plus I/M injection with 1m1 of distilled water /male. The 2nd treatment (T2) group was received the pervious basal diet plus I/M injection with 1ml of 250 mg/L of BV /male. However, the 3rd treatment (T3) group was received the pervious basal diet plus I/M injection with 1ml of 500 mg /L of BV/male. Injection process was carried out two times / week up to four weeks. Data and serum samples were collected after 2 and 4 weeks (W2 and W4) of BV injection. Goats of all treatment groups were housed separately / group in an open shaded barn under similar natural environmental condition and the ceiling of barn covered with asbestos. Fresh water and mineral mixture blocks were freely available throughout the trial period. The experimental diet covered the male goats' nutritional requirements according to **NRC (2007)**. The chemical analysis of the experimental diet was analysed according to **AOAC (2007)** as shown in Table (1).

Table 1. Chemical analysis and ingredients feeding values of basal experimental diet (on DM basis).

Chemical composition (%)	Basal experimental diets		
	*CFM	**BH	***RS
Organic matter (OM)	87.60	84.03	84.95
Crude protein (CP)	14.42	14.90	3.81
Ether extract (EE)	2.44	1.03	1.62
Crude fiber (CF)	7.33	30.83	39.91
Nitrogen free extract (NFE)	63.41	37.27	39.61
Ash	12.40	15.97	15.05
**** Ingredients feeding values			
Total digestible nutrients (TDN)	62.76	65.25	54.49
Digestible crude protein (DCP)	10.29	10.75	0.11
Digestible energy (DE) M cal/kg DM	2.77	2.88	2.40
Metabolizable energy (ME) M cal/kg DM	2.35	2.46	1.97
Net energy (NE) M cal/kg DM	1.42	1.47	1.23

* concentration feed mixture (CFM) = it consists of 26 % undecortecated cotton meal, 40 % yellow corn, 27 % wheat bran, 3.5 % molasses, 2 % limestone, 1 % common salt and 0.5 % minerals mixture.
** BH= berseem hay. ***RS= rice straws.
** ** Ingredients feeding values as total digestible nutrients (TDN) = 129.39 - 0.9419 (CF+ NFE), digestible crude protein (DCP) = 0.9596 (CP) - 3.55, digestible energy (DE) M cal/kg DM = 0.04409 (TDN %), metabolizable energy (ME) =1.01(DE) - 0.45 and net energy (NE) = 0.0245 (TDN %) - 0.12 was calculated according to NRC (2007).

Estimation of Productive performance:

Body weight (BW)

It was recorded in the morning before offered feedstuffs by digital scale.

Body weight gain (BWG)

It was calculated as following equation:

$$BWG/kg = \frac{\text{Final BW (kg)} \times 1000 - \text{initial BW (kg)} \times 1000}{\text{Duration W2 or W4 of experimental period}}$$

Feed intake (FI)

It was estimated for CFM at W2 and W4 for each male /group by served a weighed specific quantity of feed. This is mathematically expressed as:

Feed intake (g) = Specific quantity of feed offered (g) – Leftover of feed offered (g).

Feed conversion ratio (FCR)

It was calculated as following equation:

$$FCR = \frac{\text{Feed intake at W2 or W4}}{\text{The average body weight gain.}}$$

Performance index (PI)

It was calculated as following:

$$PI = \frac{\text{Final body weight (kg) at W2 or W4} \times 100}{\text{Feed conversion ratio at W2 or W4}}$$

Production efficiency factor (PEF):

It was calculated during W2 or W4 according as following equations:

$$PEF = \frac{\text{Livability} \times \text{Mass (Kg)} \times 100}{\text{FCR} \times \text{Age study (days)}}$$

Livability = 100 – Mortality rate (%). The mortality % in this study reached to zero then the livability in this study = 100 - 0.
Mass (Kg) = Final body weight at W2 or W4.
FCR = Feed conversion ratio at W2 or W4.
Age in this study= at W2 (54weeks) or W4 (56weeks).

