

A Comparative Study on the Effects of Enzymes Feed Additive “Kemzyme” and Antibiotic Growth Promoter "Salinomycin" on Haemostatic Mechanism of Rabbit Bucks

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Abstract

The present study was designed to investigate the effects of enzymes (Kemzyme) or antibiotic (Salinomycin) feed additives on haemostatic mechanism and some haemostatic parameters in rabbit bucks. Twenty four rabbit bucks of average age 4 months were used and allocated into 3 equal groups (8/ each); control un-supplemented, Kemzyme and Salinomycin supplemented groups. The supplements were mixed with the ration for 8 successive weeks. Blood samples were collected at the end of the 4th and the 8th weeks of the experiment. Results obtained revealed that: 1) Kemzyme supplementation was not associated with any significant alterations in the measured parameters and 2) at the end of the 8th week, salinomycin adversely affected the haemostatic mechanism in the form of hypocoagulable state manifested by a significant increase in PT and APTT with a significant decrease in coagulation factors VII, IX and X activities. It could be concluded that kemzyme is a more safer feed additive. While, salinomycin should not be used for more than 4 weeks to avoid its adverse effect on haemostasis that could be a cause of sudden death resulting in economic losses or rendering the rabbit meat unmarketable due to inter and intramuscular patches of bleeding.

Keywords: feed additive, haemostasis, kemzyme, rabbits, salinomycin

1. Introduction

One of the major developmental challenges facing most developing countries is their inability to adequately feed their over increasing population with the right proportion of animal protein. The development of poultry industry has been described as the fastest mean of bridging the protein deficiency problem prevailing in most developing countries.

In recent years many efforts are directed for improving poultry industry by increasing of animal performance ; using growth promoters and feed additives as well as successful disease control besides increasing attention on improving the nutritional quality of poultry meat (Bogosavljević-Bošković et al., 2012).

Since long time ago, antibiotics have been used, either alone or in combination, as growth promoters. The addition of antibiotics in poultry feed was found to improve their performance, feed conversion and daily body gain with reduced mortality rate. However, concerns were raised that the use of antibiotics for growth promotion could lead to a problem of increasing resistance for bacteria of human and animal origin (Butaye et al., 2003).

Enzymes have been used as feed supplements for more than 50 years, but the rapid growth in their use has only been within the last 10 years (Tuoyin, 2005). Enzymes are considered as an alternative to some growth promoters as hormones and antibiotics with higher profits and quality of poultry products (Officer, 2000; Cowieson, 2005), that help poultry producers to adapt the forth coming European ban on antibiotic growth promoters as they can replace them and meet the increasing consumer demand for safe and affordable food. Scientists have demonstrated the value of feed enzymes in improving nutrient digestibility and bird's performance. They attributed their effects to the fact that many feed ingredients are not fully digested by live stock (Hruby, 2005). However, adding enzymes to feed enhanced digestibility of these components, improve the efficiency of existing

formulation and lower their costs (Ghazi et al., 2002; Peter et al., 2002; Onderci et al., 2006; Cowieson & Ravindran, 2008).

Rabbits have been recognized to have a very important role to play in the supply of animal protein to humans, especially in tropical and subtropical areas. Simple biological characteristics, short breeding cycle, high prolificacy and better feed conversion efficiency logically place rabbit just below poultry (Hasanat et al, 2006). The possibility of using exogenous enzymes in non-ruminant diets, reduces environmental pollution and lower feed costs (Zakaria et al., 2010). Several studies have been attempted for incorporating exogenous enzymes into rabbit diets to improve nutrients availability, however in most trials, rabbits appeared lapsed responsive and variable effects were observed on their performances (Garcia et al., 2005; Falcao-e-Cunha et al., 2007). Rabbits are herbivores and can be successfully raised on diets that are low in grains and high in roughage. Many experiments have indicated that, high cellulose diet was favorable for depressing death rate, the mechanism of action is to stimulate ileum, appendix vigor. The poor green grass unbalanced forage based diets offered at *ad-libitum*, are usually provided to rabbits in developing countries was described by Cheeke et al. (1985). Despite these obvious advantages, improved feed formulation and strategies for enhancing the production and reproduction potentials of rabbit especially in tropical and subtropical regions of the world have not been fully exploited.

The present study was aimed to investigate and compare between the effects of antibiotic growth promoters and enzymes feed additive supplementation on haemostatic mechanism and some haemostatic elements of rabbit bucks.

2. Materials and methods

2.1 Animals, and Location:

Twenty four male New Zealand White bucks of about 4-months age and average body weight 2.400 kg were used during June to August/2013. The animals were kept in individual cages, with free rabbit chow and water. Daily lighting regime was 10-12 hour photoperiod /day through both natural and fluorescent lighting. Animals were divided randomly to three groups (8 / each).