Blood sampling:

Blood samples were collected from the jugular vein for serum separation from all treatment groups (T1, T2 and T3) after 2 and 4 weeks of BV injection. Serum samples were stored at –20ºC to be used in measuring protein profile (Total protein, albumin and globulin levels), estimating Total antioxidant capacity, measuring Immunoglobulin (IgG) concen-

tration and lysozyme activity assay.

Immunological parameters

Total protein, albumin and globulin assay:

The total protein and albumin content were estimated in serum samples using colorimetric method, kits were obtained from Spectrum. CAT. No. 310 001 and 211 001. The globulin content was evaluated by subtracting albumin from total protein.

Total antioxidant capacity (TAC) assay:

It was estimated by using colorimetric method. Kits were obtained from Biodiagnostic .CAT. No. TA 2513.

Immunoglobulin, G (IgG) assay:

It was done by Using Redial Immunodiffusion Binding site kits Ref (RN200.3) and Lot (338428).

Lysozyme activity assay:

It was determined as described by **Schultz (1987)**. The lysoplates were prepared by dissolving 1% agarose in 0.06 M PBS (pH 6.3) with 500 mg/L Micrococcus lysodeikticus. The concentrations of lysozyme were obtained from the logarithmic curve of standard lysozyme.

Economic efficiency:

The economic efficiency (EE) of the three treatment groups was calculated at the completion of trial (W4= 56 weeks of age) according to the normal costs of the experimental diets and male goats' live body weight throughout year of 2024.

$$EE = \frac{\text{price of marketing}}{\text{Total price of feed cost}}$$

Price of marketing (LE) = final weight × selling price of kg male goats.

Total price of feed cost (LE) = feed consumption (Kg/ head) × price of one Kg of feed.

Economic efficiency relative to control

It was calculated in the three treatment groups through experimental period (30days) as the following equation:

EE (%) relative to control with T2 or T3= EE of T2 or T3 – EE of T1÷ EE of T1×100 +100 (consider EE of T1 is 100%).

Statistical Analysis

The values of measured and calculated parameters were expressed as the mean values ± standard error "SE". Statistical analysis was carried out using analysis of variance (ANOVA) by using the SPSS (**SPSS Statistics version 2020**). The different letters (capital or small) mean a significant difference at $P< 0.05$.

RESULTS

The influence of BV injection on Body weight developing is displayed in Table (2). The results showed no significant differences in BW among the three treatment groups throughout the trial period. However, there was a numerical increase in BW at W4 in (T2)and (T3) groups compared with (T1) group. Male goats in (T3) group had superiority in BW among all trial groups.

Table 2. Effect of BV injection on body weight (BW) /kg.

Trial weeks	Treatment groups		
	T1	T2	T3
*W2	18.12±0.62	18.54±0.65	18.57±0.83
**W4	18.52±0.63	19.18±0.68	19.20±0.74

Means within the same column and rows without alphabet letters are non-significantly different (P<0.05).
*W2= 54weeks old. **W4= 56 weeks old.

Data of body weight gain Table (3) revealed a significant increase in BWG in (T1) group at W4 compared with W2. Those of (T2) & (T3) groups exhibited a significant increase in BWG in contrast with (T1) group throughout the experimental period (W2 & W4). A numerical increase in BWG was obvious in (T3) group compared with (T2) group all over the trial period.

Table 3. Effect of BV injection on body weight gain (BWG) /g

Trial weeks	Treatment groups		
	T1	T2	T3
*W2	19.99±5.16^{bB}	39.40±3.65^{a}	42.67±3.49^{a}
**W4	26.67±2.98^{bA}	42.67±4.00^{a}	45.33±2.94^{a}

Means within the same row bearing a, b letters and within the same column bearing A, B letters are significantly different (P<0.05).
*W2=54weeks old. **W4=56weeks old.

Concerning to the amounts of feed intake that are listed in Table (4). Data clarified the positive effect of BV injection by lowering FI in (T2) and (T3) groups comparing with (T1) group at W2 and W4.

Table 4. Effect of BV injection on feed intake (FI) /g.

Trial weeks	Treatment groups		
	T1	T2	T3
*W2	575.20±4.73^{bB}	548.40±2.51^{aB}	544.80±2.08^{aB}
**W4	696.20±9.31^{bA}	634.40±13.21^{aA}	632.00±14.34^{aA}

Means within the same row bearing a, b letters and within the same column bearing A, B letters are significantly different (P<0.05).
*W2=54weeks old. **W4=56 weeks old.

Data of Feed conversion ratio Table (5) displayed a prominent significant decrease in the values of FCR in (T2) and (T3) groups in comparing with (T1) group at W2 and W4. Values of FCR were significantly lower at W4 in all treated groups than those at W2 .

Table 5. Effect of BV injection on feed conversion ratio (FCR) %

Trial weeks	Treatment groups		
	T1	T2	T3
*W2	43.12 ±11.91^{aA}	41.11±10.55^{bA}	40.87±12.08^{bA}
**W4	26.10±9.81^{bB}	23.79±8.25^{aB}	23.70±11.64^{aB}

Means within the same row bearing a, b letters and within the same column bearing A, B letters are significantly different (P<0.05).
*W2=54weeks old. **W4= 56 weeks old.

Owing to the results of Performance index and production efficiency factor table (6), it could be summarized that BV injection at any studied doses resulting in increasing values of PI and PEF during the trail periods. The weekly age could improve positively in either PI (109.36% T2 and 125.05% T3 at W2) or (13.65% T2 and 14.50% T3 at W4) or PEF (7.39% T2 and 7.89% T3 at W2) or (13.66% T2 and 14.50% T3 at W4).

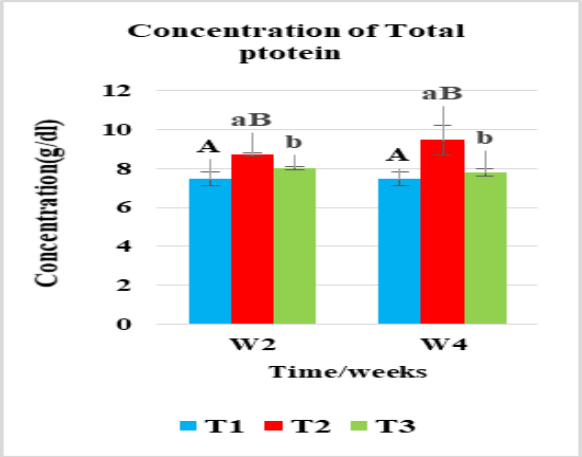
Table 6. Effect of BV injection on performance index (PI) % and production efficiency factor (PEF) %

Items	Trial weeks	Treatment groups		
		T1	T2	T3
Performance index (PI),%	*W2	46.39±11.93^{bB}	97.12±8.49^{aA}	104.40±8.34^{aA}
	**W4	71.04±2.63^{bA}	80.74±3.06^{aB}	81.34±4.50^{aB}
Production efficiency factor (PEF)	*W2	224.07±59.96^{bB}	240.63±89.52^{aB}	241.75±97.41^{aB}
	**W4	393.54±145.57^{bA}	447.31±169.88^{aA}	450.62±249.39^{aA}

Means within the same row bearing a, b letters and within the same column bearing A, B letters are significantly different (P<0.05).
*W2=54weeks old. **W4=56 weeks old.