First group; was considered as a control group(C) and fed on a commercial pelleted diet (Atmida Feed Company, Egypt).

The second group (K), the animals were supplemented with 0.1% “Kemzyme”, a multi-enzyme blend (Liu, 2001).

The third group (S), the animals were supplemented with Salinomycin powder in a dose equal to 100 mg/kg ration (Chapman et al., 1993).

2.2 Feed Additives:

Kemzyme: a multi-enzyme feed additive containing proteases, α amylase, β glucanas, Proteas, lipase, cellulase and xylanase, produced by "Kemin Agrifood Europe" and obtained as a powder from "Kemin Company, Egypt".

Salinomycin: it is an ionophore antibiotic growth promoter obtained as a powder, containing 4% active drug, from "Hoechst-RousselAgri-Vet. Co. Frankfurt, Germany. Doses of supplemented additives were mixed with the basal ration pellets.

2.3 Feed

All animal were raised on basal pelleted diet consisted of a concentrate mixture adequate in protein, energy, vitamins and minerals to meet the nutrient requirements of rabbit A commercial pelleted diet of 16.7% crude protein, 13.7% crude fiber and 2590 kcal of digestible energy per kg (Atmida Feed Company, Egypt) was used. In addition, animal were offered green darrahwa (green corn) and they had free access to fresh water. The diet subjected to chemical analysis according to AOAC (1999).

2.4 Blood Sampling

Blood samples were collected at the end of the 4th and the 8th weeks of experimental time from the ear vein of all animals at the morning before accesses to feed and water. Citrated plasma (in a ratio of 1 volume sodium citrate (3.8%): 9 volumes blood) was obtained for determination of certain haemostatic parameters.

2.5 Measurements

For monitoring of the haemostatic mechanisms, both extrinsic and intrinsic pathways were investigated through measurement of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) according to Poller (1980) and Munteau et al. (1992), respectively. Fibrinogen concentration and activities of individual coagulation factors VII, IX and X were determined according to Dacie and Lewis (1984) using a commercial reagents

manufactured by "Diamed-Swiss" obtained from "Biogen-Company, Egypt" and coagulation analyzer manufactured by "bio-Mereux company-France". Clotting factors activities were expressed as a % of normal pooled plasma collected from rabbits of different ages and sexes.

2.6 Statistical analysis:

Data for all variables were subjected to ANOVA as a completely randomized design according to Snedecor & Cochran (1982). Means were compared by Least Significant Difference (LSD) test at $P < 0.05$ (Steel & Torrie, 1980).

3. Results

3.1 Effects of feed additives on prothrombin time (PT) and Activated partial thromboplastin time (APTT)

Table 1. Shows that no significant differences were recorded in PT and APTT of both supplemented groups at the end of 4th week of supplementation when compared to control. However, a significant increase in the values of PT and APTT were detected in salinomycin supplemented group vs control and kemzyme supplemented groups at the end of the 8th week of supplementation.

Table 1. Prothrombin time (PT) and activated partial thromboplastin time (APTT) of rabbit bucks which received Kemzyme or Salinomycin feed additives for 8 successive weeks

Parameter	PT (sec.)		APTT (Sec.)	
	4w	8w	4w	8w
Control	14.40±0.51	13.0±0.42 ^a	48.5±2.41	44.4±2.50 ^a
Kemzyme	15.20±0.90	12.01±0.20 ^a	50.0±3.02	45.10±1.37 ^a
Salinomycin	15.00±0.95	17.0±0.30 ^{b,c}	52.0±3.00	57.1±2.20 ^{b,c}
LSD	N.S	2.90	N.S	7.00

Values represent mean ±SE, means within the same column having different letters are significantly different at $P < 0.01$; N.S: non-significant.

3.2 Effects of Feed Additives on Fibrinogen Concentration.

Table 2 demonstrates that no significant change in fibrinogen concentration at the end of the 4th week of the experiment was recorded in all supplemented groups vs control. Meanwhile, at the end of the 8th week, fibrinogen concentration was significantly higher in kemzyme supplemented group vs control and salinomycin supplemented groups.

Table 2. Fibrinogen concentration in rabbit bucks which received Kemzyme or Salinomycin feed additives for 8 successive weeks

Parameter	Fibrinogen (mg %)	
	4w	8w
Control	285.0±10.20	285±9.020 ^a
Kemzyme	292.0±11.50	310±9.30 ^{b,c}
Salinomycin	278.0±9.10	290±8.90 ^a
LSD	N.S	20.00

Values represent mean ±SE, means within the same column having different letters are significantly different at $P < 0.01$; N.S: non-significant.