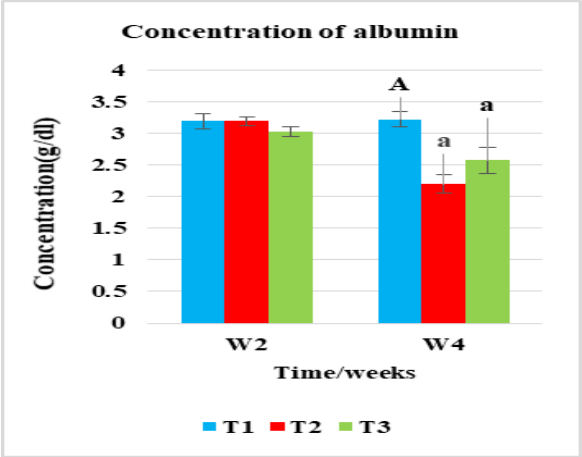
Data of serum total protein (TP), albumin (Alb.), globulin (Glob.) and albumin/globulin (A/G) ratio figures (1) ,(2) ,(3) & (4) revealed a prominent and significant increase in total proteins accompanied by a significant increase in globulins levels in (T2) group comparing with those of (T1) and (T3) groups at both times (W2 and W4). T3 group illustrated no effect on total protein and globulin all over the period of experiment compared with (T1) group.

Fig 1. Effect of BV injection on total protein concentration.



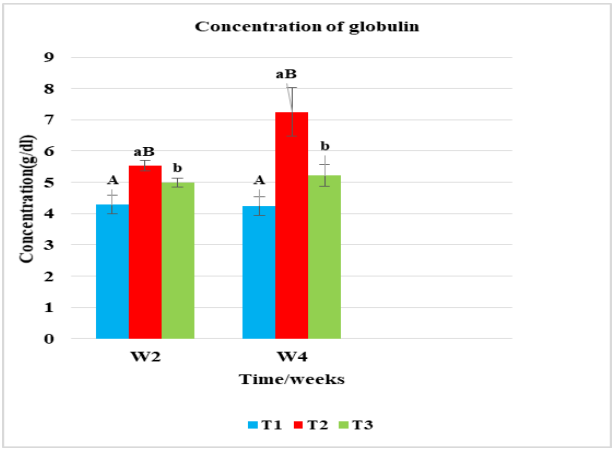
Results expressed as mean ± SE
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM

Fig 2. Effect of BV injection on albumin concentration.



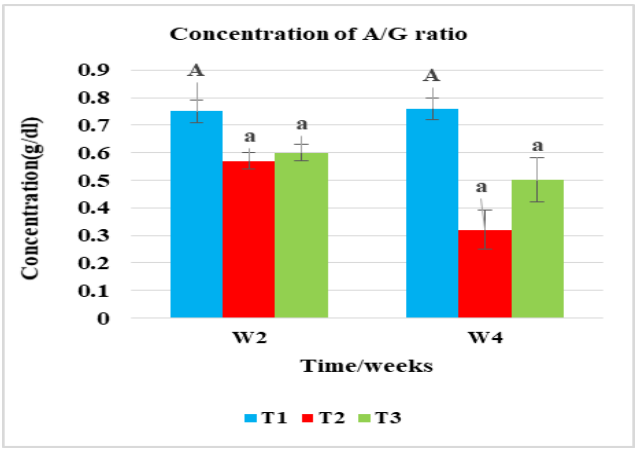
Results expressed as mean ± SE
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM

Fig 3. Effect of BV injection on globulin concentration.



Results expressed as mean ± SE
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM

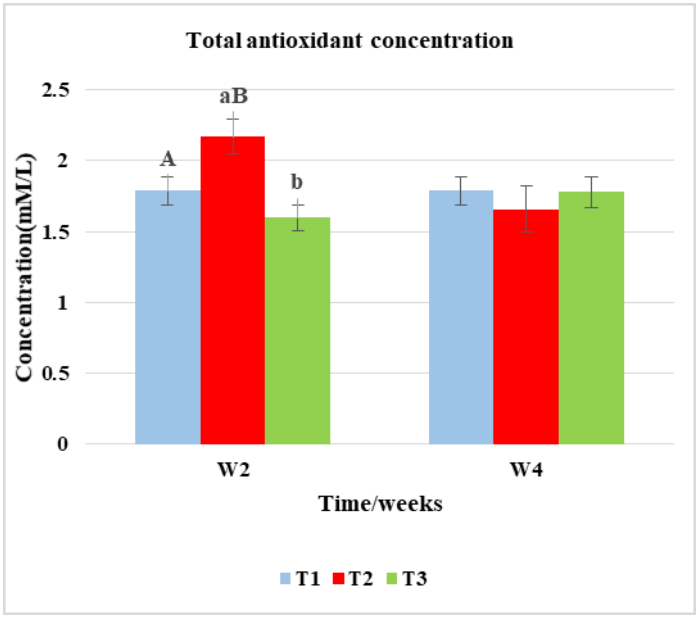
Fig. (4): Effect of BV injection on A/G ratio.



Results expressed as mean ± SE
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM

Regarding to total antioxidant capacity (TAC) fig. (5). It was clear that (T2) group revealed a significant increase in TAC compared with (T1 and T3) groups at W2. Whereas, BV injection has no significance among the three treatment groups during W4 period

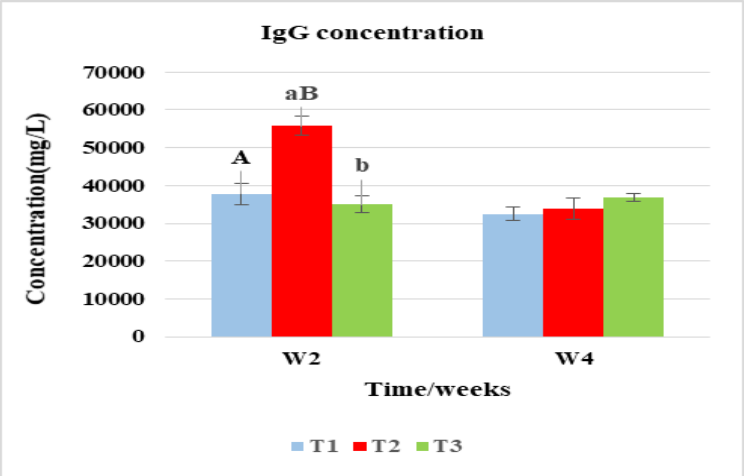
Fig 5. Effect of BV injection on total antioxidant capacity



Results expressed as mean ± SE.
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM.

Data of Immunoglobulin,G (IgG) fig. (6) illustrated that (T2) group resulted in a remarkable highly significant level in IgG concentration compared with (T3) and (T1) groups at W2. Whereas (T3) group has no effect on (IgG) concentration at W2. BV had no effect on (IgG) concentration at W4 in all treatment groups.

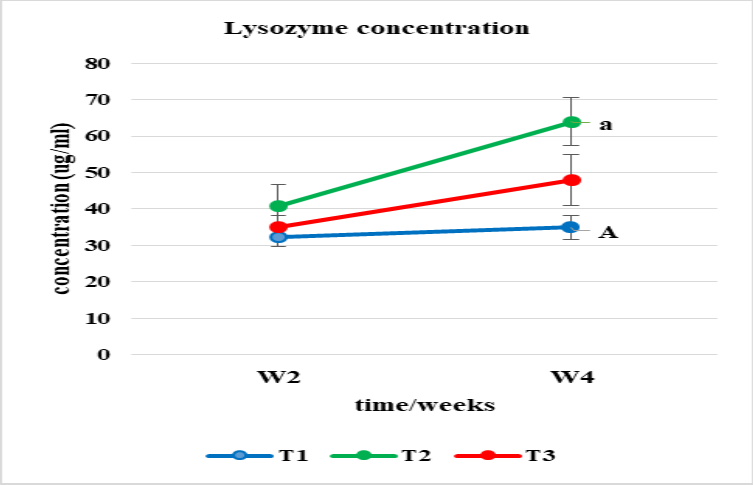
Fig 6. Effect of BV injection on Immunoglobulin,G (IgG) concentration



Results expressed as mean ± SE.
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM

From fig. (7) It could be summarized that treated groups (T2 or T3) expressed a numerical increase in lysozyme concentration throughout the experiment at all times (W2 and W4) compared with (T1) group. A significant increase in lysozyme concentration was recorded in (T2) group at W4 in comparison with (T1) group.

Fig 7. Effect of BV injection on lysozyme activity



Results expressed as mean ± SE.
Small and capital letters of the same letter indicate significant different between treatment groups at (P<0.05).
Error bars represent the SEM.

The influence of BV injection on economic efficiency (EE) was shown in Table (7). The highest values of EE and EE (%) relative to control were observed in (T2) group compared with (T3) group and (T1) group. Low cost of BV (6 LE / goat) in (T2) group resulted into much better values of EE compared with (T3) group that costs (12 LE / goat) of BV throughout the experimental period .The price of marketing was higher in (T2) group and (T3) group in comparing with (T1) group (4795, 4800 and 4630 LE respectively).

Table 7. Economic efficiency calculation of bee venom injection at end of experimental periods.

Feed consumptions / 30 days/male	Experimental groups		
	T1	T2	T3
Average daily of total feed intake (ADTFI), g	696.20	634.40	632.00
*Total of feed intake during experimental period, kg [A]	20.89	19.03	18.96
Total injection option of BV during 8 times, mg	-	2000	4000
Cost of feed intake= (A × **price of kg), LE	344.93	323.51	322.32
***Cost of BV, LE	-	6.00	12.00
Total price of feed consumed , LE[B]	344.93	329.51	334.32
Final body weight at end of experimental period, kg [C]	18.52	19.18	19.20
****Price of marketing= (final weight × sole of male goats kg), LE[D]	4630.00	4795.00	4800.00
Economic efficiency			
Feed efficiency [C/B]	0.05	0.06	0.06
Feeding cost of producing male goats [B/C]	18.62	17.18	17.41
Economic efficiency (EE) amount, [D/B]	13.42	14.55	14.36
EE (%) relative to control	100.00	108.42	107.00

* Total of feed intake during experimental period =ADTFI× 30 days.
** Price in year 2024 for CFM was 17000 EL / ton
***Cost of one BV option=0.75 LE / ml.
****Price of sale kg of live body weight of male goat is 250 (LE).

DISCUSSION

There is a lack of tangible information noting the effect of BV injection on the BW of male goats. Results of Table (2) are in harmony with that of **Han et al. (2013)** in guinea pigs and rats, and of **Ali and Mohanny (2014)** in broiler chickens. The numerical improvement in BW of (T2) group and (T3) group may be attributed to the presence of proteins, peptides, enzymes and other substances like amino acids, catecholamines, sugars and minerals in BV **(Teoh et al. 2017)**. The role of BV in enhancing growth performance results from its immunological, antioxidative responses and its ability to reduce the growth of pathogenic bacteria **(Elkomy et al. 2023)**. The positive impact of BV on BWG Table (3) is in agreement with **Han et al. (2010) (**in broiler chickens and with **Adel et al. (2022)** in rabbits. BV plays a vital role in carbohydrate metabolism by increasing insulin hormone secretion which leads to improving growth rate (**Abd El-Aziz et al. 2024)**. Also, melittin from BV does not cause harm to the immune system which reverberates on body weight gain.

The lowering levels of Feed intake in BV treated groups (T2 & T3) Table (4) are matched with **Kim et al. (2018)**. Also, **Elkomy et al. (2023)** reported that FI was less up to 5522, 5338 and 5290 g in rabbits received BV in drinking water against 5552 g in control.