3.3 Effects of Feed Additives on Individual Coagulation Factors Activities

Table 3 shows the effects of different feed additives on the activities of blood clotting factors VII, IX and X of rabbit bucks during the 4th and the 8th weeks of the experimental period. No significant changes were recorded in the activities of all measured clotting factors at the end of the 4th week of the experiment. While at the end of 8th

week, the activities of clotting factors VII, IX and X were significantly lowered ($P < 0.01$) in bucks received salinomycin as compared to control and kemzyme supplemented groups.

Table 3. Factors VII, IX and X activities in rabbit bucks which received Kemzyme or Salinomycin feed additives for 8 successive weeks

Parameter	Fac. VII (% activity)		Fac. IX (% activity)		Fac. X (% activity)	
	4w	8w	4w	8w	4w	8w
Control	63.6±2.80	66.8±2.40 ^a	55.9±1.90	58.8±2.40 ^a	66.2±1.28	73.5±2.90 ^a
Kemzyme	66.5±2.90	69.7±2.01 ^a	58.0±2.00	58.4±3.04 ^a	70.0±2.00	72.10±3.30 ^a
Salinomycin	69.5±3.01	52.4±2.10 ^{b,c}	54.0±2.02	44.3±2.70 ^{b,c}	68.0±1.80	57.10±3.01 ^{b,c}
LSD	N.S	10.50	N.S	13.00	N.S	12.60

Values represent mean ±SE, means within the same column having different letters are significantly different at $P < 0.01$. N.S: non - significant clotting factors activities were expressed as a % of normal pooled plasma.

4. Discussion

The present study was carried out to investigate the effect of some feed additives (Kemzyme and Salinomycin) on haemostatic mechanism and some haemostatic parameters in bucks.

Results obtained indicated that the haemostatic mechanism of all supplemented bucks was not influenced by the supplementation during the first four weeks of the experiment. Later on, and at the end of the 8th week of the experiment a state of hypocoagulability was detected in bucks supplemented with Salinomycin. Hypocoagulability was manifested by a significant elongation in PT and APTT, decreased activities of coagulation factors, VII, IX and X.

Kemzyme supplementation was not associated with any significant alterations concerning haemostatic mechanisms or coagulation factors activities. Such finding, did not agree with that of Saleh et al. (2008) who found that supplementing ration of growing turkey toms with Kemzyme was associated with an obvious blood hypercoagulability condition that was accompanied by an accelerated PT and APTT together with a significant activation of coagulation factors I, VII, IX and X.

The difference in the obtained results could be attributed to breed difference and the differences in gut environments between turkey and rabbits. Meanwhile, the significant increase that recorded in PT and APTT in Salinomycin supplemented group in the present study might be a reflection of decreased clotting factors activities regulating their values as described by Belleville et al. (1982).

Barragry (1994) reported that Salinomycin is one of polyether antibiotic group and was found to exert its ionophorous properties on the cations transport through the cell membrane of animal tissues, bacteria and protozoa. Heil et al. (1984) determined the tissue residues of *Salinomycin* in rabbits. They reported that at zero withdrawal day, the liver contained 10 ppm *Salinomycin*. Moreover, Atef et al. (1993) concluded that the highest concentration of *Salinomycin* was in liver, kidneys, muscles, fat and heart. They also mentioned that the toxic effect induced by Salinomycin may be due to its action on cellular cations homeostasis (Westley, 1983) or residual effects on tissue cells (Dimenna et al., 1990).

The adverse effect induced by salinomycin on the haemostatic mechanism of bucks which was recorded after 8 weeks of treatment might be due to the toxic effect of Salinomycin and / or its residues on tissue cells, particularly liver cells, which affect its capability for protein synthesis, including coagulation proteins, leading to insufficient coagulation mechanism (hypocoagulability) that could be facilitate internal bleeding disorders which is fatal if occurred in vital organs. Also, intramuscular bleeding might be unacceptable which renders the rabbit meat unmarketable causing economic losses. On the other hand, Abaza and Omara (2011) were found that Supplementation of Kemzyme to the diet of rabbit improved the liver functions keeping AST and ALT values within normal. The same results were confirmed by Abd El-Fattah et al. (2003) and Ibrahim and Saleh (2005). These findings may explain the protective role of multi-enzymes feed additive on liver function maintaining normal haemostatic mechanism.

In conclusion, enzyme feed additive (Kemzyme) was more safer to be used as a growth promoters in rabbits. Salinomycin feed additive should be not used more than 4 weeks to avoid its adverse effect on haemostasis and the subsequent economic losses.

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