Decreasing Feed conversion ratio Table (5) is in harmony with **Adel et al. (2022) and Ali and Mohanny (2014)** .They also declare that FCR could be reduced by advancing in age. The boosting ability of BV in converting feed to meat through improvement in FCR , increas-

ing BWG and decreasing FI is recorded by **Elkomy et al. (2023)**.

The improving in performance index (PI) and production efficiency factor (PEF) (table 6) is orchestrated with many authors **(Ali and Mohanny, 2014, Adel et al. 2022, Elkomy et al. 2023 and Abd El-Aziz et al. 2024)** . They revealed that BV has a positive influence on animal health and blood parameters which refluxed on all productive performance.

The increasing of total protein and globulin levels (fig.1&3) is well-matched with those of **El-Hanoun et al. (2020)** where they recorded an increasing levels of total protein and globulin in rabbits treated with BV. Such increase may be attributed to the activation of amino acid in BV **(Mohammed and Hassan, 2019)**, the pheromones **(Wehbe et al. 2019)** and the active peptide content of BV that stimulate the immune system **(Elkomy et al. 2021)**. High levels of globulins may play a vital role in improving health status and BWG of male goats.

TAC has a central role in maintaining the intracellular redox balance and removing free radicals **(Gajski et al. 2024)**. Low dose of BV in (T2) group could enhance the values of TAC more than (T3) group (fig. 5). These results settled with that of **Elkomy et al. (2021)**. Increasing TAC concentration may be endorsed to immune system activation **(Adel et al. 2022)**. Likewise, **Bava et al. (2023)** recoded that the significant positive effect of BV on TAC is related to the presence of antioxidant substances include apamin, melittin and phospholipase A2. The improvement of TAC reflects the antioxidant role of BV against oxidative damage, resulting in diminishing in the concentration of lipid peroxidation markers such as malondialdehyde (MDA) **(Kim et al. 2019)**.

The results of (IgG) concentration (fig. 6) is compatible with **Elkomy et al. (2023)** where they stated that low dose of BV is talented in powering the immunoglobulin production , while high dose has a reversible effect. **Lischer et al. (2021)** suggested that the activation of (IgG) may be attributed to melittin which has the ability to bind the cell membrane giving rise to immunogenicity for IgG. As BV components are presented on the surface of antigen presenting cells to initiate the action of Th2 , which in turn activated and produce IL4 and IL13, that subsequently directs B cells to produce IgG antibodies **(Appel 2009). Carpena et al. (2020)** also noticed that melittin of BV stimulate IL1 production leading to production of IgG.

Lysozyme is a protein plays a vital role in the innate immune response, giving protection against bacteria, virus and fungi. It exists in tissues of animals and plants and in many secretions such as tears and saliva **(Ferraboschi et al. 2021)**. The increasing level of lysozyme concentration (fig. 7) may be attributed to the presence of Melittin and phospholipase A2 (PLA2) in bee venom. These components have positive effect on antimicrobial and anti-inflammatory features **Sameh et al.(2023)** and **Shafiga and Elmar (2017)** . Also **Han et al. (2010)** stated that the unique structure of bee venom, is capable of performing multi-biological functions in the animal body, as antimicrobial agent.

Results of economic efficiency (table 7) are in harmony with those of **Elkomy et al. (2021) and Adel et al. (2022)** who reported high values of EE in rabbits that received BV. Also, **(Abd El-Aziz et al. 2024)** revealed that both pharmacologically active substances and antioxidant in BV can provide oxidative stability status which reflected on growth performance of animals. Actually, improving of performance index (PI) (table 6) in either (T2) group or (T3) group compared with (T1) group reflected on economic efficiency reality.

CONCLUSION

This study proved that injection of male goats with BV has a beneficial effect on the productive performance and immune response. Using of 250 mg/L of BV for 2 weeks is adequate to improve the health of animal immune status. Hence, using less dose of BV induced positive effect on economic efficiency. It could be concluded that BV can be used as a natural growth promoter in small ruminants to augment growth performance traits, immunological and anti-oxidative responses.

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